



Optical Biopsy with Probe-based Confocal Laser Endomicroscopy for Prediction of Residual Barrett's Esophagus after Complete Radiofrequency Ablation (RFA)– A Pilot Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SP, KM, CMW and HN designed the study, wrote the protocol and performed the indicated procedures. Authors AG and GN performed the data analysis. Author KPC edited the manuscript and performed literature searches. All authors approved the final manuscript.

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ABSTRACT

Introduction: Radiofrequency ablation (RFA) of Barrett's esophagus (BE) is associated with a high rate of complete eradication and a reduced risk of disease progression. Nevertheless, recent data indicate that about one third of patients had disease recurrence after reaching complete remission.

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Aim: To evaluate whether probe-based confocal laser endomicroscopy (pCLE) can determine complete eradication of BE as compared to histopathology from biopsy after complete RFA for optimized diagnosis in real-time and guide subsequent therapy.

Materials and Methods: Consecutive patients undergoing RFA for treatment of BE were prospectively included. pCLE was performed after complete eradication (CE) of dysplasia (CE-D) or intestinal metaplasia (CE-IM) was reached. CE was defined as complete eradication of BE as documented by histopathology obtained from mucosal biopsies. Residual BE was defined as the presence of intestinal metaplasia or dysplasia in standard surveillance biopsies. Two experienced gastrointestinal pathologists confirmed pathology findings.

Results: Based on histopathological analysis 33% of patients (3/9) had high-grade dysplasia, and 67% (6/9) had low-grade dysplasia. RFA was successfully performed in all patients (median age 60±10 yrs.). Three (33%) patients underwent endoscopic mucosal resection (EMR) followed by RFA. Patients received a median of 3±0.6 treatment sessions of RFA after which EGD with biopsies and pCLE were performed. pCLE documented CE-D and CE-IM in 78% and 44% of patients, while histology did in 90% and 67% respectively. Overall sensitivity, specificity, and accuracy of pCLE for real time diagnosis of residual BE after completed RFA treatment was 80% (95% CI 0.43–0.98), 75% (95% CI 0.28–0.98), and 78% (95% CI 0.36–0.98), respectively. Positive and negative predictive values were 80% (95% CI 0.42–0.98) and 75% (95% CI 0.28–0.98).

Conclusion: pCLE is yet not reliable for *In vivo* diagnosis of residual BE after complete RFA in real time. Larger, prospective studies are now highly warranted to further proof this initial concept.

Keywords: Barrett's esophagus; confocal endomicroscopy; radiofrequency ablation; imaging.

1. INTRODUCTION

Barrett's esophagus (BE) is a premalignant condition whereby there is replacement of the normal stratified squamous epithelium by intestinal metaplasia. Patients with BE have a 30-50 fold increased risk of esophageal adenocarcinoma (EAC) compared to those without the condition and early detection of dysplasia and treatment improves outcomes preventing progression to malignancy [1].

Radiofrequency ablation (RFA) is safe and effective in eradicating dysplasia in BE and usually requires multiple treatment sessions [2,3]. Ablation sessions are continued in 2-3 monthly intervals until no columnar epithelium is seen in the distal esophagus. Various studies have reported their absolute rates of complete eradication of dysplasia (CE-D) and complete eradication of intestinal metaplasia (CE-IM) with estimates of durability of neo-squamous epithelium after RFA [4]. Results from a U.S. multicenter consortium have reported a 33% chance of recurrence at the end of two years post RFA [5]. A recent meta-analysis of 18 studies showed that CE- IM was achieved in 78% and CE- D in 91% [6]. After eradication, IM recurred in 13%. Another study using the U.S. RFA nationwide registry showed that BE recurred in 20% of patients followed for an average of 2.4 years after CE-IM [7].

