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Stratification of the dysplasia and neoplasia risk using autofluorescence endoscopic surveillance of Barrett's esophagus

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ABSTRACT

Background: This study assessed the efficacy of autofluorescence endoscopy (AFE) using the Onco-LIFE system and numerical color value (NCV) estimation in comparison to white light endoscopy (WLE) in endoscopic surveillance for identification of early dysplasia in Barrett's esophagus (BE) to aid in real-time image elucidation and minimize the overreliance on biopsy and histology.

Methods: AFE, performed simultaneously during WLE, with biopsy was performed among 24 patients with BE. None of these patients had any obvious mucosal abnormalities in WLE. A total of 376 biopsies were taken, include 325 randomly collected according to Seattle Protocol and 51 additional biopsies, taken from the sites with pathological AF and NCV. All biopsy sites were assessed *in vivo* using WLE, AFE and NCV and compared to histological examinations, to estimate the efficacy of these methods in dysplasia assessment in BE.

Results: In the case of 248 biopsies taken from sites with NCV below 1.0, two cases of unspecified dysplasia were recognized; in 14 biopsies with NCV above 2.0 in all cases the various grades of dysplasia were documented. Dysplasia was found in 42% of AFE + NCV- guided biopsy specimens, and in 7.1% of WLE-guided biopsy specimens. AFE + NCV detected high-grade dysplasia in 7 patients, 6 more than according to Seattle Protocol in WLE. The expected odds of dysplasia detection in a sample increases almost 1.9 times, if it was selected by the AFE method ($p < 0.001$), when compared to WLE and with accordance with Seattle Protocol guided biopsy.

Conclusion: The above results indicate that AFE + NCV using the Onco-LIFE system leads to improved BE lesion visualization for targeted biopsy with accurate histologic correlation compared to WLE and Seattle Protocol guided biopsy alone, and can serve to minimize additional biopsies.

1. Introduction

The development of esophageal adenocarcinoma (EAC), the incidence of which is increasing worldwide [1–4], is almost always preceded by Barrett's esophagus (BE). Patients with BE are predicted to be thirty to over one hundred twenty times more likely to develop adenocarcinoma than those without the disease. The pathogenesis of BE is associated with the presence of metaplastic epithelium in which malignant transformations appear, and cancer risk seems to be limited to patients with specified columnar epithelium with an increased rate of cellular proliferation. Development of carcinoma from dysplasia is not predetermined, and the morbidity of EAC in patients with BE is 0.5-0.8% over the course of one year and is many times higher than in the general population [5–7].

Treatment of BE can be performed using endoscopic eradication therapy, photodynamic therapy, and by reduction of gastroesophageal reflux. Until a few years ago, it was well established that intestinal metaplasia occurs in about 10% of chronic gastroesophageal reflux disease (GERD) patients, and 10% of these patients have a risk of EAC development. Very recent studies have shown that this risk can be

Abbreviations: AFE, autofluorescence endoscopy; ASGE, American Society for Gastrointestinal Endoscopy; BE, Barrett's esophagus; BING, Barrett's International NBI Group; CE, chromoendoscopy; CLE, confocal laser endomicroscopy; EAC, esophageal adenocarcinoma; ETMI, trimodal imaging; GERD, gastroesophageal reflux disease; HGD, high grade dysplasia; HRE, high-resolution endoscopy; LGD, low grade dysplasia; NBI, narrow-band imaging; NCV, numerical color value; OAC, esophageal adenocarcinoma; VCE, virtual chromoendoscopy; WL, white light; WLE, white light endoscopy

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lower (between 0.1 and 0.4% for over the course of one year) yet despite this, many attempts to modify endoscopic surveillance in patients with diagnosed BE has been routinely recommended [6,8,9]. One significant problem concerning endoscopic surveillance of BE is the difficulty in detecting early neoplastic lesions. Surveillance guidelines for Barrett's esophagus recommend taking random biopsies in conjunction with white light endoscopy (WLE) analysis for all macroscopic changes detected during examination, such as papules, erosions, scars and stenosis [7,10]. The Seattle Protocol, which is generally recommended and used in BE diagnosis, is based on classical WLE with random sampling of quadrant biopsies each 1–2 cm in total length of affected tissue. However, this procedure requires taking many random biopsies in which dysplasia may be not recognized and scars stemming from biopsies may lead to a search of new dysplastic changes, thus the method is inexorably associated with sampling errors [11].

