Prostate Cancer

Evaluation of Fluorescent Confocal Microscopy for Intraoperative Analysis of Prostate Biopsy Cores

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Abstract

Background: Diagnosis of prostate cancer is based on histopathological evaluation, which is time-consuming. Fluorescent confocal microscopy (FCM) is a novel technique that allows rapid tissue analysis.

Objective: To determine if FCM could be used for real-time diagnosis of prostate cancer and evaluate concordance with traditional analysis.

Design, setting, and participants: From January 2019 to March 2020, 182 magnetic resonance imaging–targeted prostate biopsy cores from 57 consecutive biopsy-naïve men with suspected prostate cancer were taken. These were intraoperatively stained with acridine orange for analysis using FCM (VivaScope; MAVIG, Munich, Germany) and subsequently sent for traditional haematoxylin-eosin histopathological (HEH) examination. Two expert uropathologists analysed the FCM and HEH cores blinded to the counterpart results in a single institution.

Outcome measurements and statistical analysis: Agreement between FCM and HEH analysis in terms of the presence of cancer was analysed at biopsy core and region of interest (ROI) levels, considering HEH as the reference test.

Results and limitations: FCM allowed intraoperative assessment of prostate biopsy cores with strong histopathological evaluation agreement: Cohen's κ for agreement was 0.81 at the biopsy core level and 0.69 for the ROI level. Positive predictive values (85% and 83.78%) and negative predictive values (95.1% and 85.71%) were high at the biopsy core and ROI levels. These initial results are encouraging, but given the single-centre and preliminary nature of the study, further confirmation is required.

Conclusions: FCM allowed rapid evaluation of prostate biopsy cores. This technique is feasible and achieves rapid closure with a reliable diagnosis, parallel to the gold standard analysis. Initial results are promising but further studies are needed to validate and define the role of this technique.

Patient summary: A novel microscopic technique reduces the time needed to obtain a prostate cancer diagnosis by speeding up biopsy processing. Although the initial results are promising; this development needs to be confirmed in further studies.

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1. Introduction

Prostate cancer diagnosis is based on histopathological evaluation [1]. Traditional preparation of cores for histopathological analysis is time-consuming [2]. In attempts to achieve "real-time" diagnosis, the intraoperative frozen section (IFS) technique has been used, with variable results for radical prostatectomy specimens [3,4]. IFS for prostate biopsy cores is not recommended, as the Gleason grading is extremely unreliable [5] and the technique can impair further pathological analysis of the cores.

Confocal microscopy is a real-time imaging tool first described in 1957 [6]. 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 visualisation of fluorophores to characterise cellular details [7]. Ex vivo FCM has largely been used to evaluate margins on Mohs surgery, for which it shows good concordance with gold-standard frozen sections [8]. More recently, Puliatti et al [9] reported the first FCM analysis of prostate biopsy cores taken from radical prostatectomy specimens to evaluate concordance between confocal and traditional analysis for men with known prostate cancer. FCM showed strong concordance and the authors concluded that it could be used for intraoperative decision-making. We sought to further explore this technique in intraoperative analyses of targeted biopsy cores from biopsy-naïve men with suspected prostate cancer. To the best of our knowledge, this is the first attempt to perform intraoperative real-time prostate cancer diagnosis.

2. Patients and methods

2.1. Study population and clinical data

Patient recruitment and sampling procedures were carried out in accordance with all local regulatory requirements and laws and the Declaration of Helsinki, and after approval from the Fundacion Instituto Valenciano de Oncologia ethics committee. All patients signed written informed consent before study inclusion.

A total of 57 consecutive biopsy-naïve men with prostate cancer suspicion because of raised prostate-specific antigen (PSA; >3 ng/ml) or abnormal digital rectal examination underwent transperineal targeted and systematic prostate biopsy at our institution. In total, 65 regions of interest (ROIs) defined as magnetic resonance imaging (MRI)–visible lesions (Prostate Imaging-Reporting and Data System [PI-RADS] score ≥3) were targeted.

Demographic, diagnostic, staging, and pathological data were prospectively collected.