Yet, it is difficult to assess whether all BE epithelium has been eradicated specially at the neo squamo-columnar junction. The durability is also limited due to sampling errors during biopsy acquisition, possibility of buried metaplasia and adequate targeting of ablation especially in areas such as the gastroesophageal junction. Advanced imaging technologies have emerged over the last decade which aid in targeted biopsies and for preventing disease progression as well as surveillance with variable success [8]. The use of probe-based confocal laser endomicroscopy (pCLE) in the setting of BE has been established, however the role of pCLE for prediction of completion of ablation therapy or surrogate testing for establishing eradication has yielded conflicting results in prior multi-center trials [9]. Additionally, pCLE could serve as a reliable detection tool for residual intestinal metaplasia in real time. We therefore evaluated whether pCLE can detect residual BE after complete RFA for optimized diagnosis and to direct subsequent therapy in a pilot series.

2. PATIENTS AND METHODS

After Institutional Review Board (IRB) approval, consecutive patients undergoing RFA for treatment of Barrett's esophagus at the University of Alabama at Birmingham (UAB) between 2011- 2012 were prospectively included. Currently, RFA is being performed on patients with BE who have histological evidence

of dysplasia. All patients provided written informed consent to undergo RFA and pCLE. Data abstracted for analysis included patient demographic characteristics, medical history, indication for RFA, pathological findings, endoscopic findings, endoscopic procedures, adverse events, follow up endoscopic findings, treatment and biopsies with histopathology findings on surveillance. Patients with prior endoscopic treatment for Barrett esophagus, active malignancy or less than 19 years old or older than 85 years old were excluded.

2.1 Procedure Description

Patients were placed in the decubitus supine position. All procedures were performed with patients under monitored anesthesia care (MAC). Measurements of BE was done using the Prague Classification [10]. Patients underwent ablation using the circumferential device (HALO³⁶⁰ system) or a focal device (HALO⁹⁰ both from Covidien GI Solutions) according to extent of disease and investigator preference as previously described². Subsequent ablation sessions were performed every 2 months, until complete endoscopic and histological eradication of Barrett's esophagus. At each ablation session, the gastro-esophageal junction was ablated circumferentially, irrespective of its endoscopic appearance. Endoscopic Mucosal Resection (EMR) was performed using the Band ligation technique for nodular lesions using the Duette Multi-Band Mucosectomy Device (Wilson-Cook, Winston-Salem, NC, USA).

pCLE was performed after complete eradication of intestinal metaplasia (CE -IM) was reached. The Miami classification was used to describe findings of pCLE for evaluation of IM and dysplasia [11]. For the purpose of this study, the probe-based confocal laser endomicroscopy system (pCLE; Cellvizio, Mauna Kea Technologies, Paris, France) was used. pCLE is based on tissue illumination with a lower power laser after application of fluorescence agents. First, 5 ml of 10% fluorescein sodium (Alcon Laboratories, TX) were intravenously injected. Afterwards, the handheld pCLE probe was advanced through the working channel of a standard endoscope and gently applied to the esophageal tissue. In order to obtain real time videos, confocal images are streamed at a frame rate of 12 frames per second. In our study, the GastroFlex UHD probe was used. Technical features of the probe include a lateral resolution of 1 µm, a field of view of 240 µm and an imaging

plane depth of 55-65 µm. In order to maintain adequate image quality, a clear distal cap was placed at the tip of the endoscope and mild suction was performed during confocal imaging at 1000-fold magnification.

CE-IM and CE-D were defined as complete eradication of IM and dysplasia, respectively, as documented by histopathology from mucosal biopsy obtained by white-light endoscopy (WLE) (GIF Q 160 Olympus, Tokyo, Japan). Residual BE was defined as the presence of IM or dysplasia in optical or standard surveillance biopsies. The neo squamo-columnar junction was assessed in every case by both WLE with biopsies and pCLE. For surveillance, 4-quadrant biopsies were performed at every 2-cm interval of the original extent of the Barrett esophagus, starting at 1 cm proximal to the top of the gastric folds. In addition, any suspicious visible lesions were targeted, biopsied, and placed in separate jars. All biopsy sites were first examined by pCLE with subsequent mucosal biopsy following, with pCLE analysis and mucosal biopsies taken from similar sites to enable comparison. All pCLE sequences were analyzed in real-time in addition to off-line analysis post-procedure. These findings were then compared to the gold standard diagnostic method, histopathology, which was determined by an experienced gastrointestinal pathologist blinded to endoscopic and pCLE data. Eradication of intestinal metaplasia/ dysplasia was confirmed with endoscopic findings and 4 quadrant biopsy protocol.