Novel imaging techniques that use computer-based detectors to reconstruct endoscopic images can increase diagnostic accuracy in detection of dysplasia and early cancers reducing the number of random biopsies. These new methods include autofluorescence endoscopy (AFE), narrow-band imaging (NBI), high-resolution endoscopy (HRE), trimodal imaging (ETMI), chromoendoscopy (CE), and confocal laser endomicroscopy (CLE). High-resolution endoscopic methods allows for sharper images with fewer artifacts due to higher pixel density and faster line scanning on the monitor [12]. In NBI the tissue surface is irradiated using special filters that narrow the respective red-green-blue bands and simultaneously increase the relative intensity of the blue band. This method reveals particularly the tissue microvasculature mainly as a result of the differential optical absorption of light by hemoglobin in the mucosa [13]. Chromoendoscopy is a method in which contrast or absorptive dyes are sprayed on the mucosal surfaces to highlight the abnormal areas and to unmask flat and subtle appearing lesions. Its modality is virtual chromoendoscopy (VCE) in which a tissue image obtained as a result of white light reflection is reprocessed by software algorithm to VCE to avoid laborious and non-uniform application of stains and contrast medium. However, CE with intravital staining is regarded as more specific because it reacts directly with various epithelial entities [12]. The detectability of high-grade dysplasia and early cancer in BE with use of HRE, NBI and CE is similar [14]. The American Society for Gastrointestinal Endoscopy (ASGE) considered the pooled sensitivity and specificity for CE by using acetic acid and methylene blue, electronic chromoendoscopy by using NBI, and CLE for the detection of dysplasia [15].

AFE is a new technique based on the fluorescence of endogenous mucosal molecules after excitation with specific light wavelength [16,17]. Autofluorescence endoscopy allows for biopsies to be taken only for dysplastic areas revealing autofluorescence, and enables avoidance of methodological problems connected with staining methods for EAC that are time consuming and can be unreliable. Nevertheless, AFE requires a knowledgeable and experienced endoscopist. The detectability of high grade dysplasia in BE using AFE may approach 100% [18]. However, the limitation of this technique is a large number of false-positive results and new data suggest that AFE improved the detection of high grade dysplasia/esophageal adenocarcinoma (HGD/OAC), with 50% sensitivity, 61% specificity, and 71% negative predictive value (NPV) [19]. Therefore, AFE may be used for primary inspection to highlight possible dysplasia areas which should be re-inspected again with use of WLE or NBI [20].

In view of the above findings, the aim of this study was to establish the effectiveness of AFE, using the Onco-LIFE system and Numerical Color Value (NCV) in comparison to WLE and Seattle Protocol guided biopsies, in endoscopic surveillance to identify early dysplasia in Barrett's esophagus and to aid in real-time image elucidation targeted biopsy and to minimize overreliance on biopsy and histology.

2. Material and methods

2.1. Autofluorescence diagnostic based technique

In the Center for Laser Diagnostics and Therapy at the Medical University of Silesia in Bytom (Poland), the Onco-LIFE (Light-Induced Fluorescence Endoscopy) (Xillix, Richmond, Canada) was used to assess abnormal, potentially-cancerous tissue [21–23]. The Onco-LIFE system is based on autofluorescence and provides both white light illumination and fluorescence excitation. Autofluorescence imaging is based on light-induced changes in the absorption of porphyrins. The fluorescence of normal tissue contrasts from that of inflamed or neoplastic tissue characterized by an increased nuclear/cytoplasmic ratio, decreased collagen, and neovascularization. Blue light is used to illuminate and excite natural fluorophores presented in the tissue. Features of the light source include dual-mode operation for white light and fluorescence endoscopy with a main 150 W super-high-pressure mercury (Hg) arc lamp with a backup halogen lamp. The red (λ = 650–700 nm) and green (λ = 470–560 nm) wavelengths of the autofluorescence image are filtered and amplified by image-intensifying cameras. The images are analyzed and presented as a single real-time image on a monitor. The advantage of this system is the possibility of intensity evaluation in certain spectral ranges between the blue light-induced (λ = 395–445 nm) green autofluorescence and the red autofluorescence, followed by calculation of a ratio called a numerical color value (NCV) [22]. The NCV was developed by Xillix Technologies and defined as the red-to-green ratio in the center of an autofluorescence endoscopy image and in each pixel of the monitor [23].