2.2. Acquisition and processing of biopsy cores

Patients were positioned in the lithotomy position under general or regional anaesthesia. A transrectal ultrasound probe was inserted to obtain a prostate scan for subsequent software fusion of MRI targets (BiopSee; MedCom, Darmstadt, Germany). A median of three biopsy cores were taken from each ROI. Subsequently, all patients underwent systematic biopsies.

A total of 182 targeted cores from 65 ROIs were taken at the start of the procedure and placed on a biopsy foam sponge pad. These were immersed in acridine orange 1/100 for 30 s and rinsed with physiological saline; tissue preparation does not require specific training, as it is fairly easy and reproducible. Samples were then transferred to a crystal slide and placed on the FCM VivaScope (MAVIG, Munich, Germany) mount located in the operating theatre for scanning. VivaScan software was used to obtain colour images similar to samples stained with haematoxylin-eosin (HE). In our case, one expert pathologist (A.C.) prepares and scans the biopsy cores in theatre.

2.3. Analysis of biopsy cores

Targeted biopsy cores were intraoperatively analysed by one expert uropathologist (A.C.) with more than 10 yr of experience in analysing prostate biopsy cores. Meanwhile, systematic biopsy was being carried. All biopsy cores were subsequently sent for traditional histopathological evaluation by a second uropathologist blinded to the FCM results.

Biopsy cores were reported as positive or negative for the presence of cancer both on FCM and HE analysis. Gleason grade or other variables were not evaluated on FCM at this preliminary stage.

The criteria for identifying cancerous tissue via FCM were similar to those used for histopathological evaluation, although FCM evaluation was more challenging because of the lower image definition and the inability to perform complementary techniques such as immunohistochemistry.

For analysis purposes, identification of cancerous areas was prioritised. Thus, if cancerous tissue was detected for any of the ROI cores, the region was considered positive.

2.4. Statistical analysis

Statistical analysis was performed using R 3.4.0 [10] with the Mann-Whitney U test for comparison of means/medians, and χ^2 and Fisher's exact tests for categorical data. Statistical significance was set at $p < 0.05$. The statistical analysis was prespecified before data analysis.

The presence of cancer was expressed as a binary variable for both FCM and HE analysis at core and ROI levels. Concordance between the tests was then evaluated, considering HE analysis as the reference test.

The sensitivity, specificity, and negative and positive predictive values were calculated. Concordance was measured using Cohen's κ index, as well as the likelihood ratio for negative and positive tests [11].

3. Results

3.1. Patient characteristics

From January 2019 to March 2020, 182 targeted biopsy cores from 57 biopsy-naïve men were analysed. The median patient age was 68 yr and the men had median PSA of 7.29 ng/ml and median prostate volume of 50 cm^3. The patient characteristics are presented in Table 1. No serious adverse events were registered.

Regarding MRI, 23 (40.4%), 24 (42.1%), and ten men (17.5%) had a PI-RADS score of 3, 4, and 5, respectively.

3.2. Outcomes

The median time for FCM processing and analysis was 5 min. FCM was performed while systematic biopsies were being taken, so the operating time was not increased. No significant complications were registered.

Table 1 – Clinical and demographic data

Variable	Result
Patients (*n*)	57
Prostate volume (cm^3)	
Mean (standard deviation)	62.96 (35.77)
Median (interquartile range)	50 (40–76)
Prostate-specific antigen (ng/ml)	
Mean (standard deviation)	9.25 (8.10)
Median (interquartile range)	7.25 (4.54–10.00)
Age (yr)	
Mean (standard deviation)	65.98 (8.83)
Median (interquartile range)	68 (58–73)
Digital rectal examination, *n* (%)	
Normal	55 (96.50)
Suspicious	2 (3.50)
Magnetic resonance imaging findings, *n* (%)	
PI-RADS 3	23 (40.40)
PI-RADS 4	24 (42.10)
PI-RADS 5	10 (17.50)

PI-RADS = Prostate Imaging-Reporting and Data System score.

FCM and HE concordance was evaluated at biopsy core (Tables 2 and 3) and ROI (Tables 4 and 5) level. Cohen's κ for agreement between the techniques was 0.81 for biopsy core level and 0.69 for ROI level; the concordance was lower for ROI level because if any core within the ROI was positive, the ROI was considered positive.

