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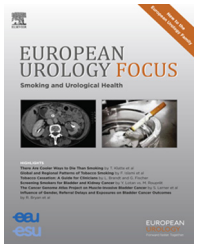

Bladder Cancer

Validation of Confocal Laser Endomicroscopy Features of Bladder Cancer: The Next Step Towards Real-time Histologic Grading

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Abstract

Background: Cystoscopy enables the visualisation of suspicious bladder lesions but lacks the ability to provide real-time histopathologic information. Confocal laser endomicroscopy (CLE) is a probe-based optical technique that can provide real-time microscopic images. This high-resolution optical imaging technique may enable real-time tumour grading during cystoscopy.
Objective: To validate and adapt CLE criteria for bladder cancer diagnosis and grading.
Design, setting, and participants: Prospectively, 73 patients scheduled for transurethral resection of bladder tumour(s) were included. CLE imaging was performed intraoperatively prior to en bloc resection. Histopathology was the reference standard for comparison.
Intervention: Cystoscopic CLE imaging.
Outcome measurements and statistical analysis: Three independent observers evaluated the CLE images to classify tumours as low- or high-grade urothelial carcinoma (UC), or benign lesions. Interobserver agreement was calculated with Fleiss kappa analysis and diagnostic accuracy with 2×2 tables.
Results and limitations: Histopathology of 66 lesions (53 patients) revealed 25 low-grade UCs, 27 high-grade UCs, and 14 benign lesions. For low-grade UC, most common features were papillary configuration (100%), distinct cell borders (81%), presence of fibrovascular stalks (79%), cohesiveness of cells (77%), organised cell pattern (76%), and monomorphic cells (67%). A concordance between CLE-based classification and histopathology was found in 19 cases (76%). For high-grade UC, pleomorphic cells (77%), indistinct cell borders (77%), papillary configuration (67%), and disorganised cell pattern (60%) were the most common features. A concordance with histopathology was found in 19 cases (70%). In benign lesions, the most prevalent features were disorganised cell pattern (57%) and pleomorphic cells (52%), and a concordance with histopathology was found in four cases (29%).
Conclusions: The CLE criteria enable identification of UC. CLE features correlate to histopathologic features that may enable real-time tumour grading. However, flat lesions remain difficult to classify.
Patient summary: Confocal laser endomicroscopy may enable real-time cancer differentiation during cystoscopy, which is important for prognosis and disease management.

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1. Introduction

Bladder cancer is the most common malignancy of the urinary tract in both men and women [1]. Currently, cystoscopy is the cornerstone for the diagnosis and follow-up of bladder cancer, enabling the identification of abnormalities of the bladder mucosa. However, white light cystoscopy (WLC) lacks the ability to provide histopathologic information, which is essential for diagnosis and prognosis [2]. In recent years, optical imaging techniques have been developed, which may overcome this limitation.

Confocal laser endomicroscopy (CLE) is a high-resolution optical imaging technique that allows for probe-based in vivo optical sectioning of tissue during endoscopy. The contrast is based on fluorescence that is excited by a laser. The contrast can be enhanced by administering a fluorescent label that binds to the cells, thereby allowing visualisation of the cellular microarchitecture of the tissue. CLE imaging was first introduced in gastroenterology to diagnose Barrett's oesophagus [3–6]. Shortly thereafter, applications were explored in pulmonology, otolaryngology, and urology [7–9]. By advancing a fibre-based probe through the working channel of a cystoscope, the bladder wall is visualised on a cellular level, providing "optical biopsies" of the tissue. Sonn et al [9,10] were the first to perform ex vivo and in vivo CLE imaging of the urinary tract. Nonetheless, translation of the images into a diagnosis is not straightforward. Diagnostic criteria for bladder cancer diagnosis were proposed; however, these criteria have not yet been validated [11,12].

Owing to the high recurrence rate, relatively long-term survival, adjuvant treatment modalities, and stringent follow-up, bladder cancer is currently one of the most expensive malignancies per patient [13,14]. An improved cost benefit of disease management could become possible when direct histopathologic information during cystoscopy becomes available, as it could potentially lead to advances in diagnosis and treatment of bladder cancer. To achieve such developments, the present study primarily aims to validate and adapt the proposed CLE criteria for bladder cancer grading. Secondary objectives are to investigate preliminary diagnostic accuracy of CLE-based grading and also in conjunction with WLC.