Although AFE using the Onco-LIFE system is a helpful tool with the highest sensitivity for early dysplasia and cancer detection, its diagnostic specificity is lower because false positive results, especially in the case of inflammatory tissue which is difficult to distinguish from dysplastic tissue. Another limitation with AFE using the Onco-LIFE system is that only the surface tissue is assessed because light scattering and absorption seriously hinders imaging of deep tissue layers. There is also no possibility of a spatial assessment of the tumor and its fluorescence. Fluorescence imaging within biological tissues is one of the most important research tools, but due to the optical properties of the tissue, the focal excitation of fluorescence is limited to a depth of approximately 1 mm. The AFE is also influenced by the vascularity and thickness of the mucosa, as well as the amount of mucus. This is related to the reduction of submucosal fluorescence by hemoglobin and the change in collagen structure due to proteolytic enzymes. Therefore, a research on the application of new methods is underway, such as spectroscopy, or ultrasound-assisted techniques, such as photoacoustic-guided and time-reversed ultrasonically encoded (TRUE) optical focusing techniques, that employ a focused ultrasound beam as a "virtual guidestar".

Standards of diagnostic procedures have been introduced basing on more than 20 years of experimental observations and research using the Onco-LIFE system for pathological evaluation of fluorescence in different precancerous and cancer diseases based on the use of AFE with NCV methods for oncological diagnosis, including BE (Fig. 1) [21,22,24].

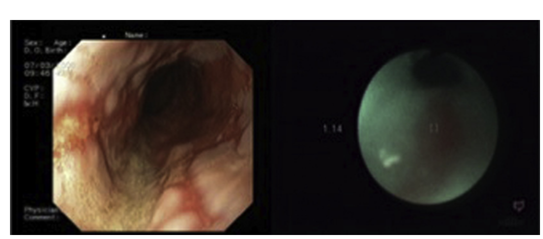

Fig. 1. The images present dysplasia in a patient with BE using WLE (left) and AFL with NCV (right).

2.2. Patients and procedures

Between the years 2005 and 2017, 24 patients with BE (19 men and 5 women, at average age of 38.7) were observed in the Center of Laser Diagnostics and Therapy. In 2005, 11 patients, in 2006 - 5 and in 2007 another 8 patients were included. This presented work concerns all endoscopic and biopsy examinations performed up until December 31, 2017. The research obtained ethical approval for BE AFE procedures.

Two hundred and sixty four WLE + AFE assessments with targeted biopsies in 24 patients with BE were performed using the Onco-LIFE system with simultaneously the measurement of NCV. Barrett's esophagus were recognized and histologically confirmed in this group previously. None of these patients had any obvious mucosal abnormalities in WLE. The frequency of endoscope investigations was estimated individually for each patient depending on clinical and histological results, and the average number of procedures was 11 endoscopies (simultaneously WLE and AFE + NCV) per patient during more than 10 years of follow up. Through this over 10 years observational period, in 24 patients with BE, a total of 376 biopsies were taken, include 325 randomly taken according to Seattle Protocol (standard endoscopic procedure that cannot be omitted- quadrantic biopsies every 2 cm). In addition to the biopsies and AFE measurements taken at sites according to Seattle protocol, many other normal appearing sites were measured using AFE-NCV and any that showed a value of NCV greater than 1.0 were biopsied. Typically about 0–9 of such sites, depended on pathological AFE-NCV, different for each patient in addition to the Seattle protocol sites. Overall, 51 such sites with an abnormal AFE - NCV were identified and biopsied. All biopsy sites were assessed *in vivo* using AFE and NCV and compared to histological examinations, to estimate the efficacy of these methods in dysplasia assessment in BE. Then the histopathological result was correlated with the NCV.

The histological estimation of BE was performed according to the Vienna classification of gastrointestinal epithelial neoplasia: (1) negative for neoplasia/dysplasia, (2) indefinite for neoplasia/dysplasia, (3) non-invasive low grade neoplasia (low grade adenoma/dysplasia), (4) non-invasive high grade neoplasia (high grade adenoma/dysplasia, non-invasive carcinoma and suspicion of invasive carcinoma), and (5) invasive neoplasia (intramucosal carcinoma, submucosal carcinoma or beyond).