The positive predictive value (85% and 83.78%) and negative predictive value (95.1% and 85.71%) were high at biopsy core and ROI levels. Five cores were reported as negative on FCM and tumour was found on the corresponding final HE evaluations, with Gleason grade of 6 (3 + 3) in three cases, 7 (4 + 3) in one case, and 8 (4 + 4) in one case (Table 6).

The likelihood ratio for positive versus negative tests showed that FCM accurately predicted the presence of cancer on final histopathological evaluation at biopsy core (8.46 vs 0.08) and ROI (4.43 vs 0.14) levels.

4. Discussion

To the best of our knowledge, this is the first use of FCM for intraoperative diagnosis of prostate cancer after Puliatti et al [9] described FCM-based analysis of prostate biopsy cores. In our case, we went a step further by analysing biopsy cores from men who had not yet been diagnosed with prostate cancer.

As with any novel diagnostic approach, a new test must be compared with a reference test for validation. In our case,

Table 2 – Analysis at biopsy core level

FCM	Haematoxylin-eosin staining, *n* (%)		
	Positive	Negative	Total
Positive	68 (85.00)	12 (15.00)	80 (43.96)
Negative	5 (4.90)	97 (95.10)	102 (56.04)
Total	73 (40.11)	109 (59.89)	182 (100.00)

FCM = fluorescent confocal microscopy.

Table 3 – Concordance evaluation at biopsy-core level

	Estimate (95% CI)
Prevalence (%)	40.11 (32.93–47.62)
Properly classified patients (%)	90.66 (85.47–94.46)
Sensitivity (%)	93.15 (84.74–97.74)
Specificity (%)	88.99 (81.56–94.18)
Positive predictive value (%)	85.0 (75.26–92.0)
Negative predictive value (%)	95.1 (88.93–98.39)
Likelihood ratio for a positive test	8.46 (4.94–14.48)
Likelihood ratio for a negative test	0.08 (0.03–0.18)
Cohen's κ	0.81 (0.72–0.90)

CI = confidence interval.

Table 4 – Analysis at region-of-interest level

FCM	Haematoxylin-eosin staining, *n* (%)		
	Positive	Negative	Total
Positive	31 (83.78)	6 (16.22)	37 (56.92)
Negative	4 (14.29)	24 (85.71)	28 (43.08)
Total	35 (53.85)	30 (46.15)	65 (100.00)

FCM = fluorescent confocal microscopy.

Table 5 – Concordance evaluation at region-of-interest level

	Estimate (95% CI)
Prevalence (%)	53.85 (41.03–66.3)
Properly classified patients (%)	84.62 (73.52–92.37)
Sensitivity (%)	88.57 (73.26–96.8)
Specificity (%)	80.0 (61.43–92.29)
Positive predictive value (%)	83.78 (67.99–93.81)
Negative predictive value (%)	85.71 (67.33–95.97)
Likelihood ratio for a positive test	4.43 (2.14–9.15)
Likelihood ratio for a negative test	0.14 (0.06–0.37)
Cohen's κ	0.69 (0.51–0.87)

CI = confidence interval.

FCM showed strong concordance with traditional HE examination, with high positive and negative predictive values, as well as likelihood ratios for positive and negative tests [12]. The degree of disparity between tests in our study is within the expected interobserver variability for evaluation of prostate biopsy cores [13]. Subjective perception of FCM resolution by our pathologist reflects good image quality that allows for confident diagnosis of prostate cancer, as recently described by Bertoni et al [14] for an FCM image atlas obtained from radical prostatectomy specimens for which they evaluated benign and malignant prostatic tissues. Furthermore, identification of extraprostatic tissue allows recognition of nondiagnostic biopsy cores, which can improve the diagnostic yield (Fig. 1).