2.2 Data Analysis

Categorical variables were summarized as frequencies and percentages. Continuous variables were summarized as mean and standard deviation when normally distributed and as median and interquartile range (25th and 75th percentiles) when not normally distributed. All analyses were performed using SPSS Statistics version 22IBM®.

3. RESULTS

A total of nine patients were studied for eradication using white light and pCLE after RFA. 33% of patients (3/9) had high-grade dysplasia and 67% (6/9) had low-grade dysplasia and all underwent RFA (median age 60±10 yrs., males= 7) (Table 1). The length (median, IQR) of circumferential BE (C) was 4 (6.5) cm and maximum BE (M) was 5.5 (5.5) cm.

Radiofrequency ablation (RFA) was successfully performed in all patients. Three (33%) patients underwent EMR followed by RFA. Patients received a median of 3.3±1.1 treatment sessions of RFA.

When repeat endoscopy using white-light endoscopy showed CE- D or CE- IM, EGD with biopsies and pCLE were performed. pCLE documented CE-D and CE-IM in 78% and 44% of patients, while histology did in 90% and 67% respectively. The patients who had persistent dysplasia on histology underwent further RFA treatment sessions.

The sensitivity, specificity and accuracy of pCLE for real time diagnosis of residual BE after completed RFA treatment was 80% (95% CI 0.43 – 0.98), 75% (95% CI 0.28–0.98), and 78% (95% CI 0.36–0.98), respectively. Positive and negative predictive values were 80% (95% CI 0.42–0.98) and 75% (95% CI 0.28 – 0.98) (Table 2).

Table 1. Baseline patient characteristics

	Barrett's and RFA n=9
Age, mean±(SD), years	60.1±(10.5)
Men, No. (%)	7 (77.8)
White race/ethnicity, No. reported (%)	9 (100.0%)
BMI, mean±(SD)	31.8±(7.8)
Circumferential Barrett's esophagus, median (IQR), cm	4 (6.5)
Maximum Barrett's esophagus, median (IQR),cm	5.5 (5.5)
History of gastroesophageal reflux disease, No (%)	9 (100)
Tobacco/ smoker, No. reported (%)	4 (44.4)
Number of RFA sessions, mean±(SD)	3.3±(1.1)
Number of patients with high grade dysplasia	3 (33.3%)

Table 2. Comparison of findings between pCLE and histopathology

	pCLE (n=9)	Histopathology (n=9)
CE- IM	7 (78%)	8 (90%)
CE- D	4 (44 %)	6 (67%)

On follow up, based on histopathology, 6 patients were followed for 1 year after RFA with surveillance endoscopy and had no recurrence of

BE, 2 patients had focal IM and had further RFA, and 1 patient had high grade dysplasia with recurrence and underwent chemoradiation therapy.

4. DISCUSSION

In this study we have evaluated the potential of pCLE for *In vivo* prediction of residual BE after complete radiofrequency ablation (RFA). We found that pCLE is yet not reliable for *In vivo* diagnosis of residual BE and could therefore not replace standard biopsies.

Radiofrequency ablation (RFA) is a safe and effective treatment of Barrett's esophagus and associated neoplasia. One initial multicenter, sham-controlled trial randomly assigned 127 patients with dysplastic Barrett's esophagus in a 2:1 ratio to receive either radiofrequency ablation (ablation group) or a sham procedure (control group) [2]. Overall, 77% of patients in the ablation group had complete eradication of intestinal metaplasia, and significantly less disease progression and fewer cancers as compared to the control group. Complications were rare and include upper gastrointestinal hemorrhage, and esophageal stricture. Those initial results were also confirmed by a recent meta-analysis including 18 studies describing a complete eradication of intestinal metaplasia and dysplasia in 78% and 91%, respectively [6].

