SCANDINAVIAN JOURNAL OF UROLOGY
2021, VOL. 55, NO. 1, 36–40
https://doi.org/10.1080/21681805.2020.1841285

ARTICLE

Diagnostic value of probe-based confocal laser endomicroscopy versus conventional endoscopic biopsies of non-muscle invasive bladder tumors: a pilot study

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ABSTRACT

Purpose: The standard procedure for diagnostics and follow-up for non-muscle invasive bladder cancer (NMIBC) is cystoscopy in the outpatient clinic. Suspicious lesions are biopsied for histopathological assessment. This pilot study aimed to evaluate the ability of Confocal Laser Endomicroscopy (CLE) to rule out High Grade Urothelial Carcinoma (HGUC) to select patients for in-office fulguration.

Materials and methods: We performed a prospective non-randomized, single surgeon study. Intraoperative CLE was performed independently by the surgeon and a blinded on-site uropathologist. Following the procedure, a CLE evaluation was performed by another blinded urologist. Lesions were classified as normal/inflammatory, Low Grade Urothelial Carcinoma (LGUC) or HGUC. With the histological evaluations as the gold standard we calculated sensitivity, specificity, PPV and NPV for HGUC and the accuracy for each CLE assessor. The primary outcome was the NPV for HGUC for the surgeon.

Results: Twelve patients with a total of 34 lesions were included. Six lesions were flat and 28 were exophytic. On histopathology, 25 lesions were classified as normal/inflammatory or LGUC, while nine were classified as HGUC. For the surgeon, the uropathologist and the second urologist, the sensitivity was 44%, 78% and 22%, respectively. Specificities for the three observers were 84%, 68% and 96%. This corresponded to PPVs for HGUC of 50%, 47% and 67% and NPV for HGUC of 81%, 89% and 77%.

Conclusions: In our hands the NPV of CLE is not high enough for it to be considered an alternative to histopathological assessment of bladder lesions.

ARTICLE HISTORY
Received 5 June 2020
Revised 16 September 2020
Accepted 16 October 2020

KEYWORDS
Urothelial carcinoma; bladder cancer; confocal laser endomicroscopy; non-muscle-invasive bladder carcinoma; optic biopsy; negative predictive value

Introduction

Bladder cancer is the ninth most common cancer and as such it represents a substantial burden on both patients and healthcare systems [1]. Approximately 75% of patients present with non-muscle invasive bladder cancer (NMIBC) and about 50% of these tumors show a growth pattern termed low grade (LG) [2]. The standard initial diagnostic procedure is white light cystoscopy performed in the outpatient clinic and the same method is used for regular follow-up of patients who have received local treatment for their tumors. However, it is not possible to determine the growth pattern with this procedure and all suspicious lesions need subsequent confirmation by biopsy and histopathological assessment. This means that patients with bladder tumors exhibiting a LG pattern may be subjected to relative overtreatment. To reduce this problem, variant technologies aiming for instant diagnosis are investigated. These include optical coherence tomography [3], Raman spectroscopy [4] and a technique called Confocal Laser Endomicroscopy (CLE), that has been suggested as an alternative to biopsy. With CLE, the bladder mucosa is first subjected to a fluorescent substance (fluorescein) through an intravenous injection or an intravesical instillation [5]. Through a cystoscope, the mucosa is subsequently subjected to the CLE system which is comprised of a fiberoptic bundle with an integrated distal lens that is connected to a laser scanning unit. With this system, a non-invasive low-power laser light (wavelength 488 nm) illuminates the tissue or interest at a precise depth [6]. The light is reflected back and refocused through a pinhole comprised of the individual optical fibers to the CLE system by the same lens. Because light reflected at other angles from the tissue is excluded, the spatial resolution of CLE is increased significantly, thus producing an enhanced two-dimensional cellular image with a diameter of 240 μm allowing for microscopic evaluation of the cellular structures of the tissue of interest during cystoscopy. In theory, this constitutes an 'optical biopsy', which allows for histopathological assessment without the removal of tissue. With current technology, the use of a fluorescent substance is needed to generate high quality images. A further limitation of current CLE technology is that it has a fixed focal length, which means that it can only scan in a single plane. With the CystoFlex probe used in the urinary tract, the depth of the imaging from the surface is 55–65 μm, making it unsuitable for use in tumor staging.