One important limitation to this technique is that we lack the expertise to confidently assign Gleason grading to the cores analysed via FCM; thus, we have not specifically analysed these data and limited the evaluation to a binary outcome of cancer versus no cancer. This might be overcome with further development. Furthermore, although the

Table 6 – Concordance between FCM and haematoxylin-eosin Gleason grading

FCM	Gleason grading on haematoxylin-eosin staining, *n* (%)							
	No cancer	3 + 3	3 + 4	4 + 3	4 + 4	4 + 5	5 + 4	Total
Positive	12 (15.00)	26 (32.50)	29 (36.25)	6 (7.50)	3 (3.75)	2 (2.50)	2 (2.50)	80 (43.96)
Negative	97 (95.10)	3 (2.94)	0 (0.00)	1 (0.98)	1 (0.98)	0 (0.00)	0 (0.00)	102 (56.04)
Total	109 (59.89)	29 (15.93)	29 (15.93)	7 (3.85)	4 (2.20)	2 (1.10)	2 (1.10)	182 (100.00)

FCM = fluorescent confocal microscopy.

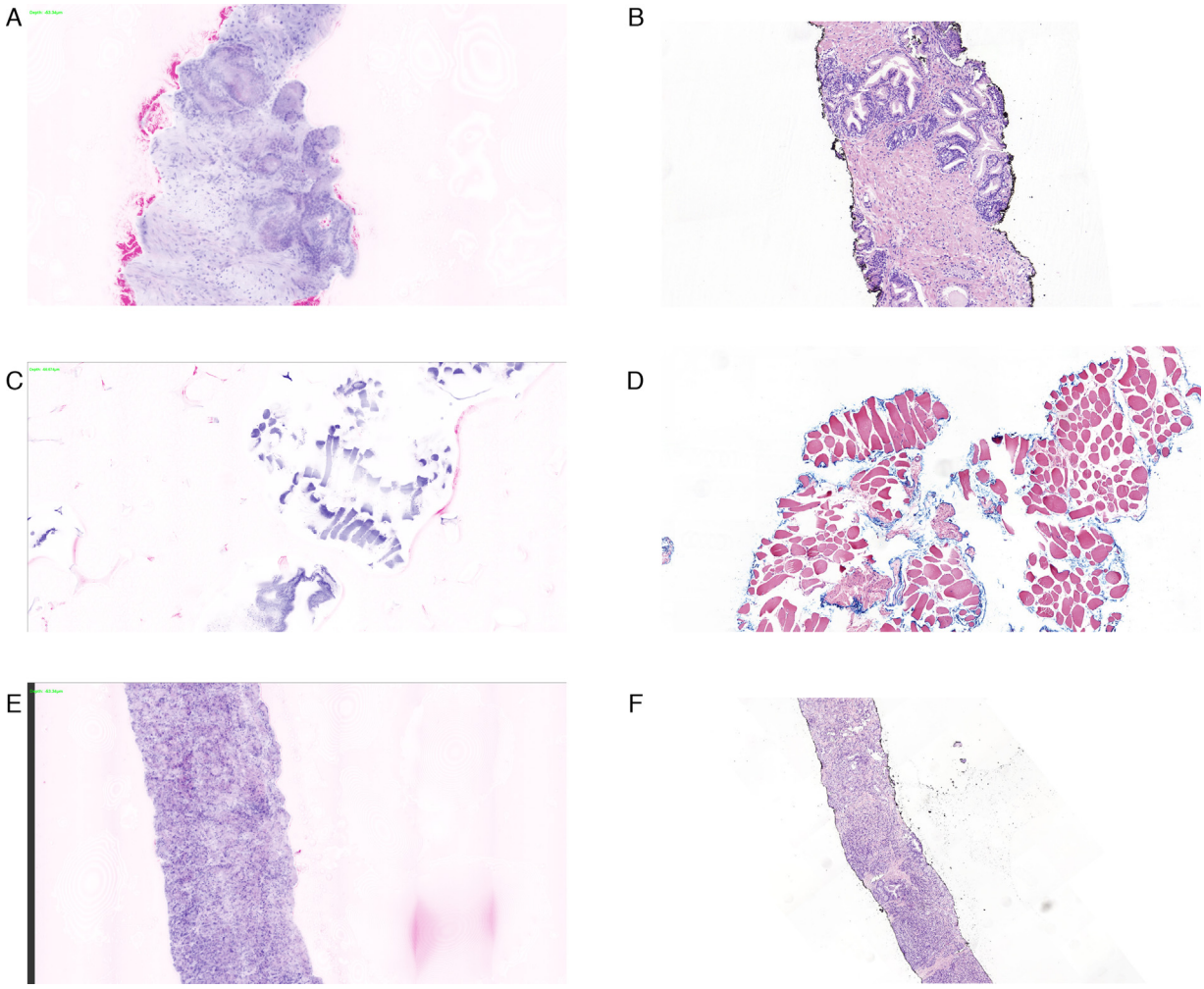

Fig. 1 – Comparison of VivaScan fluorescent confocal microscopy (FCM) images and haematoxylin-eosin (HE) stained samples. Benign glands in (A) FCM and (B) HE images; striated muscle (nonviable biopsy core) in (C) FCM and (D) HE images; and prostatic adenocarcinoma in (E) FCM and (F) HE images.

initial experience is encouraging, internal and external validation is needed to confirm these findings, as in our case a single pathologist analysed all the FCM cores. Another factor that might be a deterrent for reproducibility of this technique is that the pathologist needs to be available if not on site, as is the case in our institution, for intraoperative analysis. Lastly, in our opinion this approach is only advised for pathologists who are highly experienced in evaluating prostate biopsy cores, as FCM reduces image quality and might add difficulty to the already challenging task of prostate cancer diagnosis.

Intraoperative FCM-based analysis is relevant as prostate cancer diagnosis invariably relies on pathological examination of biopsy cores. In men with MRI-visible prostate lesions and targeted biopsy data, FCM showed high concordance with traditional analysis, with much faster closure on diagnosis. Furthermore, FCM analysis did not add time to the biopsy acquisition process or hinder

traditional analysis for the biopsy cores. In our opinion, FCM is superior to IFS for intraoperative prostate core evaluation as tissue can be used for subsequent analysis. Furthermore, in our experience FCM requires less preparation time and expertise given that slicing of frozen sections is technically challenging, reduces the amount of tissue for further analysis, and can even lead to sample destruction if inappropriately handled.

