Histological Validation of in-vivo VERDICT MRI for Prostate using 3D Personalised Moulds

Elisenda Bonet-Carne[1,2], Maira Tariq[1], Hayley Pye[3], Mrishta Brizmohun Appayya[2], Aiman Haider[4], Colleen Bayley[1], Joseph Jacobs[1], Alexander Freeman[4], David Hawkes[1], David Atkinson[2], Greg Shaw[5], Hayley Whitaker[3], Daniel C Alexander[1], Shonit Punwani[2], and Eleftheria Panagiotaki[1]

[1]Centre for Medical Image Computing, University College London, London, United Kingdom, [2]Centre for Medical Imaging, University College London, London, United Kingdom, [3]Research Department for Tissue & Energy, University College London, London, United Kingdom, [4]Department of Pathology, University College London Hospital, London, United Kingdom, [5]Division of Surgery and Interventional Science, University College London, London, United Kingdom

Synopsis

VERDICT analysis can successfully distinguish benign from malignant prostate tissue *in-vivo* showing promising results as a cancer diagnostic tool. However, the accuracy with which model parameters reflect the underlying tissue characteristics is unknown. In this study, we quantitatively compare the intracellular, extracellular-extravascular and vascular volume fractions derived from *in-vivo* VERDICT MRI against histological measurements from prostatectomies. We use 3D-printed personalised moulds designed from *in-vivo* MRI that help preserve the orientation and location of the gland and aid histological alignment. Results from the first samples using the 3D mould pipeline show good agreement between *in-vivo* VERDICT estimates and histology.

INTRODUCTION

VERDICT aims to provide microstructural information from solid cancer tumours non-invasively using mathematical models linking the diffusion MRI signal to microscopic tissue features[1]. The VERDICT model for prostate cancer characterisation shows promising results in differentiating benign and cancerous tissue and correlates with different Gleason patterns[1,2]. However, few attempts have been made to validate the clinical VERDICT for prostate using histological ground truth[3] and rigorous validation of the *in-vivo* parameters has never been done.

MRI validation is challenging in matching its registration with histology. During *in-vivo* imaging, the prostate is deformed (due to voluntary and involuntary body movements) which makes it difficult to match even the corresponding tissue planes. Using personalised-moulds from *in-vivo* MRI[4] with landmarks has demonstrated consistent tissue positioning and minimised misalignment between *ex-vivo* VERDICT MRI and histological features[5]. This study goes one step further and uses 3D-personalised-mould, with additional *ex-vivo* MRI for alignment to aid the registration of *in-vivo* MRI and histopathology.

The aim of this study is to perform a direct quantitative validation of the in-vivo VERDICT parametric maps for prostate cancer[1,6] against histological sections obtained from radical prostatectomies.

METHODS

Imaging

Three different candidates for prostatectomy with a previous clinical multiparametric prostate MRI (mpMRI) were scanned for VERDICT analysis as part of the INNOVATE clinical trial[7]. For each VERDICT scan, diffusion-weighted (DW) MRI was performed using a 3T scanner (Achieva, Philips Healthcare, Netherlands) as in[2,8]. The VERDICT model was fitted to the data in each voxel using the AMICO[9] framework to obtain the parameter maps as previously described in[8]. The VERDICT parameters that we are validating here are the volume fractions (*vf*): intracellular (f_{IC}), extracellular-extravascular (f_{EES}) and vascular (f_{VASC}).

The clinical mpMRI was used to create patient-specific 3D-printed prostate moulds with anatomical landmarks to preserve the original shape of the organ and to be able to identify areas of interest[4]. After the prostatectomy, prostates were placed in the mould, following the protocol described in[4] and scanned again fresh (*ex-vivo*) in the 3T scanner. We used landmarks (red arrows in Figure 1,3a) in the 3D-mould to select the same slice.

Histological analysis

Samples were cut in 5mm sections using the mould guides. From each section, three consecutive 3μm slices were obtained, stained with (1) hematoxylin and eosin (H&E), (2) cytokeratin and smooth muscle actin (MNF-SMA) and (3) CD31 marker (CD31), respectively and digitised (Hamamatsu NanoZoomer) using a 20x objective. Slices were aligned with manual rotation and translation. We segmented SMA-MNF images to obtain the proportion of epithelial cells and CD-31 images for quantifying the vasculature.

We analysed different regions of interest (ROI) in the VERDICT-DWI image and in the corresponding histological slice.