All sites of collected biopsies were assessed using AFE and by the measurement of NCV. These sites were divided into four groups depending on NCV score: I. NCV < 1.0–248 biopsies, II. NCV: 1.0–1.49 - 90, III. NCV: 1.5–1.99 - 24, and IV. NCV > 2.0-14. Dysplasia was found in 45 biopsies. In group I, two cases of unspecified dysplasia were found. In group II- 11, in III group – 18, and in group IV- 14 cases of various grades of dysplasia were found. In the course of observation, dysplasia of various grades was identified in 9 patients, while dysplasia was not present in 15 patients (Table 1).

Thus, in the case of 248 biopsies taken from sites with NCV below 1.0 - only 2 cases of undetermined dysplasia were identified; in 14 biopsies with NCV above 2.0 in all cases, dysplasia was recognized (Table 2).

Table 1
The different grades of 45 dysplasias detected in 9 patients from the group of 24 patients subjected to AFE with NCV over a 10 year surveillance of BE.

BE Patients:	Indefinite dysplasia	Low grade dysplasia (LGD)	High grade dysplasia (HGD)
1.	4	2	0
2.	1	1	0
3.	2	1	1
4.	5	0	0
5.	3	0	2
6.	3	3	3
7.	3	0	2
8.	0	1	2
9.	1	2	3

2.3. Statistical methods

Mixed logistic models were used to investigate the relationship between the presence of dysplasia in the samples and method of sample selection as well as the NCV value measured at the sample extraction site. This type of regression is used to model dichotomic variables in which the log odds of the outcomes are modeled as a linear combination of predictor variables when the data are clustered or include both fixed and random effects. In this case the explained variable was detection of dysplasia based on a singular biopsy while individual differences between patients were taken into account as a random effect (as there were multiple biopsies taken from the same patients).

In order to evaluate the relationship between dysplasia (histology) and NCV values in biopsy, as well as to compare NCV values in samples collected using WLE and AFE methods, linear mixed effects models were constructed.

For mixed logistic models statistical significance of coefficients was tested with Wald's test and Tukey contrasts (from lsmeans package, version 2.27) were used to compare NCV values in biopsy samples and between samples collected using WLE and AFE methods. These results are presented as least squares means (lsmeans) that are calculated from model estimates.

The level of significance was assumed to be 0.05. All calculations were made using R statistical package (version 3.4). Mixed models were constructed and evaluated using LME4 library (version 1.1) [25,26].

3. Results

In the case of 248 biopsies taken from sites with NCV below 1.0, two cases of indefinite dysplasia were recognized, while in 14 biopsies with NCV above 2.0, dysplasia was documented in all cases (Table 2). Autofluorescence endoscopy detected high-grade dysplasia in 7 patients, 6 more than with using WLE.

Additional biopsies guided using the pathological AF had a significantly higher NCV (lsmean 1.45, 95% confidence interval; CI: 1.3–1.6) than those guided by WLE/AFE according to Seattle Protocol (lsmean 0.74, 95% CI: 0.67-0.83; $p < 0.001$, Fig. 2). In addition, biopsies, in which dysplasia has been detected (lsmean 1.81, 95% CI 1.68–1.95) had more than two and half times higher NCV values than biopsies where no dysplasia was present (0.72, 95% CI 0.66-0.77, $p < 0.001$, Fig. 3).

For biopsies in which dysplasia was detected but its degree had not been determined, the NCV value was on average about 0.7 higher than for those in which dysplasia was not detected (Table 3). When the degree of dysplasia was determined to be low, the NCV was on average higher by around 1.0 than in cases where dysplasia was not found (Table 3). A high degree of dysplasia was associated with higher (by around 1.4 NCV) values than those that determined biopsies without dysplasia (Table 3).

Table 4 presents predictors of dysplasia detection based on a logistic mixed models method: Model 1 – detection of dysplasia based on the NCV value, Model 2: Comparison of AFE to WLE. For model 1, the odds ratio refers to change of NCV value by 0.1 unit, while model 2 this relates to the number of times the chance of dysplasia was higher with the AFE method compared to the WLE method. The results indicate that with an increase of NCV by 0.1 unit, the odds of detecting dysplasia grow about 2.2 times (95% Confidence Interval 1.6–3.0; Table 4). The odds of dysplasia detection grew approximately 6-fold when the AFE method was compared to WLE (95% CI 2.8–15; Table 4). In both models, coefficients of logistic regression were statistically significant based on Wald's test ($p < 0.001$).