Despite its effectiveness, recurrence of intestinal metaplasia after complete eradication of the Barrett's segment is remarkable. One recent study by Gupta and coworkers [5] analyzed data from 592 patients with BE treated with RFA. Fifty-five percent of patients underwent endoscopic mucosal resection before RFA. Twenty-four months after complete remission of intestinal metaplasia was reached, the incidence of recurrence was 33%. Importantly, 22% of all recurrences observed were dysplastic BE thereby suggesting the importance of continued surveillance after RFA [3]. The remarkable recurrence after RFA has also been confirmed in various other studies reporting on recurrence rates of 13-20% within 2-years after complete eradication of BE [6,8].

Various factors have been shown to be associated with the recurrence of BE after RFA. These include regeneration of the endoscopic resection wound with Barrett's instead of squamous epithelium, the absence of squamous islands in the Barrett's segment [12] and

aggressive neoplastic Barrett's cells acquiring certain genetic abnormalities rendering it resistant to RFA [13,14].

Therefore, continued surveillance with multiple biopsies of the neosquamous epithelium after RFA is essential for optimized diagnosis and individual patient's management.

Confocal laser endomicroscopy has been established as a potentially valuable tool for diagnosis of Barrett's esophagus allowing real time *in vivo* optical biopsies at 1000-fold magnification during ongoing endoscopy. The initial study by Kiesslich et al. described the potential of pCLE to predict BE and associated neoplasia with an accuracy of 97% [15]. Those results have been confirmed by a variety of studies [9,16-18]. The main potential advantage of pCLE is the possibility to obtain real time tissue diagnosis thereby allowing immediate decision making without the need to wait for the final histopathological diagnosis. Accordingly, pCLE could be an ideal tool to evaluate the esophagus for residual BE after complete radiofrequency ablation to guide subsequent therapy.

In this pilot study we have prospectively evaluated this question. However, we found that pCLE is not reliable for *In vivo* assessment of residual Barrett's tissue after RFA. The recent introduced ASGE PIVI statement of BE proposed that for an imaging technology to eliminate the need for random mucosal biopsies during the endoscopic surveillance a sensitivity of at least 90% and a negative predictive value of at least 98% is needed. In addition, the new imaging technology should have a specificity that is sufficiently high (80%) to allow a reduction in the number of biopsies (compared with random biopsies). In our study we report on a sensitivity and negative predictive value of 80% and 75%, respectively. Potential explanation for the limited effectiveness of pCLE in this setting might be the fixed imaging plane depth of the confocal probe which is 55-65 μm . Therefore, pCLE might miss Barrett's tissue located below the neosquamous epithelium [19]. In this context it has also been shown that biopsies taken with jumbo forceps significantly improve dysplasia detection and adequate tissue sampling in patients with BE [20]. As the tissue penetration is based on the laser light of the confocal system, which is yet restricted to 488 nm, one could not expect to solve this issue with the currently available pCLE-systems. However, it might be interesting to study the potential of optical coherence

tomography in the described setting as OCT allows tissue interpretation of up to 3 mm in depth. One additional explanation for the limited value of CLE for prediction of residual tissue after RFA might be the aggravated imaging at the squamocolumnar junction according to motion artefacts. However, in our series we did not observe any significant limitation during confocal imaging, which is also in line with other studies demonstrating the potential of CLE to visualize the gastroesophageal junction [21]. Another potential limitation of our study is that our cohort included both LGD and HGD, whereas previous pCLE studies solely investigated detection of HGD.



Fig. 1. BE segment WLE



Fig. 2. BE after RFA

Potential limitations of our study have to be addressed. First, we are describing a single center pilot study at a tertiary referral center with a limited sample size. Accordingly, the results should be validated in a prospective study including a larger cohort of patients. In addition, examiners had extensive knowledge of CLE. Therefore, the results of this study may not be transferred to the general practice. However,

even pCLE is not yet established in the community setting and the aim of the study was to assess the potential value of pCLE to predict

complete eradication and enhance potential detection of residual BE after successful and complete RFA.