This finding opens a new scenario in which men with suspected prostate cancer can obtain a rapid diagnosis, which would reduce patient anxiety and eventually provide an opportunity for intraoperative decision-making.

In our opinion, there are two main fields in which FCM evaluation could have a role. The first is evaluation of surgical margins during radical prostatectomy to improve functional outcomes while not compromising oncological safety [9]. Many intraoperative histological evaluations have been proposed to address this issue, with varying results; the advantage of FCM is that it can be applied to whole tissue sections with rapid and relatively easy preparation.

The second role is a potential novel one-stop diagnosis and focal treatment concept for localised unifocal prostate cancer. This approach would be feasible if three requirements are met: (1) a high negative predictive value of prostate multiparametric MRI [15] to rule out significant prostate cancer; (2) a reliable intraoperative biopsy core analysis via FCM provided there is further validation of the technique; and (3) a low impact of focal therapy on subsequent radical therapies [16–18] if focal ablation at the time of diagnosis is considered. In any case, this simultaneous diagnosis and treatment concept for prostate cancer is appealing but is not yet an advisable approach.

5. Conclusions

Our preliminary data suggest that fluorescent microscopy could be used for intraoperative evaluation of prostate biopsy cores from men with suspected prostate cancer. We observed strong concordance with histopathological evaluation compatible with known interobserver variation for prostate cores analysis.

This is a novel and exciting line of research and, to the best of our knowledge, is the first-in-man intraoperative diagnosis of prostate cancer. These results should be interpreted with caution given their preliminary nature, and further studies are needed to confirm our findings.

Author contributions: Jose Marenco 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: Calatrava, Casanova, Barrios, Martin.
Acquisition of data: Calatrava.
Analysis and interpretation of data: Marenco, Claps.
Drafting of the manuscript: Marenco.
Critical revision of the manuscript for important intellectual content: Calatrava, Casanova, Rubio.
Statistical analysis: Marenco, Mascaros, Claps.
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