The method may potentially present a non-invasive way to diagnose LG bladder lesions without the need for a biopsy. This means that CLE may potentially help identify

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patients, who can safely be offered in-office fulguration without anesthesia. In addition, the technique may help to reduce the number of necessary samples in patients who undergo standard biopsies.

The purpose of this pilot study is to evaluate the ability of CLE to rule out high grade (HG) bladder lesions and thereby serve to select patients for in-office fulguration or laser treatment.

Materials and methods

We performed a pilot study as a prospective non-randomized, single center, single surgeon study with within-lesion comparisons of CLE and histology in the grading of bladder lesions in a transurethral resection of the bladder (TURB) setting. Patients were eligible for inclusion if they were scheduled for a TURB at the Department of Urology, Herlev and Gentofte Hospital, Denmark, based on cystoscopic suspicion of primary bladder tumor in the outpatient clinic.

All procedures were performed with photodynamic diagnosis (PDD) guidance under general anesthesia by the same experienced urologic surgeon (SB) using a 22 Fr, 0 degree, Karl Storz optics cystoscope and a 26 Fr resectoscope (either Olympus or Karl Storz)., Karl Storz PDD equipment was part of our department's standard equipment, the CLE platform was borrowed from the company (Cellvizio® System Mauna Kea Technologies, France) and the probes were purchased (Cystoflex™ UHD, Cellvizio® Confocal Miniprobes™ Mauna Kea Technologies, France).

White light cystoscopy was performed first followed by PDD. Suspicious lesions were located and marked laterally by the cautery electrode. Then 2.5 mL Fluorescein 100 mg/mL was given intravenously. After 5 min, the suspicious lesions were examined with the CLE technique by introducing the Cystoflex UHD probe through the working channel of the cystoscope. Intraoperative CLE evaluations and registrations were performed independently by the surgeon (SB) and subsequently by an on-site uropathologist (AT) who was blinded to the surgeon's assessment as well as to the patient history and the endoscopic findings. Subsequently, 1-min CLE videos were recorded for each lesion. After image recording, the suspicious lesions were resected (en-bloc if technically possible). Following the procedure, the CLE videos were used for a third CLE evaluation performed by a second urologist (MF) who was also blinded to both the patient history and the previous findings. All three assessors had undergone an online Confocal Laser Endomicroscopy course (Joseph C. Liao, M.D. Stanford University School of Medicine). CLE assessment was based on organization of cells, cellular morphology and definition of cell borders (Figures 1 and 2). Histopathological evaluations were performed by two independent uropathologists in accordance with the 2004 World Health Organization guidelines. On both CLE evaluations and histopathology, the lesions were classified as normal/inflammatory, low grade urothelial carcinoma (LGUC) or high-grade urothelial carcinoma (HGUC).

As this was a pilot study only, we did not perform a formal power analysis and results are provided as crude values and percentages similar to previous studies on CLE in urothelial lesions. With the histological evaluations as the gold standard we calculated the sensitivity and specificity for HGUC as well as the positive predictive value (PPV) and the negative predictive value (NPV) for each CLE assessor. The accuracy of each assessor, defined as correct discrimination between HGUC and non-HGUC lesions, and the agreement between the analyses by the two urologists (SB and MF) was also calculated. The primary outcome was defined as the NPV for HGUC for the surgeon, as this represents the main parameter for intraprocedural distinction between tumors requiring resection and tumors eligible for in-office fulguration or laser treatment.

We performed descriptive statistics using SAS version 9.2 (Institute Inc., Cary, NC, USA). The study was approved by the Danish Capital Region ethics committee (H-15020548) and the Danish Data Protection Agency (2012-58-0004) and all patients provided verbal and written consent before inclusion.

Results

Twelve eligible patients with a total of 34 lesions were included in the study in June 2018. These included seven men and five women with a median age of 74 years (range = 52–94). Overall, 29 of the lesions were visible in white light and of these 28 were PDD positive. The remaining five were visible with PDD only. Six lesions were flat and 28 were exophytic. Further tumor characteristics are shown in Table 1.