Table 5 presents a comparison of NCV values and the frequency of dysplasia depending on biopsy site selection method (AFE vs WLE vs Overall). Descriptive statistics were calculated for the histological

Table 2
Correlation of NCV with different degrees of dysplasia assessed in histology biopsies.

Results of 376 biopses	NCV < 1.0	NCV 1.0–1.49	NCV 1.5–1.99	NCV > 2.0
Negative for neo/dysplasia	246	79	6	0
Indefinite dysplasia	2	8	7	5
Low grade dysplasia (LGD)	0	2	7	1
High grade dysplasia (HGD)	0	1	4	8

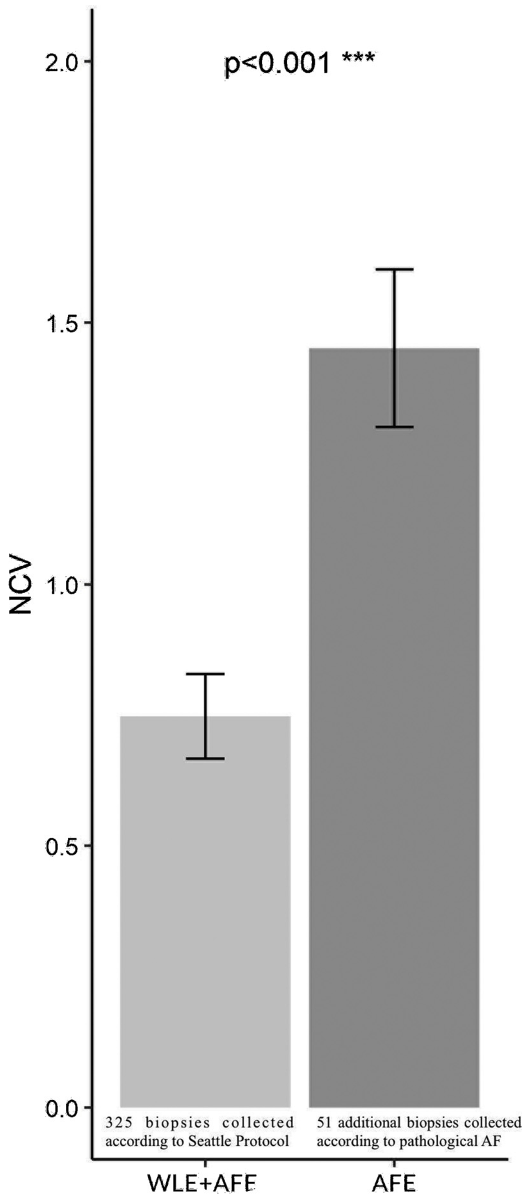

Fig. 2. Comparison of NCV from biopsy sites randomly designated according to the Seattle Protocol using WLE/AFE, and from additional sites with pathological AF/NCV (51biopsies*)*. Height of the bar represent lsmean, while error bars represent 95% confidence intervals.

