

MR spectroscopic imaging of prostate cancer: metabolism or morphology?

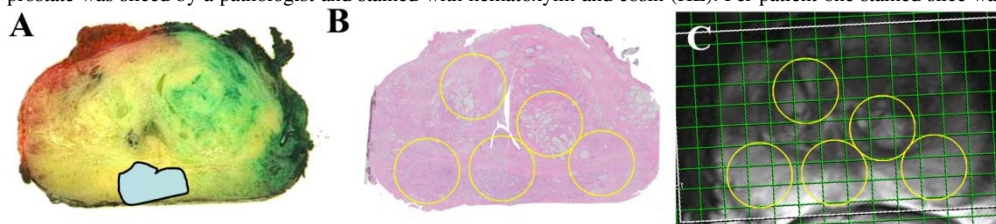
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Target audience: Scientists and clinicians involved in (prostate) cancer research.

Introduction and purpose: With ¹H MRSI the spatial distribution of the metabolites citrate (Cit), choline (Cho), creatine (Cr) and spermine (Spm) can be studied in the prostate. In spectra of prostate cancer (PCa) tissue, an increase in the Cho+Cr/Cit ratio is observed compared to normal tissue spectra, in particular because the intensity of the Cit signal decreases [1]. In healthy epithelial cells Cit oxidation is inhibited due to inactivation of aconitase by high Zn levels, however cellular Cit concentrations remain moderate (< 1mM), because Cit is transported into the luminal space of the prostate wherein it can accumulate to concentrations of more than 50 mM [2]. It has been suggested that the lower Cit signal in PCa is caused by increased Cit oxidation due to decreased Zn levels as part of the malignant process [2]. However, in PCa the luminal space decreases due to tumor growth, which may be a better explanation for the decreased signal of Cit, as it has a high luminal level. In this study we test the hypothesis that the intensity of the Cit signal depends on the volume of the luminal space. To this end the relative Cit signal in ¹H MRSI voxels was correlated with the relative luminal volume in these voxels, which was objectively obtained by digital segmentation of representative histopathological slices.

Methods: The institutional review board approved this study and waived the need for informed consent. 55 PCa patients were retrospectively included who received a 3T MR exam, including MRSI, and a prostatectomy between January 2009 and June 2012. The MR exam included high resolution T2w imaging in three directions and PRESS based MRSI with MEGA pulses for water and lipid suppression. The voxel size after elliptical weighted sampling and hamming filtering varied between 0.37 and 1.0 cc, and depended on the size of the prostate and whether merely body array coils were used or also an endorectal coil for better SNR. After prostatectomy, the prostate was sliced by a pathologist and stained with hematoxylin and eosin (HE). Per patient one stained slice was chosen and digitized with 10x magnification. Two



← Figure 1: A. Prostate slice annotated by the pathologist. B. Corresponding HE-slice with the regions of interest indicated. C. The matching T2w image with the spectroscopic grid overlain and voxels of interest indicated.

readers matched independently the stained slice with a T2w imaging slice and reached consensus for the cases of disagreement. The T2w imaging slice including MRSI grid was matched with the HE-slice (Fig. 1). Per patient 1 to 7 non-overlapping regions of interest (ROIs) on the HE-slice could be chosen that matched MRSI voxels (Fig.1). Care was taken to place these ROIs completely in the tissue and not in fragmented parts of the tissue. The HE-slice was segmented based on a color-based segmentation algorithm in Matlab (R2011b, Mathworks). The identified components were nuclei, stroma/cytoplasm, and lumen (Fig. 2). For each ROI the %area-of-nuclei, %area-of-lumen and %area-of-stroma was determined. To obtain the corresponding metabolite ratio, the MR spectra were fitted with an LC-Model basis set based on simulated signals for Cho, Cr, Spm and Cit. From this the Cho+Spm+Cr/Cit ratio was derived. Only ROIs were included that had a Cramér-Rao Lower Bound of $\leq 20\%$ for the fit of Cit and the combined fit of Cho, Spm and Cr. The Cho+Spm+Cr/Cit ratio was related to the %area-of-nuclei divided by %area-of-lumen as Cho and Cr only occur in cells and Cit dominantly in lumen. The data was fitted with a linear mixed-effect model to correct for multiple ROIs per patient.

