Fluorescence Confocal Microscopy in Urological Malignancies

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Fluorescence confocal microscopy (FCM) represents a novel diagnostic technique able to provide real-time histological images from non-fixed specimens. As a consequence of its recent developments, FCM is gaining growing popularity in urological practice.

Keywords: confocal microscopy ; prostate neoplasms ; bladder neoplasms ; ureteral neoplasms ; kidney neoplasms

1. Introduction

Fluorescence confocal microscopy (FCM) is an imaging technique that provides real-time digital images of fresh tissue, without the need for further conventional pathology. It allows real-time microscopic examination with the high-resolution visualization of cells and structures.

Confocal microscopy was first described by Marvin Minsky in 1957 [1]. The key to confocal approach is the elimination of out-of-focus light (also known as flare) by scanning a point source of light across the specimen and using a pinhole to eliminate the out-of-focus light from the detector. When compared to a conventional wide field light microscope, the confocal microscope provides an increase in both the maximum lateral resolution (0.5 µm vs. 0.25 µm) and the maximum axial resolution (1.6 µm vs. 0.7 µm) [2].

It can be used in reflectance (RCM) or fluorescence mode (FCM): RCM is based on the reflection of light from different components of cellular structures, while FCM involves the visualisation of fluorophores to characterise cellular details. FCM harnesses external dyes to obtain fluorescence contrast. To date, the most widely used is Acridine Orange, which binds specifically to DNA thus allowing a clear visualization of the nuclei under the fluorescent laser. Images are obtained in a haematoxylin and eosin (H&E) digital staining, which facilitates the interpretation by pathologists and surgeons. CFM has been approved for clinical use in gastroenterology and pulmonology, specifically for the evaluation of Barrett's oesophagus, pancreaticobiliary diseases, gastric cancer, and other pathological conditions [3][4][5]. It has also been applied in dermatology, where it is currently used to determine positive margins of basal cell and squamous cell carcinoma during Mohs surgery [6].

The use of fluorescence confocal microscopy is also spreading in urological practice. Over the last ten years various applications have been explored in a bid to validate a useful diagnostic tool able to aid both intraoperative decision making and office followup [7][8].

Considering the urothelial carcinoma (UC) scenario, FCM has been investigated in both bladder cancer (BC) and upper-tract urothelial carcinoma (UTUC). Confocal laser endomicroscopy (CLE) is a unique optical imaging technology that can provide real-time and high-resolution imaging of the cellular architecture and the morphology of mucosal lesions. Its use during transurethral resection of bladder tumours (TURBT) or cystoscopy provides the surgeon with useful histological information and represents a promising technique for conservative BC management [9][10][11]. CLE is a reliable and accurate technique in BC diagnosis [12]. Furthermore, CLE can be performed in patients with UTUC during ureteroscopy [13]. In regard to prostatic specimens' interpretation, CFM has been applied both in the office setting to study biopsy cores as well as intraoperatively to evaluate surgical margins during radical prostatectomy [14][15]. CFM has also been successfully applied for a real-rime diagnosis of renal cell carcinoma (RCC) [16].

2. Fluorescence Confocal Microscopy in Urological Malignancies

2.1. Bladder Cancer

BC is a heterogeneous disease encompassing non-muscle-invasive (NMIBC) and muscle-invasive BC (MIBC) and entailing very heterogeneous managements and prognoses [17][18][19][20][21]. The results regarding CFM applications in BC detection are reported in **Table 1**.

Table 1. Confocal microscopy in BC.

Author	Year	Pat. (n.)	Setting	CFM System	Procedure	Se. (%)	Sp. (%)	PPV (%)	NPV (%)	Main Outcomes
Lee [22]	2019	75	In vivo	Cellvizio	TURB	91.7 (mal. vs. ben.) 94.5 (LGUC vs. HGUC) 71.4 (CIS vs. IT)	73.9 (mal. vs. ben.) 66.7 (LGUC vs. HGUC) 81.3 (CIS vs. IT)	93.6 (mal. vs. ben.) 89.7 (LGUC vs. HGUC) 83.3 (CIS vs. IT)	68.0 (mal. vs. ben.) 80.0 (LGUC vs. HGUC) 68.4 (CIS vs. IT)	CLE represents a promising technology to provide real-time reliable diagnosis and grading of UC. Moreover, it might improve RFS.
Lucas [23]	2019	53	In vivo	Cellvizio + AI-image analysis	TURB	NR	NR	NR	NR	CLE accuracy regarding malignant vs. benign tissue distinction was 79%, while the HGUC vs. LGUC differentiation accuracy was 82%.
Liem [24]	2018	53	In vivo	Cellvizio	TURB	76.0 (LGUC) vs. 70.0 (HGUC)	76.0 (LGUC) vs. 69.0 (HGUC)	NR	NR	Concordance between CLE-based classification and final histopathology was found in 19 LGUC cases (76%), 19 HGUC cases (70%), and 4 benign lesion cases (29%).
Chang [10]	2013	31	Ex vivo	NR	TURB	50.0 (LGUC) vs. 75.0 (HGUC)	94.0 (LGUC) vs. 64.0 (HGUC)	NR	NR	Novice CLE observers achieved a diagnostic accuracy comparable to WLC-images-only observation after a short training. An expert CLE observer achieved higher accuracy rates compared to WLC-image-only analysis.