On histopathology, 25 lesions were classified as normal/inflammatory or LG and nine were classified as HG lesions. There was complete agreement between the two uropathologists. The sensitivity, specificity, PPV and NPV regarding HGUC for the three CLE assessors are listed in Table 2. Notably, the intraoperative NPV of CLE evaluation by the surgeon was 81%. This corresponded to him missing 5/9 HG tumors. The two urologists had the same grading of the tumors in 25/34 lesion.

Discussion

CLE was originally developed for basic research and initially evaluated in gastrointestinal tumors [7–9]. Sonn et al. [10] reported the first *in vivo* microscopic evaluation of bladder urothelium using CLE finding marked visual differences between low and high grade tumors. Chang et al. [11] evaluated CLE interobserver agreement and defined the CLE features used for the assessment of bladder lesions. Liem et al. [12] extended this work and validated it showing a moderate-to-substantial interobserver agreement for the features of papillary configuration, organization of cells, cellular morphology and definition of cell borders.

In our study we evaluated CLE's ability to rule out HG bladder lesions as a way to select patients for in-office fulguration. We found wide variation in both sensitivity and specificity between the three observers and with a PPVs around 50% our study suggests that the method is not sufficient to confidently identify HG lesions. The accuracy of CLE

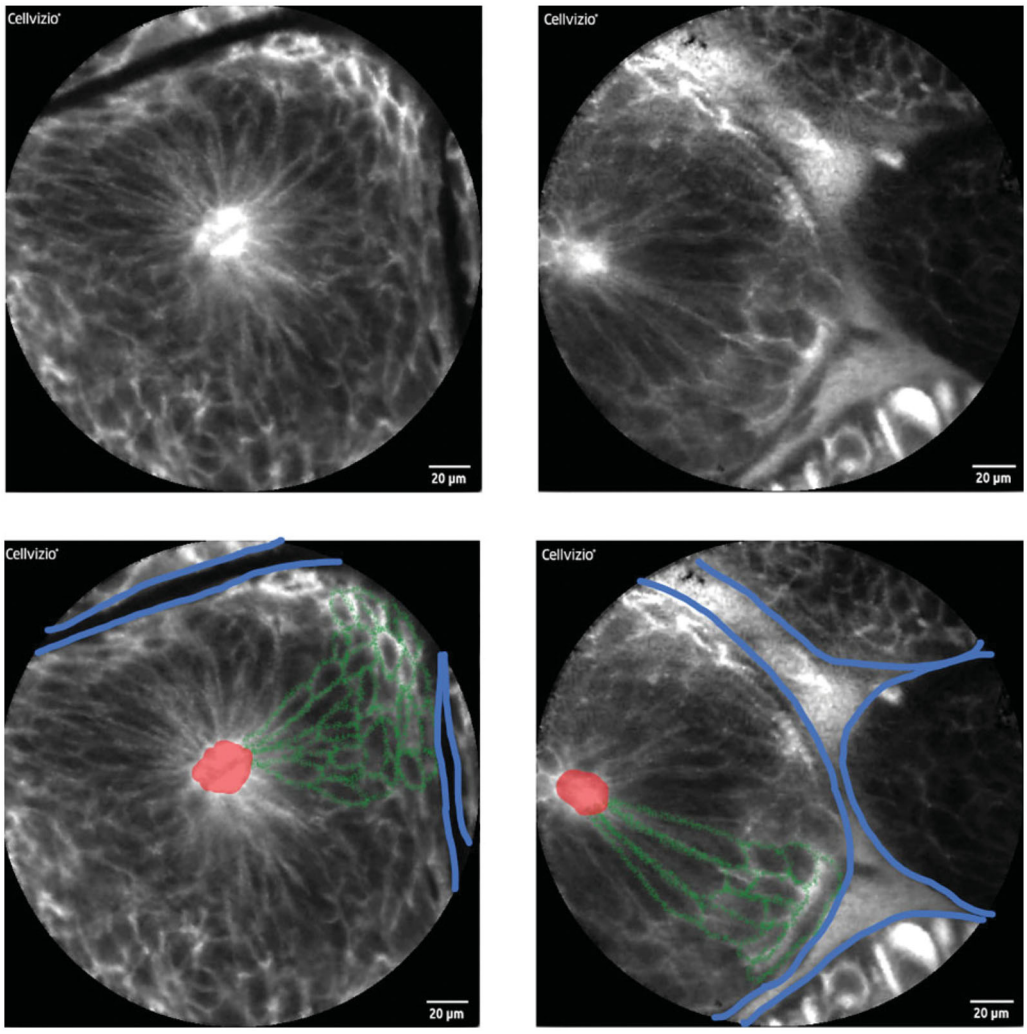

Figure 1. Two examples of LGUC. (a, b) As displayed by the CLE system. (a1, b1) With illustrations by the authors. Clear papillary configuration. Well defined cell borders. Well organized urothelial architecture with monomorphic cohesive cells. Thin fibrovascular stalk.