2. Patients and methods

2.1. Study design

The study protocol was approved by the institutional review board and was registered in the Dutch Central Committee on Research involving humans (NL55537.018.15) and on Clinicaltrials.gov (NCT03013894). The study was carried out according to the guidelines of good clinical practice. Written informed consent was obtained from all participants. This prospective clinical trial was in agreement with the IDEAL stage 2b recommendations and was carried out as described previously [15,16].

2.2. Patients

Patients were prospectively recruited in the Academic Medical Center (Amsterdam, The Netherlands). Adult patients, with a primary or recurrent bladder tumour or suspicion of carcinoma in situ (CIS), who were scheduled for transurethral resection of the bladder tumour (TURB), were eligible for the study. Main exclusion criteria were fluorescein allergy and pregnancy.

2.3. Study procedure

CLE imaging was performed during TURB using a low-power 488 nm laser system (Cellvizio 100 series; Mauna Kea Technologies, Paris, France) in conjunction with the Cystoflex UHD-R probe (Mauna Kea Technologies) with a 2.6 mm outer diameter, a field of view of 240 μm, a 1 μm lateral resolution, and an imaging depth of 50–65 μm.

CLE imaging was performed during TURB, prior to the resection of the suspect lesion. After cystoscopy, at least one suspicious lesion was marked using a cautery electrode. To stain the extracellular matrix of the bladder mucosa, ~300 ml fluorescein 0.1% was administered intravesically via a Foley catheter and left indwelling for 5 min [17]. The CLE probe was introduced through the working channel of 22 Fr rigid cystoscope with 0° optics. After placing the probe in direct perpendicular contact with the marked region of interest (ROI), images of the cellular microarchitecture were recorded (8–12 frames/s; Supplementary video) [18]. In general, two recordings of 1 min were obtained per ROI. After CLE imaging, the imaged lesion was resected en bloc. Histopathologic workup and analysis were performed according to standard clinical protocol by a uropathologist (C.D.S.H.), blinded to CLE images.

2.4. CLE image evaluation

Prior to the CLE image analysis, three observers (E.I.M.L.L., J.E.F., and C.D.S.H.) were trained with a CLE training programme of Chang et al [12]. The CLE images of the current study were analysed offline frame by frame with the Cellvizio Viewer software (Mauna Kea Technologies) by the three observers, who were blinded to clinical information and histopathology. For the CLE image analysis, the presence of the proposed CLE features (papillary configuration, organisation of cells, cohesiveness of cells, cellular morphology, definition of cell borders, and vasculature) by Chang et al [12] and an additional feature, polarity of the cells, were assessed (Fig. 1). Cellular polarity was defined as the relative orientation of cells and nuclei in the same direction. Based on the identified CLE features, the observers classified the ROI according to the World Health Organization (WHO) 2004 classification (low-grade urothelial carcinoma [UC], high-grade UC, or benign lesion). After individual analysis, consensus was reached through a two-step process. First, consensus for classification based solely on CLE images was reached. Thereafter, corresponding WLC images were added to account for the potential additional value of endoscopic evaluation adjunct to CLE imaging. With the additional information of the WLC images, a second joint consensus for the CLE-based classification was formed. To determine the concordance of the CLE-based classification with histopathology, CLE images were compared with the corresponding histopathology of the en bloc resected specimen (Supplementary Fig. 1).

2.5. Endoscopic tumour evaluation

During TURB, pictures and short videos of the CLE-imaged tumours were recorded. After a washout time of at least 4 wk, these images were presented to three urologists (T.M.d.R., J.B., and G.K.), blinded to any clinical information, to predict the histologic grade of the lesions according to the WHO 2004 classification. After individual prediction, a joined consensus was reached.