Abbreviations are as follows: Pat. = patients; CFM = confocal microscopy; Se. = sensitivity; Sp. = specificity; PPV = positive predictive value; NPV = negative predictive value; TURB = transurethral resection of the bladder; CLE = confocal laser endomicroscopy; LGUC = low-grade urothelial cancer; HGUC = high-grade urothelial cancer; CIS = carcinoma in situ; mal. = malignant; ben. = benign; IT = inflammatory tissue; AI = artificial intelligence; RFS = recurrence free survival; WLC = white light cystoscopy; NR = not reported.

2.2. Upper Tract Urothelial Cancer

The results regarding CFM applications in UTUC's detection are reported in **Table 2**.

Table 2. Confocal microscopy in UTUC.

Author	Year	Pat. (n.)	Setting	CFM System	Surgery	Se. (%)	Sp. (%)	PPV (%)	NPV (%)	Main Outcomes
Prata [25]	2023	46	Ex vivo	VivaScope 2500	ORC	53.8 (vs. H&E)	90.9 (vs. H&E)	90.9 (vs. H&E)	83.3 (vs. H&E)	CFM showed similar results compared to frozen section analysis for ureteral margins evaluation.
Sanguedolce [26]	2021	7	In vivo	Cellvizio	URS	71.4 (total) 100.0 (HG lesions)	57.1 (for HG lesions only)	NR	NR	Real time concordance with definitive histology in UTUC biopsy: 71.4% (5/7 cases)
Villa [27]	2016	11	In vivo	Cellvizio	f-URS	NR	NR	NR	NR	CLE allows clear recognition of UTUC histological features.
Bui [28]	2015	14	In vivo	Cellvizio	f-URS	NR	NR	NR	NR	CLE provided images of papillary structures, fibrovascular stalks, and pleomorphism. Lamina propria was identified in normal areas.

Abbreviations are as follows: UTUC = upper tract urothelial cancer; pat. = patients; CFM = confocal microscopy; Se. = sensitivity; Sp. = specificity; PPV = positive predictive value; NPV = negative predictive value; H&E = haematoxylin and eosin; CLE = confocal laser endomicroscopy; ORC = open radical cystectomy; URS = ureteroscopy; f-URS = flexible ureteroscopy; HG = high-grade; NR = not reported.

2.3. Prostate Cancer

Prostate cancer (PCa) represents a clinical scenario, where novel technologies have the potential to guide a tailored treatment and personalized management [29][30][31]. The results regarding CFM applications for PCa detection are reported in **Table 3**.

Table 3. Confocal microscopy in PCa.

Author	Year	Pat. (n.)	Setting	CFM System	Se. (%)	Sp. (%)	PPV (%)	NPV (%)	Main Outcomes
Gobbo [32]	2023	NR (75 biopsy slides)	Biopsy	VivaScope	NR	NR	NR	NR	Almost complete agreement was obtained for ISUP/WHO grade group I, IV, and V (k = 0.85). For the remaining noncancer stains, agreement was nearly complete (k = 0.81).
Marenco [8]	2020	57 biopsy-naive men	Biopsy	VivaScope	NR	NR	85.0 (biopsy core) 83.8 (ROI level)	95.1 (biopsy core) 85.7 (ROI level)	CFM and H&E concordance was evaluated on the biopsy core and ROI level; Cohen's k for agreement between the techniques was 0.81 for the biopsy core level and 0.69 for the ROI level. The PPV and NPV were high at biopsy core and ROI levels.

Author	Year	Pat. (n.)	Setting	CFM System	Se. (%)	Sp. (%)	PPV (%)	NPV (%)	Main Outcomes
Rocco [33]	2020	8	Surgical margins (periprostatic tissue) during RP	Vivascope	NR	NR	NR	NR	7/8 patients had overall negative SM in the sampled areas. The agreement between CFM and H&E in regard to the discrimination between cancerous and noncancerous tissue was 100%.
Puliatti [14]	2019	13	Biopsy (on RP surgical specimen)	VivaScope	83.3	93.5	NR	NR	The overall diagnostic agreement between CFM and histopathological diagnoses was substantial with 91% correct diagnosis and an AUC of 0.884 (95% CI 0.840–0.920).

Abbreviations are as follows: Pat. = patients; CFM = confocal microscopy; RP = radical prostatectomy; SM = surgical margins; PCa = prostate cancer; ROI = region of interest; H&E = haematoxylin and eosin; AUC = area under the curve; k = Cohen statistic coefficient; PPV = positive predictive value; NPV = negative predictive value; Se. = sensitivity; Sp. = specificity; NR = not reported.

2.4. Renal Cell Carcinoma

Results regarding CFM applications in renal cell carcinoma cancer (RCC) are shown in **Table 4**. To date, only three papers have investigated CFM in RC diagnosis. Mir et al. reported a concordance of 100% between ex vivo CFM analysis and definitive H&E assessment [7]. Liu et al. reached an overall 89.2% accuracy rate as compared to H&E-stained samples [34].