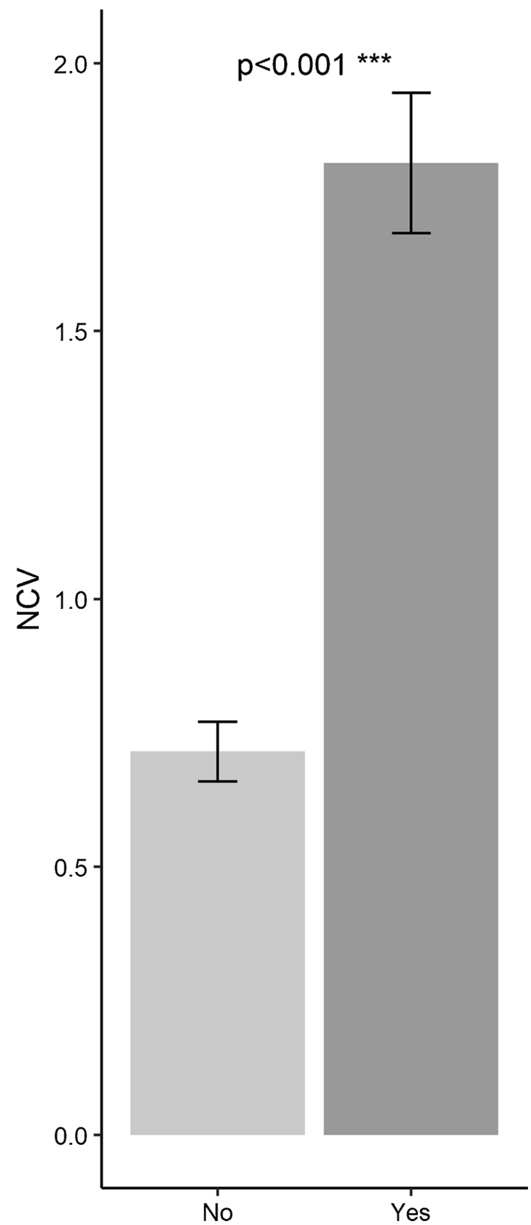

Fig. 3. Comparison of NCV in samples with (YES) and without dysplasia (NO). Height of the bar represent lsmean, while error bars represent 95% confidence intervals.

biopsies and assessment collected under WLE, AFE and NCV endoscopic control. The mean NCV was 0.84, with 1.52 for histological biopsies selected by AFE method and 0.74 for samples selected by WLE method (Table 5, Fig. 2). Dysplasia was detected in 42% of autofluorescence guided biopsy specimens significantly more often than in the case of WLE-guided biopsy specimens with this finding (7.1%).

Table 6 describes the dependence of NCV values on the presence or absence of dysplasia in histopathological examination of all collected biopsy specimens. For histological biopsies, when the dysplasia was detected, the mean NCV was 1.84, while for other histological biopsies it was 0.71 (Table 6). These results confirm that the NCV coefficient correlates with the presence of dysplasia, so the AFE is more useful compared to WLE (Table 6, Fig. 3). Fig. 3 presents the comparison of NCV in samples with and without dysplasia, and confirms that AFE using NCV is the useful tool for easy and sensitive dysplasia detection.

Table 3
Linear mixed model coefficients describing NCV values in respect to results of histology. Fixed effects.

	Estimate (95% CI)	Pr (> |t|)*
Dysplasia which degree was not detected	0.7152 (0.4814–0.9489)	0
LGD (low grade dysplasia)	1.054 (0.88–1.229)	0
HGD (high grade dysplasia)	1.419 (1.203–1.635)	0

* This is the two-tailed p-value evaluating the null against an alternative that the mean is not equal to 50. It is equal to the probability of observing a greater absolute value of t under the null hypothesis. If p-value is less than the pre-specified alpha level, the mean is statistically significantly different from zero.

Table 4
Predictors of dysplasia detection based on logistic mixed models method: Model 1 – detection of dysplasia based on NCV value, Model 2: Comparison of AFE to WLE. For model 1, odds ratio refers to change of NCV value by 0.1 unit, while model 2 relates to the number of times the chance of dysplasia is higher with the AFE method compared to the WLE method.

	Odds ratio (95% CI)	Pr(> |z|)*
Model 1: NCV value	2.205 (1.6–3)	< 0.001
Model 2: AFE vs WLE	6.443 (2.8–15)	< 0.001

* The z value is the Wald statistic for testing the hypothesis that the corresponding parameter (regression coefficient) is zero. Under the null hypothesis it has an approximately N(0,1) distribution. P(> |z|) is the tail area in a 2-tail test, i.e. the test within a 2-sided outer hypothesis.

Table 5
Descriptive statistics. Comparison of NCV values and the frequency of dysplasia depending on biopsy site selection method (AFE vs WLE vs Overall).

Variables	Parameter	Overall (N = 376)	AFE (N = 51)	WLE (N = 325)
NCV	N	376	51	325
	Mean (SD)	0.84 (0.54)	1.52 (0.4)	0.74 (0.48)
	Median (IQR)	0.67 (0.4–1.25)	1.45 (1.25–1.72)	0.65 (0.36–1.01)
	Range	0.25–2.53	0.9–2.53	0.25–2.42
Dysplasia	No	88.3% (N = 332)	58.8% (N = 30)	92.9% (N = 302)
	Yes	11.7% (N = 44)	42% (N = 21)	7.1% (N = 23)

Table 6
Descriptive statistics. Dependence of NCV values on dysplasia (D) presence (Yes D) or absence (No D) in histopathological examination of all collected biopsy specimens.