2.6. Sample size and statistical analysis

The sample size was based on prior publications and conformed to the IDEAL recommendations for explorative studies [16]. In 62 consecutive

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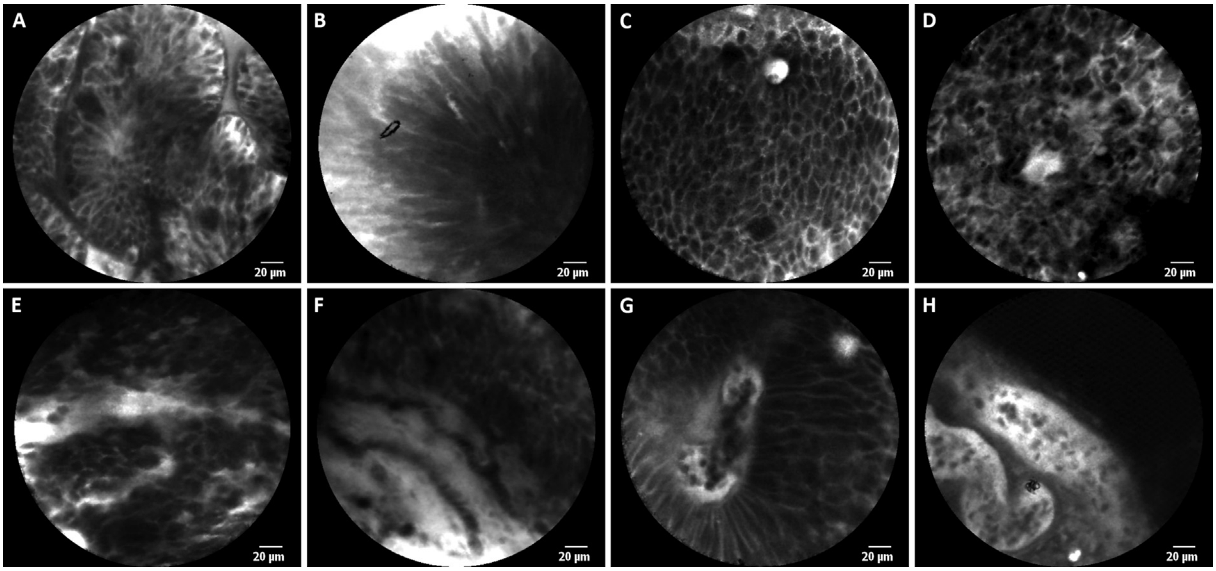

Fig. 1 – Examples of the different CLE features that were evaluated. (A) Presence of papillary configuration. (B) Polarity of urothelial cells, that is, alignment and orientation in the same direction. (C) Organised cell pattern, with cohesive and monomorphic cells, and distinct cell borders. (D) Disorganised cell pattern, with pleomorphic cells and indistinct cell borders. (E) Disorganised cell pattern, with discohesive and pleomorphic cells, and indistinct cell borders. (F) Capillary network. (G) Fibrovascular stalk is visible. (H) Large vessel. CLE = confocal laser endomicroscopy.

patients with a bladder tumour or suspicion of CIS, CLE imaging was performed.

Statistical analyses were performed using SPSS Statistics version 24 and Matlab R 2017b. Descriptive statistics were used to determine demographic and disease-specific characteristics. For the primary objective, interobserver agreements with regard to the endoscopic evaluation, CLE features, and CLE-based classifications were determined using Fleiss kappa analysis. The diagnostic accuracy for CLE, WLC, and CLE and WLC combined, including sensitivity and specificity, was calculated with 2×2 tables.

3. Results

3.1. *Patient characteristics*

Seventy-three consecutive patients were included in the study between March 2016 and September 2017. CLE imaging was performed in 62 patients, with a total of 82 suspicious lesions (Fig. 2). Lesions of which more than half of the CLE feature assessments were non-diagnostic were excluded. In total, 66 suspicious lesions were included for final analysis, yielding a diagnostic rate of 86%. Histopathology of the 66 lesions revealed 25 low-grade UCs, 27 high-grade UCs (including two cases of CIS), and 14 benign lesions (two normal, eight reactive, and two inflammatory lesions, one inverted papilloma, and one urothelial proliferation of uncertain malignant potential). Patient and tumour characteristics are summarised in Table 1.

