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Real-time assessment of surgical margins during radical prostatectomy: a novel approach that uses fluorescence confocal microscopy for the evaluation of peri-prostatic soft tissue

The intra-operative control of surgical dissection continues to be an important issue [1]. The correct plane of dissection is sometimes difficult to define, therefore, real-time pathological examination could be useful to assess whether benign or malignant prostate tissue is still present at the prostatic fossa [1–4].

Frozen-section approaches and the 'NeuroSAFE' technique have been developed for this purpose [1–4]. These require a fully equipped laboratory, a pathologist inside the institution and take overall >30 min to perform. This has limited the diffusion of NeuroSAFE, which is currently performed only in facilities with a dedicated set-up.

Superimposed three-dimensional imaging is another solution to address the problem of surgeon confidence with the plane of dissection [5]; however, as is the case with NeuroSAFE, not all institutions can afford such a technological advance and it relies on multiparametric MRI, whose sensitivity in the detection of extracapsular extension is generally low [6].

The surgeon may have also concerns about the safety of the dissection in an imaging-safe area. Inadvertent incision into glandular tissue may occur and awareness of persistent benign prostate tissue at the margin of dissection is also important [7].

Given the importance of tailored and controlled dissection, the aim of the present study was to test the ability of a novel technique, fluorescence confocal microscopy (FCM), to detect residual prostate tissue in the peri-prostatic environment, differentiating it from extra-prostatic soft tissue.

Ex vivo FCM (VivaScope®2500M-G4; Caliber I.D., Rochester, NY, USA) is an optical technology allowing examination of freshly excised tissue using fluorescence and reflectance techniques, providing a histologic-like appearance of tissues in few minutes. We previously tested the level of agreement between FCM and the conventional haematoxylin and eosin (H&E) approach to discriminating between prostate cancer (PCa) and normal prostatic tissue, finding an agreement of 91% (Cohen's $\kappa = 0.75$) [8]. That trial [8] was designed as an exploratory study, with samples obtained from 18G biopsies of a prostate specimen, in order to test the feasibility of prostate tissue reading with FCM.

We are currently evaluating FCM in a real-life setting, the intra-operative analysis of peri-prostatic tissue retrieved from suspect areas. To this end, we sought to explore the level of agreement between FCM and H&E in discriminating patterns typical of peri-prostatic tissue.

A total of 20 patients undergoing partial or full nerve-sparing robot-assisted laparoscopic prostatectomy (RALP) between January and June 2019 were enrolled in this prospective study, which was approved by the local ethics committee (Protocol 0018091/18). No inclusion criteria were predefined, except for the need for signed informed consent from the patient. During RALP, one or more samples from the peri-prostatic tissue were retrieved in cases of proximity of a mpMRI-detected lesion or in cases where the surgeon suspected extracapsular extension; all interventions were performed by a single surgeon (B.R.).

Specimens were analysed according to our previously described technique [8] (specimens were immersed in 95% alcohol for 5–10 s, rinsed in 0.9% NaCl, soaked for 30 s in acridine orange 0.6 mmol/L; Sigma-Aldrich, Milan, Italy; samples were then embedded between two glass slides). FCM digital images were acquired within 1–2 min per sample, and sent for microscopical reading by a single pathologist outside the institution (blinded to the identity of the patient, to his clinical data, and to the subsequent H&E analysis). After FCM, the specimen underwent H&E analysis and official reporting was provided by the same pathologist.

Of the 20 patients, 19 had localized disease (≤pT2); one patient had a macroscopical site of extracapsular extension, proximal to a PI-RADS-5 lesion, recognized intra-operatively. Overall, two patients had a positive surgical margin (pT3a disease, $n = 1$; pT2c disease, $n = 1$).

A total of 41 samples from peri-prostatic tissue were immediately available for FCM (median of 2 per patient). PCa was detected in one patient (the patient with macroscopical pT3a disease), prostatic benign glands were

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detected in one patient, and for the remaining patients only soft tissue was present.

During the FCM, basic assessment of the extra-prostatic components AS fatty tissues (Fig. 1a), muscle bundles and nerves (Fig. 2a) was carried out reflectance mode and they are shown in a pink-colour resembling eosin staining (Fig. 1); prostate glandular patterns are clearly different, with a predominant purple colour provided by acridine-stained nuclei (Fig. 3a). PCa is depicted in Fig. 4a. The same H&E features are reported in Figs 1b, 2b, 3b and 4b, respectively. The sample containing PCa was obtained from the patient with extensive extracapsular extension. FCM diagnostic performance in distinguishing between non-prostatic tissue, benign prostatic tissue and PCa was high; FCM demonstrated almost perfect agreement with H&E in its depiction of all tissue types.

The advantages of FCM analysis of peri-prostatic tissue demonstrated in the present study are: fast and simple tissue handling and no need for a dedicated set-up; fast acquisition of high-resolution images similar in appearance to H&E stained images; and instant web availability of images. A weakness of the present study is that only one sample of peri-prostatic soft tissue contained PCa; however, the issue of PCa detection using FCM has been exhaustively addressed in our previous manuscript [8]. Another limitation of the study is the random fashion of the sampling method; a prospective study is currently ongoing to define the role of systematic sampling of the postero-lateral aspect of the prostate read by FCM to achieve real-time detection of positive surgical margins.

