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Laser-induced autofluorescence spectroscopy: Can it be of importance in detection of bladder lesions?

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Summary
Background: Non-muscle invasive bladder cancer can be missed during white light endoscopy in up to 50% of cases. We aimed to test whether or not we could find a difference between benign and cancerous tissue wavelengths using laser induced autofluorescence spectroscopy can increase cancer detection.
Materials and methods: We analysed 67 tissue samples using spectral analysis. The WavSTAT (Spectra Science) optical biopsy device was used to record fluorescence spectra from biopsied tissue enabling calculation of an AUC for each spectrum, a measure of the mean spectral wavelength ($\bar{\lambda}$ (nm)) and a dimensionless fluorescence ratio. Mann—Whitney test was used to compare the two groups.
Results: We found that 49.3% (33/67) of the tissue was benign, 44.8% (30/67) was CIS/cancerous tissue, and the remaining 4/67 samples were atypia (2) and dysplasia (2).

The median AUC for the benign tissue was 19.53 (interquartile range [IQR]: 5.35—30.39) and that for CIS/cancerous tissue was 7.05 (IQR: 2.89—14.24) ($P = 0.002$).

The median wavelengths for the benign tissue and malignant tissue were 502.4 nm (IQR: 500.3—504.3 nm) and 505.2 nm (IQR: 502.1—513.2 nm), respectively ($P = 0.003$).

The median fluorescence ratio was 0.080 (IQR: 0.070—0.088) for benign tissue and 0.096 (IQR: 0.079—0.221) for CIS/cancerous tissue ($P = 0.002$).

Abbreviations: AUC, area under the curve; CIS, carcinoma in situ; IQR, interquartile range; NBI, narrow band imaging; OB, optical biopsy; PD, photodynamic diagnostic; PDT, photodynamic therapy; PPIX, protoporphyrin IX; US, ultrasonography.

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Conclusions: We found statistical differences between the median AUC calculations and median wavelengths for the benign and cancerous tissue. We also found a statistical difference between the fluorescence ratios between the two tissue types. There seems to be a role for optical spectroscopy in verifying bladder lesions.

Introduction

Bladder cancer is the most common malignancy in the urinary tract with a worldwide incidence of 9/100,000 and 2/100,000 for men and women, respectively and a mortality rate of 3/100,000 and 1/100,000 for men and women, respectively [1]. The mortality rates from bladder cancer decreased in recent years, mainly due to the vigorous follow up regimens and improved standard of care [1]. However, despite the regular investigations and follow ups, imaging modalities such as CT scanning and ultrasonography (US) can miss superficial lesions [1]. Even cystoscopic examination can miss carcinoma in situ (CIS) and dysplastic lesions [1].

The importance of detecting CIS early is that 54% of these will progress to muscle-invasive disease, which will require more aggressive treatment [1]. To try and enhance visualisation of superficial lesions, fluorescence cystoscopy or narrow band imaging (NBI) technologies have been employed [2,3]. The role of photodynamic diagnostic (PD) cystoscopy, is still debatable, as the technique is limited by a high false-positive rate induced by inflammation, recent resection margins, or if conducted in the first 3 months after BCG instillation [1]. Furthermore an additional cost is added because of the use of the photosensitiser and equipment required for blue light visualisation which should be taken into consideration [1]. While the initial results of NBI have shown some potential to improve cancer detection, confirmation from large trials are needed [1].

New technologies continue to emerge to enhance the detection of cancer, recalling the one of which is known as optical biopsy (OB) and has shown some potential in distinguishing between benign and malignant tissues [4,5]. The optical biopsy device used for this study operates on the principle of fluorescence spectroscopy, a promising optical diagnostic technique that generates fluorescence spectra by utilising the interaction of light with different tissue types. In ex vivo study Zheng et al. studied fluorescence excitation—emission matrices (220—500 nm) instead of single or few wavelengths for spectrometry. He found bladder cancer to be characterised by increase in spectra from tryptophan and porphyrins and a decrease in spectra from collagen, NADH and FAD compared to benign tissue. He also suggested those excitation wavelengths at 280 and 330 nm can be of great importance to differentiate cancerous from benign tissue in the bladder [6].

In Anidjar et al's study excitation wavelength of 308 nm and Koenig et al's study the wavelength of 337 nm were found to be the most effective in distinguishing between benign and malignant tissue [7—9]. Anidjar et al. also suggested argon laser excitation light of 488 nm and spectra analysis in length between 550 nm and 56 nm as he found significant reduction in oxidised flavoprotein concentration in urothelial cell cancer [10]. Zaak et al. conducted in vivo spectrometry study with excitation wavelength of 380 nm to verify all fluorescent areas in the bladder during Photodynamic Diagnostic cystoscopy with 5-ALA administered into the bladder and photosensitizer [11]. They found sensitivity of 90% and specificity of 84% for combination of both techniques [11].

All the studies were very promising, but also limited as were design as ex vivo or suggested wavelength of potential carcinogenetic risk which was not fully evaluated for the bladder [12]. Protoporphyrin IX remains of great value in detection of cancerous tissues even without photosensitizer enhancement and its excitation does not require ultraviolet light [13]. Laser light at 405 nm can be used to excite endogenous fluorophores in tissue (including PPIX), which subsequently emit characteristic autofluorescence at a different wavelength and can be displayed as a spectrum [13]. Autofluorescence has been shown to decrease in cancerous tissue and thus can be used to differentiate between benign and cancerous tissue [13].

To this end, we conducted a feasibility study to evaluate the role of OB in the detection of bladder cancer with excitation wavelength of 405 nm.

Materials and methods

The study was designed as a pilot to evaluate the feasibility of 405 nm laser-induced autofluorescence spectrometry in the diagnosis of bladder cancer by optical biopsy measurements carried out in theatre during cystoscopy. No drugs were involved in the study. Patients who underwent transurethral resection of bladder tumour for confirmed bladder cancer or those who underwent routine bladder biopsies for possible malignancy were enrolled into this study if they provided informed written consent. Each participant was counselled and informed of the procedure and a written participant information sheet was provided. Patients, who were unable to consent (e.g. with learning difficulty), did not retain or comprehend information, or had a mental disability were excluded.

The study protocol was approved by the institution's Research and Development department and East of Scotland Research Ethics Committee. All data were prospectively collected. Fluorescence spectral measurements were taken from the bladder wall just before taking biopsies. These fluorescence measurements were reviewed against pathological findings for each biopsy. Biopsies were labelled as routine practice by the urological team in theatre while the pathologist was blinded to the spectral measurements and clinical information.

The WavStat System (Spectra Science, Inc.; CE 0495) was used to acquire all fluorescence measurements. WavStat incorporates a diode laser (Topica Photonics, iBeam Smart), which emits low-level laser light with a power of 0.105 mW