3.2. *Differentiating CLE features*

Percentages of the different CLE features specified per type of lesion are displayed in Supplementary Table 1. CLE features with a mean prevalence of $\geq$60% for low-grade UC were presence of papillary configuration (100%), distinct cell borders (81%), presence of fibrovascular stalks (79%), cohesiveness of cells (77%), organised cell pattern (76%), monomorphic cells (67%), and presence of polarity (61%). For high-grade UC, prevalent CLE features were pleomorphic cells (77%), indistinct cell borders (77%), presence of papillary configuration (67%), and disorganised cell pattern (60%). Benign lesions did not show any CLE features with a mean prevalence of $\geq$60%.

3.3. *Interobserver agreement*

Interobserver agreement of the different CLE features varied between fair and substantial (Table 2), with moderate or substantial agreement for the features of papillary configuration, organisation of cells, cellular morphology, and definition of cell borders. Interobserver agreement for CLE-based classification was substantial ($\kappa = 0.676$, 95% confidence interval: 0.647–0.704).

3.4. *CLE-based classification*

The concordance with histopathology was higher with the consensus-based classification compared with individual assessment by three observers. The individual CLE-based classification of the three observers was in concordance with histopathology in 38–40 cases (58.5–62.5%), whereas consensus for CLE-based classification was confirmed by histopathology in 42 of 66 cases (63.6%). In 19 cases (76%) of low-grade UC, the CLE-based classification was in concordance with histopathology (sensitivity 76%, specificity 76%). For high-grade UC, the CLE-based classification was in concordance with histopathology (sensitivity 70%, specificity 69%) in 19 cases (70%). In four cases (29%) of benign lesions, the CLE-based classification was in concordance with histopathology (sensitivity 29%, specificity 96%; Table 3).

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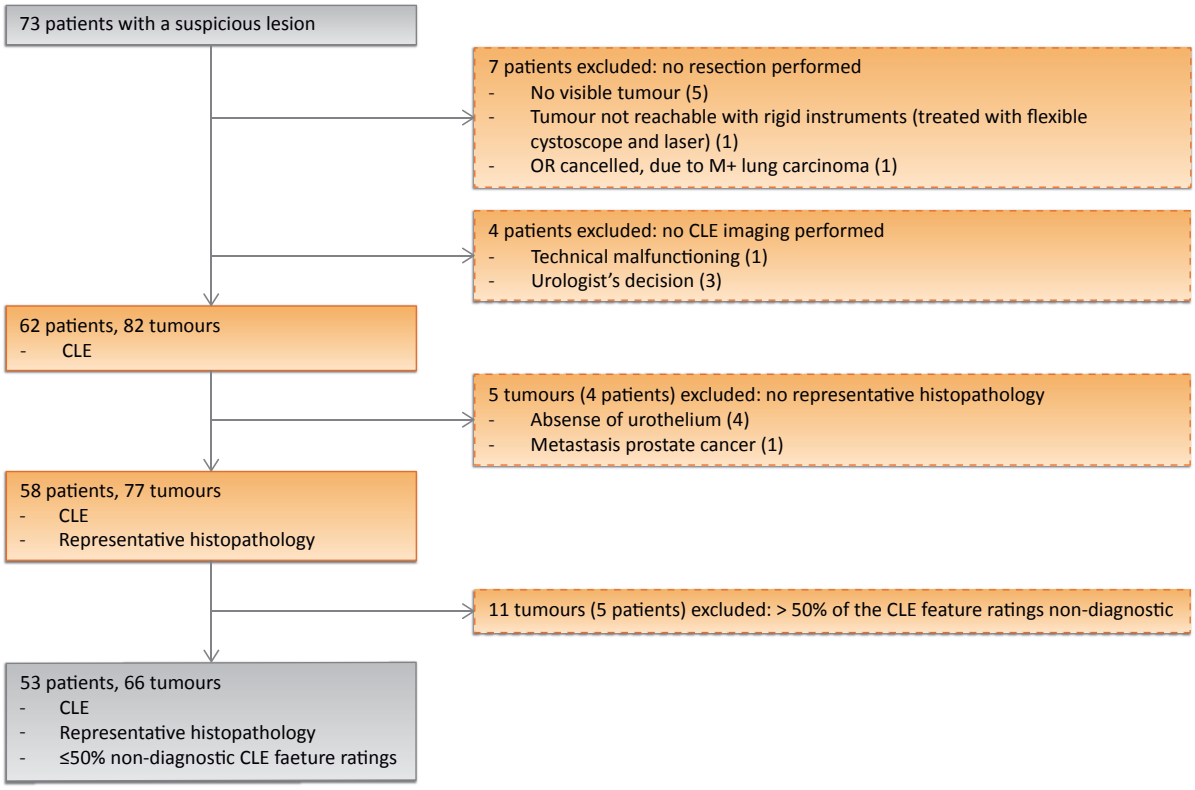

Fig. 2 – Flow diagram of inclusion. CLE = confocal laser endomicroscopy.