RESULTS

Figure 1 shows the different MRI acquisitions with the corresponding histological slices. Important intra-patient differences between *in-vivo* scans (clinical *vs.* VERDICT) highlight the challenge for accurate histological correlation.

Figure 2 shows the VERDICT maps for the three patients. Maps have been filtered removing voxels with poor fitting using the objective function. As expected, in all samples, prostate peripheral zone has lower f_{IC} than transition zone[10].

Figure 3 illustrates for one subject the 3D-mould with the prostate and the corresponding 5mm histological section. Also, it shows the three different stains and the alignment between the histological slices.

Figure 4 shows the f_{IC} distribution per voxel for different ROIs with corresponding histology. Results reveal that regions with fewer epithelial cells correspond to areas of lower f_{IC}. Table 1 shows the quantitative VERDICT-histology comparison and the correlation for the f_{IC} (r=0.96, p=0.002), f_{EES} (r=0.96, p=0.001) and f_{VASC} (r=0.386, p=0.449). We note that these samples had very low vasculature, hence the close to zero and noisy estimates of f_{VASC}. The rest of *vf* show agreement with histology.

DISCUSSION

Our study is the first to show that *in-vivo* VERDICT *vf* parameters agree well with histology. We used 3D-personalised moulds and *ex-vivo* scans to aid precise tissue comparison. However, *in-vivo* images still exhibit prostate deformations and distortions (i.e. bowel gas) that hinder accurate *in-vivo* to *ex-vivo* registrations. Future work will improve VERDICT alignment with histology by: (1) utilising the T2 images from the VERDICT acquisition, instead of mpMRI, to create 3D-personalised moulds and (2) using tissue information such as anatomical structures and lesions, prior to registration, to segment regions with similar shrinkage. With better tissue agreement, a more accurate voxel-wise comparison could be performed as in[3]. More samples are required to further validate all VERDICT parameters.

CONCLUSIONS

Early results from the first samples using personalised 3D-printed moulds from MRI show good agreement between *in-vivo* VERDICT *vf* estimates and histology.

Acknowledgements

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Figures

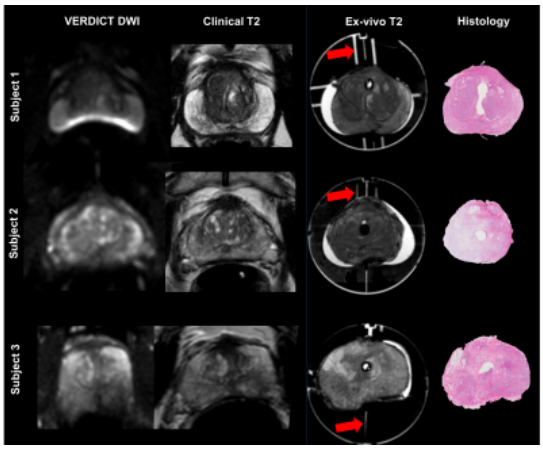


Figure 1. For each subject *in-vivo* T2 MRI images from VERDICT and clinical acquisitions, *ex-vivo* T2 image using the personalised moulds and the corresponding histology slice. VERDICT DWI: diffusion b0 image acquired as part as the VERDICT protocol. Clinical T2: T2 image acquired as a part of the mpMRI, used to create the mould. *ex-vivo* T2: 3T *ex-vivo* scan of the prostate inside the mould. Red arrows show 3D-moulds landmark. Histology: Histological slice corresponding to the other MR images.

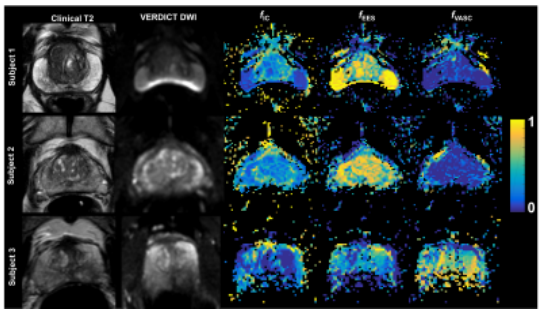


Figure 2. Clinical T2: T2 image acquired as a part of the mpMRI, used to create the mould.VERDICT parameter maps. VERDICT DWI: diffusion b0 image acquired as part as the VERDICT protocol. f_{IC}: Intracellular volume fraction, f_{EES}: Extracellular-Extravascular volume fraction, f_{VASC}: Vascular volume fraction. Black pixels in all parameter maps correspond to voxels with poor fitting.