was within a narrow range from 71–77%. This is comparable to findings in the study by Liem et al. [12], where the concordance of CLE evaluation by three observers and histopathology was 63.6%. Importantly, this study found concordance between the CLE evaluation and histopathology in 70% of HG lesions.

Meanwhile, we believe that the main potential value in CLE is its possible ability to spare patients from unnecessary surgery. Therefore, the most important parameter from a clinical standpoint is the NPV for HUCG. This showed more promising results between 77% and 89%. This corroborates previous findings that CLE may be valuable when distinguishing between HG bladder tumors and more indolent findings. Thus, Liem et al. found a CLE specificity for LG lesions of an impressive 96%. However, in our study, it is important to note that the majority of histology showed normal tissue or LG lesions, which could in part explain the high NPVs. Even with this theoretical advantage, the NPV of the intraoperative CLE evaluations only reached 81%. Considering the prognosis for HG bladder lesions, this number is not high enough to omit surgery based on CLE. The problem is highlighted as the histopathology of one biopsy showed a pT1 HG urothelial carcinoma, while all three CLE observations concluded that the lesion was non-invasive LGUC. Such a misdiagnosis would be devastating to the patient.

When evaluating our work, it is important to note that it represents a pilot study with a small number of patients and tumors. However, even in our small cohort, the surgeon missed 5/9 HG lesions, which would remain unacceptable even in a much larger sample. Since CLE is a new method, which has not made its way into clinical practice in urology at this time, the involved clinicians also have limited experience with the method. Therefore, it is possible that results can be improved with an extended educational program and clinical experience. However, discrepancies between CLE and histopathology may also be due to the limited depth of view of the laser probe, which means that imaging only captures the surface of the tumor (55–65 µm). Likewise, it is possible that tumor heterogeneity may limit the practical application of CLE as only parts of the lesions are evaluated during surgery. In addition, there could be an interference between CLE and PDD, but we are not aware of any studies investigating this *in vivo*. An *ex vivo* pilot study showed that hexyl aminolevulinic acid (HAL), used in PDD, was insufficient to allow conclusive CLE but combining PDD and CLE allowed