The reliable discrimination provided by FCM between peri-prostatic soft tissue and prostatic glandular tissue, together with the aforementioned advantages, makes FCM a promising

Fig. 1 Fatty tissue as assessed by (**a**) fluorescence confocal microscopy (FCM) and (**b**) haematoxylin and eosin (H&E). Typical features of enlarged nuclei, prominent nucleoli and absence of basal cell layer, as displayed on FCM analysis, are shown. The level of agreement between FCM and H&E was 97.14% for muscular (κ = 0.94, 95% CI 0.798–1), 97.14% for nervous, 97.14% for vascular (κ = 0.65) and 94.2% for fatty tissue (κ = 0.861, 95% CI 0.639–1). Agreement on benign prostatic glands and prostate cancer was perfect (100%).

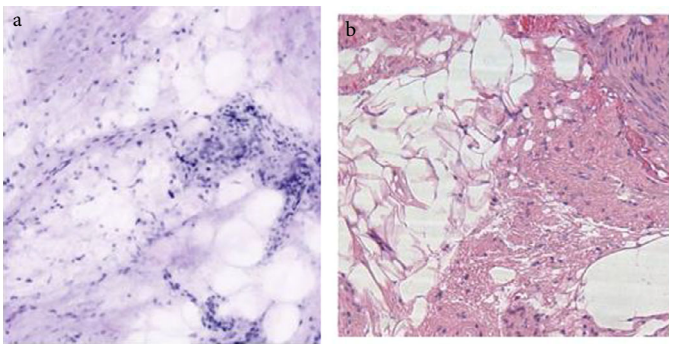


Fig. 2 Image of a nerve as assessed by (**a**) fluorescence confocal microscopy (FCM) and (**b**) haematoxylin and eosin (H&E). Typical features of enlarged nuclei, prominent nucleoli and absence of basal cell layer, as displayed on FCM analysis, are shown. The level of agreement between FCM and H&E was 97.14% for muscular (κ = 0.94, 95% CI 0.798–1), 97.14% for nervous, 97.14% for vascular (κ = 0.65) and 94.2% for fatty tissue (κ = 0.861, 95% CI 0.639–1). Agreement on benign prostatic glands and prostate cancer was perfect (100%).

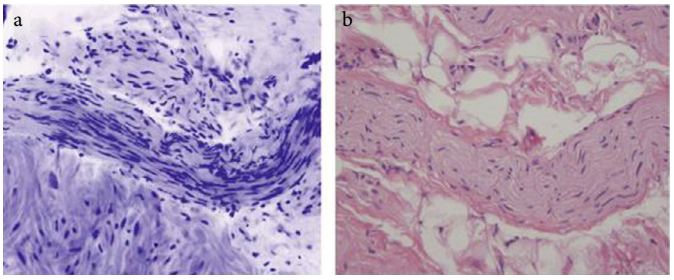


Fig. 3 Benign prostatic glands as assessed by (**a**) fluorescence confocal microscopy (FCM) and (**b**) haematoxylin and eosin (H&E). Typical features of enlarged nuclei, prominent nucleoli and absence of basal cell layer, as displayed on FCM analysis, are shown. The level of agreement between FCM and H&E was 97.14% for muscular (κ = 0.94, 95% CI 0.798–1), 97.14% for nervous, 97.14% for vascular (κ = 0.65) and 94.2% for fatty tissue (κ = 0.861, 95% CI 0.639–1). Agreement on benign prostatic glands and prostate cancer was perfect (100%).]

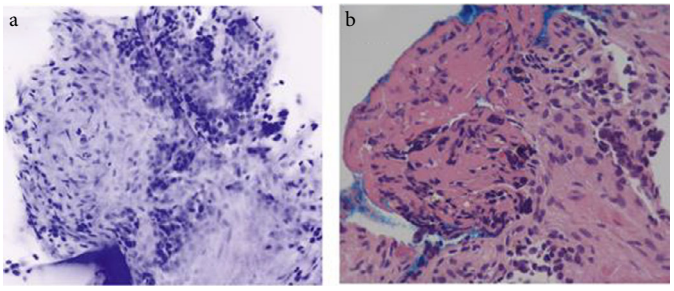
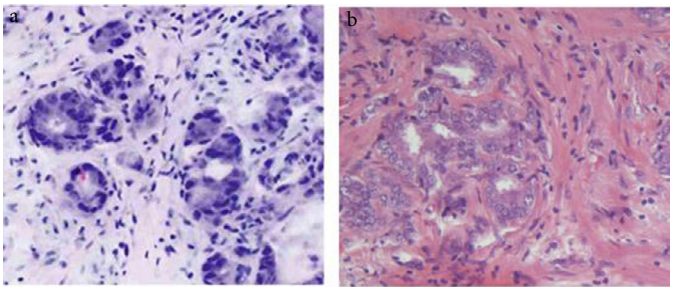


Fig. 4 Prostate cancer as assessed by (**a**) fluorescence confocal microscopy (FCM) and (**b**) haematoxylin and eosin (H&E). Typical features of enlarged nuclei, prominent nucleoli and absence of basal cell layer, as displayed on FCM analysis, are shown. The level of agreement between FCM and H&E was 97.14% for muscular (κ = 0.94, 95% CI 0.798–1), 97.14% for nervous, 97.14% for vascular (κ = 0.65) and 94.2% for fatty tissue (κ = 0.861, 95% CI 0.639–1). Agreement on benign prostatic glands and prostate cancer was perfect (100%).



prospect for real-time tailoring of surgical dissection during RALP; future applications such as systematic sampling of the prostatic surface (e.g. NeuroSAFE [3]) could be one of the most interesting.

Conflicts of Interest

Marco Sandri has a temporary contract as a statistics consultant for MAVIG GmBH.

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