3.5. *WLC-based classification*

In 38 lesions (58.5%), the WLC-based consensus classification was in accordance with histopathology. Sensitivity and specificity were 54% and 71% for low-grade UC, 67% and 61% for high-grade UC, and 50% and 100% for benign lesions, respectively (Table 3).

Table 1 – Patient and tumour characteristics.

Patient characteristics		*N* = 53	%
Age (yr), mean (SD)/med [IQR]		70 (12)	70 [62–79]
Gender, *n* (%)	Male	39	74
	Female	14	26
History of bladder cancer, *n* (%)		29	55
Previous intravesical treatment, *n* (%)	No	32	60
	Yes	21	40
Tumour characteristics		***N* = 66**	**%**
Tumour size, *n* (%)	<3 cm	54	82
	>3 cm	12	18
Tumour stage, *n* (%)[a]	T0	15	23
	CIS only	2	3
	Ta	40	61
	T1	5	8
	≥T2	3	5
Tumour grade WHO 1973, *n* (%)	Benign	15	23
	CIS only	2	3
	Grade 1	4	6
	Grade 2	32	48
	Grade 3	13	20
Tumour grade WHO 2004, *n* (%)	Benign	14	21
	Low grade	25	38
	High grade	27	41

CIS = carcinoma in situ; IQR = interquartile range; med = median; SD = standard deviation; WHO = World Health Organization.
[a] Tumour stage of one patient could not be determined.

3.6. *CLE-based classification after WLC evaluation*

The CLE-based consensus classification after viewing WLC images showed an agreement with histopathology in 44 cases (68.2%). Concordance with histopathology was found in 19 (79%), 18 (67%), and seven (50%) cases for low-grade UC, high-grade UC, and benign lesions, respectively. Sensitivity and specificity were 79% and 78% for low-grade UC, 67% and 79% for high-grade UC, and 50% and 92% for benign lesions, respectively (Table 3).

4. Discussion

This study is the first validation of the previously proposed CLE features for bladder cancer diagnosis [11,12]. The CLE-based consensus classification with and without adjunct WLC image assessment was in concordance with histopathology in 68.2% and 63.6% of the cases, respectively. Concordance of the purely WLC-based classification and histopathology was lower (58.5%), suggesting that that CLE might be of additional value to cystoscopy for real-time bladder cancer assessment. In comparison with Herr et al [19], the concordance rate of WLC-based classification with histopathology seems to be low. However, in their study, the observers were not blinded for additional clinical information. Furthermore, they limited their grading assessment to

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Table 2 – Modified CLE image characteristics and their variables for analysis. Interobserver agreement is displayed for the CLE features and CLE-based classification (low-grade UC, high-grade UC, or benign lesion).

CLE feature	Variables	Fleiss κ	95% CI	Agreement
Papillary configuration	Present | not present	0.777	0.741–0.813	Substantial
Polarity of cells	Present | not present	0.382	0.356–0.408	Fair
Organisation of cells	Organised | disorganised	0.575	0.545–0.605	Moderate
Cohesiveness of cells	Cohesive | discohesive	0.337	0.307–0.367	Fair
Cellular morphology	Monomorphic | pleomorphic	0.430	0.398–0.462	Moderate
Definition of cell borders	Distinct | indistinct	0.666	0.632–0.701	Substantial
Vasculature	Capillary network | fibrovascular stalk | large vessels	0.574	0.551–0.598	Moderate
CLE classification		0.676	0.647–0.704	Substantial

CI = confidence interval; CLE = confocal laser endomicroscopy; UC = urothelial carcinoma.