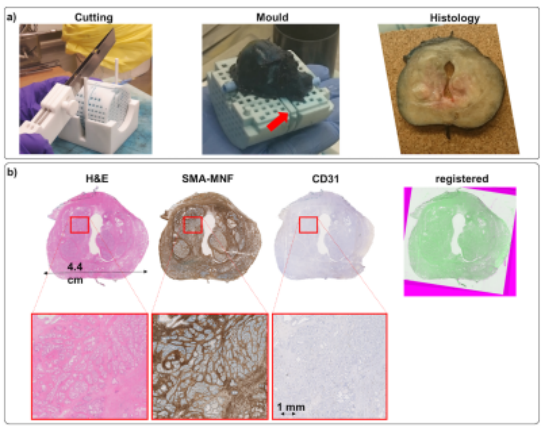


Figure 3.a) The prostate placed inside the 3D-personalised-mould and the cutting process to obtain the 5mm slice is shown. Red arrow shows the landmark in the 3D-mould. **b)**Three different stains for the first sample corresponding to region of interest 1. H&E: standard hematoxylin and eosin. MNF-SMA: cytokeratin (MNF) and smooth muscle actin (SMA) to mark epithelial cells (in blue) and stroma regions (in brown) respectively. CD31: CD31 marker for blood vessels and capillaries (in brown). Note that tissue is not exactly the same as it corresponds to consecutive micrometre slices.

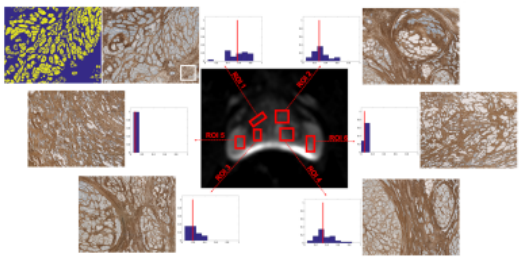
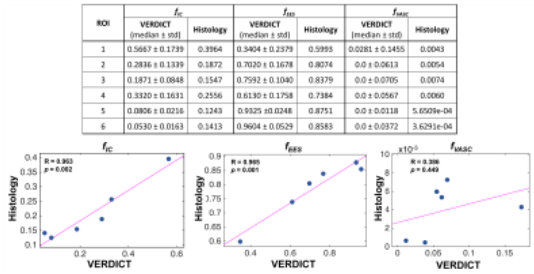


Figure 4. Voxel distribution of the intracellular volume fraction (f_{IC}) from VERDICT scan for six regions of interest (ROI). Red line indicates the f_{IC} median value for that ROI. The corresponding MNF-SMA tissue image for each ROI is shown next to the histograms, where epithelial cells appear in blue and stroma in brown. White square in MNF-SMA image for ROI illustrates the voxel size correspondence. For ROI 1 segmentation for epithelial cells is shown next to the MNF-SMA.



ROI	f_{IC}		f_{EES}		f_{VASC}	
	VERDICT (median ± std)	Histology	VERDICT (median ± std)	Histology	VERDICT (median ± std)	Histology
1	0.5667 ± 0.1739	0.3964	0.3404 ± 0.2379	0.5993	0.0281 ± 0.1455	0.0043
2	0.2836 ± 0.1339	0.1872	0.7020 ± 0.1678	0.8074	0.0 ± 0.0613	0.0054
3	0.1871 ± 0.0848	0.1547	0.7592 ± 0.1040	0.8379	0.0 ± 0.0705	0.0074
4	0.3320 ± 0.1631	0.2556	0.6130 ± 0.1758	0.7384	0.0 ± 0.0567	0.0060
5	0.0806 ± 0.0216	0.1243	0.9325 ±0.0248	0.8751	0.0 ± 0.0118	5.6509e-04
6	0.0530 ± 0.0163	0.1413	0.9604 ± 0.0529	0.8583	0.0 ± 0.0372	3.6291e-04

Table 1. VERDICT-histology comparison for the ROIs shown in Figure 4 and all the volume fraction parameters. The corresponding Pearson's correlation are also shown. Peripheral Zone ROIs (ROI 5 and 6) present lower f_{IC} than transition zone ROIs (TZ). This difference is not that clear in histology, this might be for the effects of tissue fixation on microstructure. TZ volume fractions from histological quantitative analysis fall within the VERDICT parameter ranges. f_{IC}: Intracellular volume fraction, f_{EES}: Extracellular-Extravascular volume fraction, f_{VASC}: Vascular volume fraction.