Variables	Parameter	Overall (N = 376)	No D (N = 332)	Yes D (N = 44)
NCV	N	376	332	44
	Mean (SD)	0.84 (0.54)	0.71 (0.39)	1.84 (0.4)
	Median (IQR)	0.67 (0.4–1.25)	0.65 (0.36–1.01)	1.84 (1.49–2.25)
	Range	0.25–2.53	0.25–1.75	0.92–2.53

4. Discussion

From an endoscopist's point of view, the attraction to using imaging techniques is to improve the accuracy of screening for early superficial neoplasia [27].

This study has shown that the sensitivity of WLE and Seattle Protocol guided biopsies in dysplasia detection was only 7.1%, while for AFE + NCV targeted biopsies it is set at a higher value of 42% demonstrating a statistically significant difference. Another diagnostic study has revealed that advanced imaging techniques increased the diagnostic yield for the detection of dysplasia or cancer by 34% [27]. Qumseya et al. performed a review study, using Medline and Embase to identify peer-review investigations performed for detection of esophagus dysplasia or cancer. The author's included research that comparatively evaluated both WLE/RB and either one of the new imaging modalities (MB, IC, AFE, Fujinon intelligent chromoendoscopy- FICE/AA, CE and VC) for the detection of dysplastic changes in patients with BE. Fourteen studies were analyzed, including AFE studies with a total of 843 patients based on the QUADAS tool. The results of Qumseya's meta-analysis indicate an increase in the diagnostic detection of dysplasia/cancer when using advanced imaging including AFE [27]. In turn, Sharma and Yu Ho in "Recent Updates in the Endoscopic Diagnosis of Barrett's Oesophagus" analyzing WLE, NBI, and autofluorescence imaging for the detection of HGD/OAC, revealed, that autofluorescence imaging alone had a sensitivity, specificity, and NPV of 50, 61, and 71%, respectively, with an overall accuracy of 57%. By using magnification NBI in a combined modality, the sensitivity rises respectively to 71 and 76%. Simultaneously, the author emphasizes that ASGE confirmed the pooled sensitivity and specificity for CE by using acetic acid and methylene blue, electronic chromoendoscopy by using NBI, and CLE for detection of dysplasia [15]. These findings clearly suggest the efficacy of AFE for the assessment of BE dysplasia and confirmed the adjunctive and significant role of the NCV, which correlates with histological assessment. This result implies an advantage of the AFE with NCV technique over classical WLE in detection of early dysplasia in Barrett's esophagus [16,17]. Thus, AFE can improve the detection of dysplasia/early cancer in patients with BE [18,28]. This study revealed that in accordance with AFE and NCV increase, the risk of dysplasia in patients with BE increase too. WLE is associated with imperfect sensitivity and biopsy sampling is associated with specimen error. Hence, from a practical clinical viewpoint, endoscopists should aim to use supplementary tools to help in real-time image clarification and to minimize the number of biopsy specimens for histology. In the context of our optimistic and promising results, pointing to the possibility of improving the visualization of BE suspected lesions and the possibility of a targeted biopsy, critical reports of other authors limiting the AFE method are surprising and difficult to agree with.

It is emphasized by some researchers that the drawback of the AFE method is its relatively low specificity (high false-positive rates) [11,16,17,21,22]. Also, some authors draw attention to the fact that the current AFE systems are limited by low signal-to-noise ratios. In the study of Curvers and coworkers, AFE identified 90% of the patients with neoplastic lesions in Barrett's esophagus, significantly more than the 53% identified by WLE, however, the false-positive rate was 81% [11]. Similarly, Kato et al. in a prospective blinded study systematically comparing AFE with WLE for the detection of superficial gastric neoplasia, found poor AFE specificity and its clinical value as limited [29]. Moreover, Borovicka and coworkers showed that the AFE-guided approach cannot replace the standard four-quadrant biopsy protocol [30]. This drawback may be overcome by using NBI for detailed examination, which reduces the false-positive rate to 26%. In this scenario, AFE can be used as a 'red flag' technique, aiding the endoscopist to concentrate on suspicious areas while a NBI technique as such is used to explore minute details [31]. On the other hand, the skill of the endoscopist is also important in identifying early gastric cancers [16,31]. In this study, dysplasia detection in a sample increases almost 1.9 times if it was selected by the AFE method ($p < 0.001$) compared to WLE. It seems that the main dominant affecting this advantageous result is the possibility of NCV-guided targeted biopsy. Also in previous studies, a significant correlation between lesion NCV index and the grade of dysplasia or GI tumor malignancy showed that the Onco-LIFE system is a helpful tool when performing targeted biopsies, but it was emphasized that the most important factor determining the reliability of results is the experience of the endoscopist [22,24].