G1 and G3 (WHO 1937) of recurrent tumours, which may overestimate the concordance of WLC-based grading.

The diagnostic accuracy for CLE-based bladder cancer grading of this study is in line with the results of Chang et al [12]. Nevertheless, we found higher sensitivity for low-grade UC and slightly higher specificity for high-grade UC.

Based on the interobserver agreement for the CLE analysis, we can conclude that assessment of the CLE features by independent observers yields comparable results. Evaluating the CLE images based on seven criteria can be laborious and time consuming. Considering that papillary aspect is a predominant CLE feature (≥60%) for both low- and high-grade UC, our results suggest that organisation of cells, cellular morphology, and definition of cell borders are the most discriminating features for grade differentiation (Fig. 3). Differentiation based on the presence of two or more of these three features yields similar sensitivity (low grade 75%, high grade 80%) and specificity (low grade 76%, high grade 66%; Supplementary Table 2). Importantly, these three CLE features have a moderate to substantial interobserver agreement. Image assessment based on three CLE features would simplify the interpretation and make it more accessible for clinicians, though this remains to be investigated prospectively.

Identifying differentiating CLE features for CIS was not possible since only two CIS lesions were included in the study. The higher discordance for benign lesions in comparison with low- and high-grade UC may be due to the heterogeneity of this group (two normal, eight reactive, and two inflammatory lesions, one inverted papilloma, and one urothelial proliferation of uncertain malignant potential). As a result, accurate differentiation of flat lesions remains challenging.

In this study, CLE imaging prolonged the TURB procedure for 10–15 min, including 5 min of fluorescein instillation time. To shorten the CLE procedure, the fluorescein could be administered directly onto the ROI, as applied in the upper urinary tract [15]. In daily practice, imaging time may be shorter because normal tissue does not have to be imaged, and it may not be necessary to obtain multiple recordings of multiple regions as in the extensive protocol in our study.

The use of CLE in urology is still in an early stage, and possible applications in clinical practice are being explored.

Table 3 – Diagnostic accuracy for the differentiation between benign, low-grade, or high-urothelial carcinoma. Sensitivity and specificity for CLE-based tumour evaluation, WLC-based tumour evaluation, and CLE-based tumour evaluation after reviewing endoscopy images.

	CLE evaluation (n = 66)		WLC evaluation (n = 65)[a]		CLE + WLC evaluation (n = 65)[a]	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Low grade						
Observer 1	72[b]	70[b]	42	73		
Observer 2	76	76	50	83		
Observer 3	76[c]	69[c]	71	56		
Consensus	76	76	54	71	79	78
High grade						
Observer 1	62[b]	67[b]	70	50		
Observer 2	63	67	70	53		
Observer 3	67[c]	73[c]	44	79		
Consensus	70	69	67	61	67	79
Benign						
Observer 1	29[b]	96[b]	43	100		
Observer 2	21	92	57	98		
Observer 3	25[c]	96[c]	50	94		
Consensus	29	96	50	100	50	92

CLE = confocal laser endomicroscopy; WLC = white light cystoscopy.
[a] Owing to technical problems, endoscopic images of one tumour were not recorded.
[b] It was not possible to determine CLE-based diagnosis in one case.
[c] It was not possible to determine CLE-based diagnosis in two cases.

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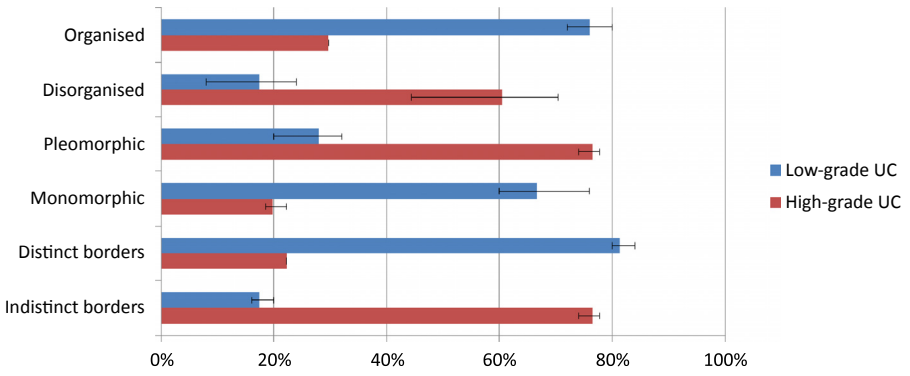

Fig. 3 – Most prominent features to differentiate between low- and high-grade urothelial carcinomas based on CLE images. Error bars represent the range how often different features were recognised by the independent CLE observers. CLE = confocal laser endomicroscopy; UC = urothelial carcinoma.