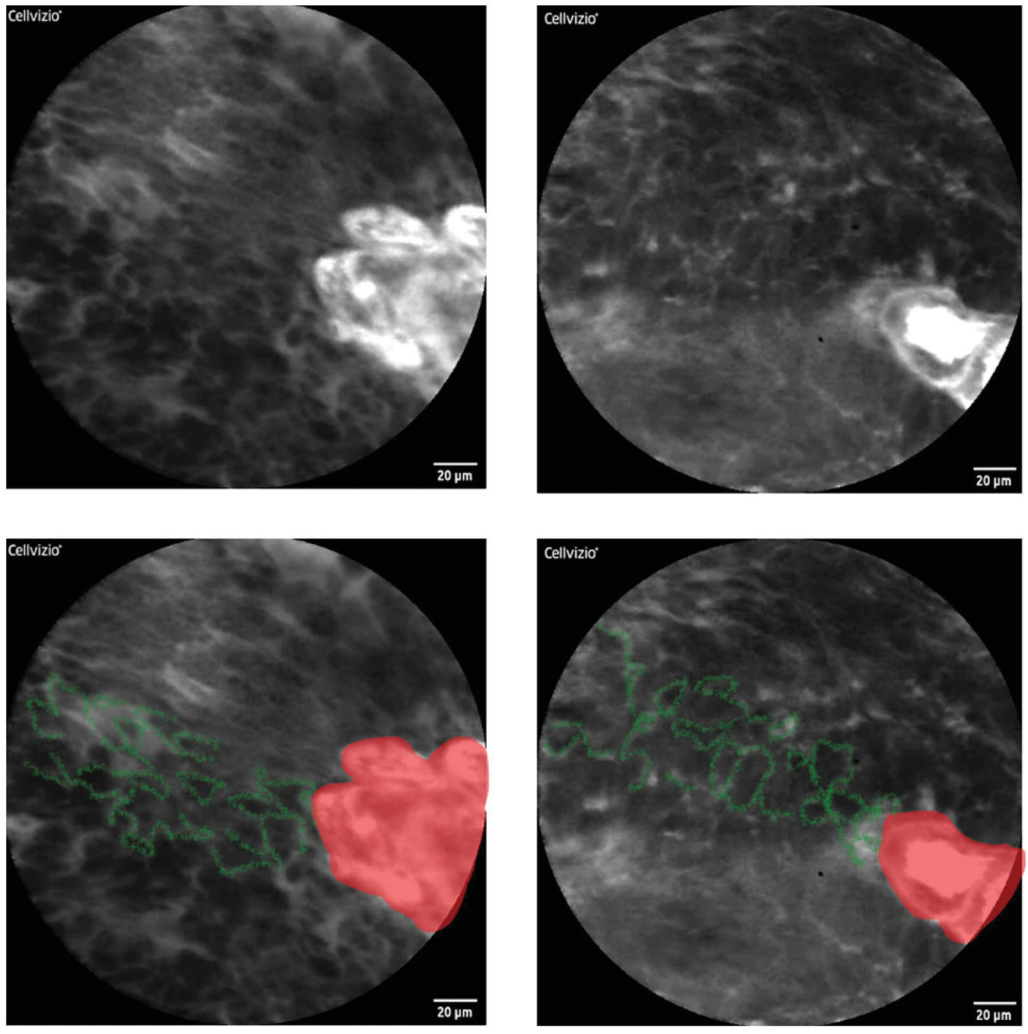

Figure 2. Two examples of HGUC. (a, b) As displayed by the CLE system. (a1, a1) With illustrations by the authors. No papillary configuration. Poorly defined cell borders. Disorganized tissue architecture with pleomorphic, discohesive cells. Hyperplastic fibrovascular stalk.

Table 1. Tumor characteristics.

	Number (%)
Tumor stage	
T0	8 (24)
Ta	23 (68)
T1	1 (3)
Carcinoma in situ	2 (5)
Tumor grade (WHO 2004)	
Benign	8 (23)
Low grade	17 (50)
High grade	9 (27)

Table 2. The ability of CLE to detect high-grade urothelial carcinoma.

	SB	AT	MF
Sensitivity %	44	22	78
Specificity %	84	96	68
PPV %	50	67	47
NPV %	81	77	89
Accuracy %	74	77	71

PPV: Positive predictive value; NPV: Negative predictive value.

guiding CLE by locating areas of interest for CLE examination [13]. As the literature on CLE in bladder lesions is very limited at the current time, more research is needed to explore these possibilities.

Conclusions

With a NPV of the intraoperative CLE evaluations of 81% CLE showed some promise as an optical biopsy technique but due to the malignancy potential of HG lesions CLE cannot be yet considered as an alternative to histopathological assessment of bladder lesions. This study is not designed to evaluate if this is due to limitations of CLE in classifying bladder lesions or a lack of experience with CLE among the investigators. Further research is needed to elucidate these questions.

Acknowledgements

The authors acknowledge Dr J. Liao, Department of Urology, Stanford University, USA, for sharing his 'Teaching set for confocal laser endomicroscopy of the bladder'. We would like to thank Dr J. Baard, department of Urology, Amsterdam UMC, for her valuable assistance and her help with the CLE setup.

Disclosure statement

The Cellvizio® System was borrowed by Mauna Kea Technologies for a period of 30 days. No potential conflict of interest was reported by the author(s).

Research involving human participants

The study was approved by the Danish Capital Region ethics committee (H-15020548) and the Danish Data Protection Agency (2012-58-0004).

Informed consent

All patients provided verbal and written consent before inclusion.

Funding

The study was funded by Beckett fund, Axel Muusfeldt fund and Medac grant for research in non-muscle invasive bladder cancer.

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