From a practical point of view, the use of the AFE method and targeted biopsy presented in the above study clearly demonstrates usefulness in the early detection of BE suspicious lesions and easily indicates regions for targeted biopsy. However, it is worth stressing that endoscopic experience in use of this technique is essential because otherwise, from a practical view, it is then necessary to note the limitations of the AFE method. Dysplasia/cancer can be oversighted or neglected both in random biopsies and in AFE. Therefore, it is not known undoubtedly if a patient has no dysplasia when there is a negative biopsy result similar to false positive results. Therefore, calculating sensitivity, specificity, positive predictive value and negative predictive value is potentially not biased. The only verification of evaluation of AFE examination in the detection of dysplasia or cancer in BE would be histopathologic examination after total resection of BE, in which all mucosa of BE would be resected endoscopically [32]. Advanced endoscopic method can overcome limitations of the present AFE system.

The Barrett's International NBI Group (BING) established a NBI classification system for categorizing dysplasia and EAC in BE. In the current web-based analysis by experts, the BING criteria were certified for their capability to predict dysplasia in BE. Experts from the working group studied 120 NBI images not previously seen and expected histopathology using the BING criteria. The experts rated their level of confidence in their predictions as high or low, and were able to predict dysplasia at 62.5%. On the basis of the expert research, the BING criteria identified patients with dysplasia with an 85% overall accuracy, 80% sensitivity, 88% specificity, 81% positive predictive value (PPV), and 88% NPV [33]. This study suggests that in like manner, AFE imaging could be used by an experienced endoscopist to predict the presence or absence of dysplasia in AFE + NCV images. The AFE + NCV simple method with prospective for use in BE surveillance in routine clinical practice can be competitive with the criteria proposed by BING. Surprising is the study of Giacchino et al., who estimated the clinical utility of AFE and magnification NBI to detect HGD and EAC in a group of 42 patients (14 with HGD/EAC) and reveled that AFE alone had a 50% sensitivity, 61% specificity, and 71% NPV and by using AFE and NBI together, the sensitivity improved to 71% and NPV to 76% [19]. Simultanously the author concluded that a multimodality endoscopy system using AFI and magnification NBI is not yet accurate enough for the detection of HGD/EAC based on results established by the American Society for Gastrointestinal Endoscopy Preservation and Incorporation of Valuable Endoscopic Innovations thresholds, but further efforts are needed to identify a new, easy-to-use endoscopic technology with a simple classification system that could improve the detection of HGD and EAC in patients with BE.

The results presented here are the first to estimate agreement between AFE and NCV with histological results of dysplasia in BE. Hence, these results confirm the clinical usefulness of AFE with the NCV estimation and this is supported by accurate statistical data. It has been demonstrated in this study that although AFE does not allow avoidance of a standard diagnostic protocol of quadrant biopsies, it delivers useful diagnostic information regarding endoscopic surveillance of BE. Moreover, the NVC significantly correlates with histology results. Many researchers are looking for reliable methods for visualizing dysplastic and neoplastic changes in BE and may have doubts about new imaging techniques. Our findings clearly demonstrate the usefulness of AFE with NCV performed by an expert endoscopist.

Future studies are necessary to compare autofluorescence endoscopy with the standard biopsy protocol (4 quadrant, 2 cm) and to assess the cost-effectiveness ratio of AFE as a method of dysplasia/early cancer diagnosis in BE patients, in relation to other imaging techniques.

When WLE + AFE + NCV images are evaluated with a high degree of certainty, this method can classify BE with highest accuracy and with a high level of histology agreement.

5. Conclusion

WLE + AFE + NCV using the Onco-LIFE system leads to improved BE lesion visualization for targeted biopsy with accurate histologic correlation when compared to WLE alone, and can serve to minimize additional biopsies, as the Seattle Protocol requires. The clinical advantage of this targeted biopsy technique is the possibility of reducing the number of biopsies to decrease the risk of new metaplastic changes associated with the scars that remain after a biopsy is taken. Thus, this method can objectively guide the endoscopist in selecting sites for biopsy with appropriate pathologic association and also is useful in the monitoring of patients with Barrett's esophagus. Hence, these results show the potential clinical usefulness of AFE with the NCV estimation which is supported by accurate statistical data.

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