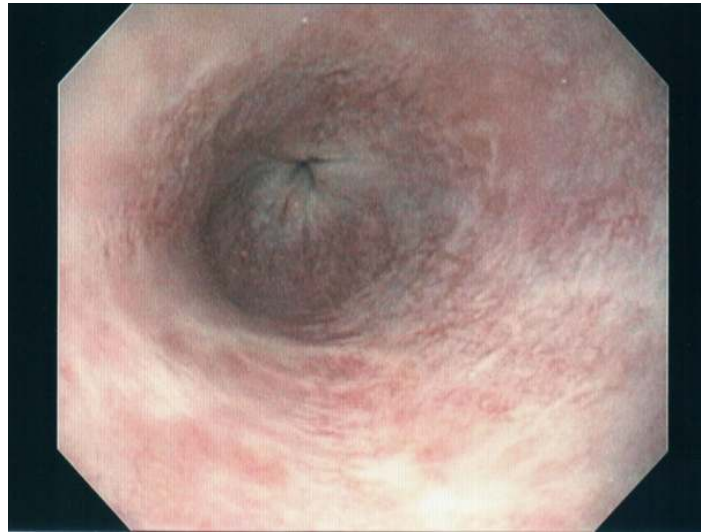


Fig. 3. WLE of normal neosquamous epithelium

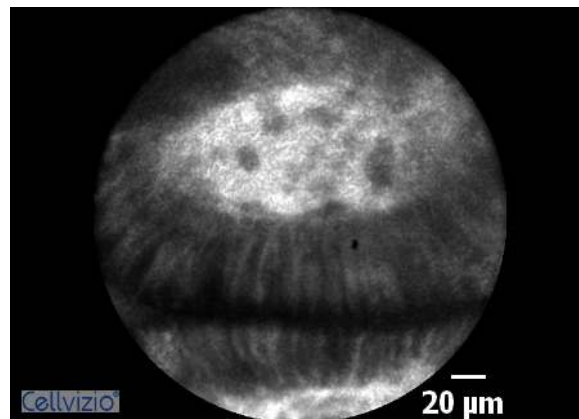


Fig. 4. pCLE of metaplasia

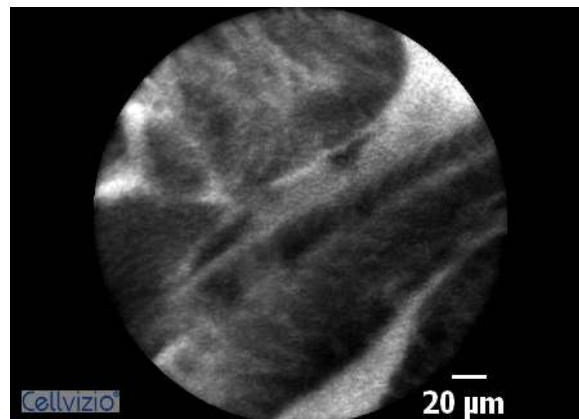


Fig. 5. pCLE of dysplasia

5. CONCLUSION

Taken together, in the present study we have evaluated the potential of confocal imaging to diagnose residual BE after complete RFA. We have shown that pCLE is yet not reliable for *in vivo* prediction of residual tissue. Therefore, the current approach of random biopsies should still be the reference standard. Based on our findings, it can be argued that pCLE was likely to detect more residual BE (and hence lower percent of CE-D and CE-IM) than histopathology and therefore may be considered a promising new technology more effective in detecting residual IM after RFA. However, this interpretation may lead to overtreatment as we still contend that histopathology is the gold standard of diagnostic techniques. Future studies should validate our findings in a larger setting and also evaluate the potential of OCT as the technique allows deeper tissue interpretation. At least, even in our pilot study we have confirmed the not deniable rate of residual metaplasia even after complete RFA. Therefore, regular endoscopic surveillance with multiple random mucosal biopsies after RFA is highly warranted.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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