Table 4. Confocal microscopy in RCC.

Author	Year	Pat. (n.)	Setting	CFM System	Se. (%)	Sp. (%)	PPV (%)	NPV (%)	Main Outcomes
Mir [7]	2020	4	Ex vivo	VivaScope 2500	NR	NR	NR	NR	Neoplastic and noncancer tissues were both detected in 100% of cases through CFM images analysis (one oncocytoma and three RCC). CFM images showed strong overlapping with traditional H&E-stained samples regarding cytoarchitectural features.
Liu [34]	2016	19	Ex vivo	VR-SIM	79.2	95.1	82.6	90.7	CFM diagnostical outcomes were compared to traditional H&E staining; final accuracy was 89.2%.
Su [16]	2015	20	Ex vivo	Cellvizio	NR	NR	NR	NR	CLE imaging properly evaluates normal renal parenchymal features. It allows a rapid distinction between cancer and normal tissue, as well as the possibility to distinguish between benign and malignant ones. Enhanced CLE images resolution was provided by topical fluorescein rather than by IV route administration.

Abbreviations are as follows: Pat. = patients; CFM = confocal microscopy; RCC = renal cell carcinoma, H&E = haematoxylin and eosin, PPV = positive predictive value; NPV = negative predictive value, IV = intravenous.

2.5. Summary

CFM represents an innovative and attractive tool, able to provide a real-time histological assessment. Despite being still experimental, urological applications are on the rise. Both in vivo and ex vivo experiences have been reported. Regarding CFM in vivo applications, the reports mainly focused on surgical margins' evaluation and real-time histological grading. The reported diagnostic outcomes were heterogeneous among the included papers. Nevertheless, CFM has shown intriguing results in various areas.

UC was the most investigated topic. The technique's applications have been reported for both BC and UTUC. Histological grade assessment represents one of the most investigated topics in the BC setting. In their paper, Chang et al. first proposed diagnostic criteria for BC grading based on CLE features [10]. Cellular, microarchitectural, and vascular characteristics in CFM images were collected and evaluated. The comprehensive evaluation of the histological pattern provided a real-time grading for BC. Interestingly, high interobserver agreement was documented after only short training sessions with optical biopsies' images. CLE was surprisingly easily performed and interpreted by novice observers.

Incomplete TURBT represents one of the main concerns in BC operative management [35]. Some reports evaluated CLE's ability to distinguish between normal urothelial mucosa and cancerous residual tissue [22][23][24]. This potential may be intraoperatively harnessed to assess resection margins' status, potentially providing survival benefits. Lee et al. reported a recurrence-free survival advantage for the CLE-aided TURBT cohort compared to the WLC-only group [22]. Nevertheless, larger studies with long-term followup are required to definitively assess the actual impact of CLE on RFS.

Currently, European Association of Urology (EAU) guidelines recommend adopting a kidney-sparing surgical approach for low-risk and selected cases of high-risk UTUC [36]. In this setting, CLE may represent a valuable supportive tool to enhance patients' conservative management. In vivo CLE experiences during ureteroscopies have been reported. As for BC, real-time CLE-based UTUC grade assessment was the most reported outcome. Variable rates have been described for diagnostic outcomes: Sanguedolce et al. reported a relatively low concordance rate between CLE and biopsy at final pathology (71.4%) [26]. Nonetheless, the same authors reported a 100% Se for high-grade lesion detection. Conversely, Freund et al. described a high concordance between CLE and the final histology for both low-grade and high-grade lesions (90% and 86%, respectively) [37]. The main CLE cytological and microarchitectural features have been reported by the same authors.

As previously reported, CFM applications have also been explored in PCa. Both in-office and intraoperative settings have been explored. Notably, the sensitivity and NPV were generally slightly higher for PCa optical biopsies as compared to BC and UTUC. Remarkably, both Marenco and Rocco reported higher NPV for CFM as compared to traditional H&E histological assessments (95.1% and 96.7%, respectively) [8][38]. Both authors evaluated concordance at prostate biopsies for PCa diagnosis. However, the Se did not reach comparably high rates.

Today, novel cutting-edge technologies have been proposed in multiple urological fields: for instance, even though recently developed, PSMA-radioguided surgery might dramatically change PCa management in the next future. On the other hand, fluorescence-guided technologies are already routinely employed to enhance BC detection at the time of TURBT [39]. Likewise, CFM might be included as part of a multimodal surgical strategy alongside with these innovative procedures. To date, successful attempts to combine fluorescence imaging and optical biopsies have been reported for BC: Gladkova et al. first described the combination of fluorescence cystoscopy and cross polarization optical coherence tomography in 2013 [40]. More recently, Marien et al. proposed the combination of CLE and PDD to enhance BC detection [41]. Therefore, optical biopsies may contribute to the ongoing paradigm shift towards precision surgery: in particular, CFM-driven real-time assessment of excisional surgical margins might provide potential survival improvements.

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