Histologic information during cystoscopy could improve the cost benefit of bladder cancer management in the long run, as it could lead to advances in diagnosis and treatment of bladder cancer. For example, laser fulguration has been performed in outpatient setting as treatment of low-risk bladder tumours. However, this technique is not commonly used due to the lack of histopathologic certainty and potential undertreatment [20,21]. CLE may enable real-time grading prior to laser fulguration to assure treatment of low-grade tumours. The shift of treatment from the operating theatre to the outpatient clinic could lead to a decrease in medical costs and shortening of waiting time for surgery. Next, CLE would be of great additional diagnostic value if it enables the identification of CIS. CLE may also be used during TURB to confirm surgical radicality or the presence of detrusor muscle in the resected tissue. By reducing histopathologic uncertainty during cystoscopy, CLE might enable active surveillance in patients with low-risk UC when subsequent surgical treatment is not preferred. CLE may also be used for upper tract UC to assist in patient selection for kidney-sparing treatment [22,23]. In addition, the combination of CLE with other optical imaging techniques (eg, photodynamic diagnosis, narrow band imaging, and optical coherence tomography) for guided or multimodal optical assessment should be investigated [24].

A limitation of this study was the impossibility to identify discriminating CLE features for benign lesions and CIS, due to heterogeneity of benign lesions and the small number of both benign lesions and CIS. In addition, heterogeneity within bladder tumours may be a limitation [25]. Considering the limited field of view of the probe (240 μm), only a fraction of the tumour surface is imaged. Therefore, the recorded image sequence may give a biased view with regard to the whole tumour, and might be responsible for discrepancies between CLE-based classification and histopathology. Additionally, variability in CLE image quality could impede CLE image evaluation. Specifically, at the start of this study, there was a learning curve with regard to probe stabilisation. Movement artefacts could have contributed to the 14% nondiagnostic rate of CLE images. Lastly, despite a washout time of several weeks to months, a recall bias might still exist for the urologists who predicted the tumour grade based on WLC images. However, this bias would have led to an overestimation; hence, the actual concordance of the WLC-based diagnoses with histopathology should be even lower.

In this study, we have extended the work of Chang et al [12] and validated CLE features for bladder cancer classification. Before CLE imaging can be used routinely for bladder cancer diagnosis, there are still some hurdles to overcome. Multicentre collaborations for larger clinical trials are required to fine-tune the established CLE criteria, develop a diagnostic nomogram, and further explore future applications. In addition, the digital data of CLE offer opportunities for automated image analysis and deep machine learning, which should be explored jointly to create big data.

5. Conclusions

This study is the first prospective validation of earlier published CLE features for bladder cancer diagnosis and grading. CLE images correlate to histopathologic features, and may enable real-time differentiation between low- and high-grade UC. Our data demonstrates that the proposed CLE features suffice to identify and grade bladder tumours. Moreover, our data suggest that bladder cancer grading might be possible based on three CLE features. Differentiation of flat lesions remains to be investigated.

Author contributions: Esmee I.M.L. Liem had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Liem, Freund, Savci-Heijink, de la Rosette, van Leeuwen, de Reijke, de Bruin.
Acquisition of data: Liem, Freund, Savci-Heijink, Kamphuis, Baard.
Analysis and interpretation of data: Liem, Freund, Savci-Heijink, de Reijke, de Bruin.
Drafting of the manuscript: Liem, Freund.
Critical revision of the manuscript for important intellectual content: Liao, van Leeuwen, de Reijke, de Bruin.
Statistical analysis: Liem.
Obtaining funding: de la Rosette, de Reijke.
Administrative, technical, or material support: Liao.
Supervision: de la Rosette, de Reijke, de Bruin.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.euf.2018.07.012.

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