Figure 2. Left: HE-staining of glandular tissue. Right: segmented tissue, in which red represents luminal space, green nuclei and blue stroma and cytoplasm.

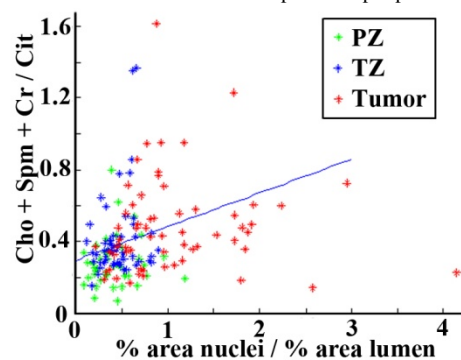
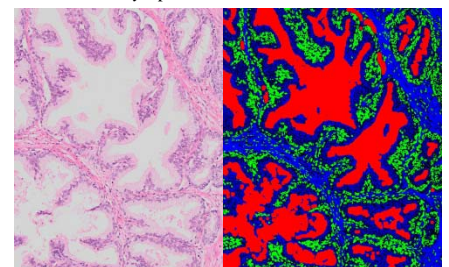


Figure 3. For each ROI the metabolic ratio is plotted against the %area-of-nuclei / %area-of-lumen. The blue line is the result of the linear mixed-effect model ($y=0.182+ 0.298x$)

Results: 55 patients were included in which a total of 215 ROIs could be selected. After quality control of the segmentation and fit of the spectra, 165 ROIs were included in the final analysis. Of these, 42 contained normal peripheral zone (PZ) tissue, 55 normal transition zone (TZ) tissue and 68 tumor tissue. The Gleason score of the tumor ROIs was 5 (n=12), 6 (n=13), 7 (n=37), 8 (n=2) and 9 (n=4). The Cho+Spm+Cr/Cit ratio was correlated with the %area of nuclei / % area of lumen (Fig. 3) and a significant relation of $y=0.182+ 0.298x$ was found.

Discussion: In this study a significant relation between the Cho+Spm+Cr/Cit and %area-of-nuclei / %area-of-lumen was found. Given that the dominant variable in this ratio is the Cit signal, this is in agreement with our hypothesis that the intensity of the Cit signal is determined by the luminal volume. Thus the value of the Cit signal to detect PCa is based on the decrease of the luminal space due to tumor growth (see Fig 3). This relation with morphology and not necessarily cell metabolism (or genetic expression [3]) also explains why occasionally false-positives are observed in the detection of PCa tissue if other prostate diseases have a strong effect on the luminal volume. Previously, the apparent diffusion coefficient in prostate was also suggested to be related with alterations in morphology (cell density and luminal space) [4]. This study was hampered by some methodological limitations, which might explain the variation in the data. Matching of histopathology with MRI may be difficult and therefore 2 readers did the matching in consensus. Both the T2w image and pathology slices were made perpendicular to the rectal wall; however, there might still be a difference in angulations. As the MRSI voxels have a volume of 0.37 - 1.0 cc and the HE-slice is 4 μ m thick, we had to

assume that the tissue in the ROIs are representative for the tissue in the voxel. Our ROIs have to be completely inside intact parts of the HE-slice, and have to have the diameter of the spectroscopy voxel. Therefore, we have partial volume effects, but as this is also present in the MRSI data, this will not affect the results.

Conclusion: This study indicates that changes in the signal of Cit (and possibly of other metabolites) in PCa result from morphological alterations, rather than changes in metabolism.

References: [1]Kurhanewicz et al. Radiology 1996 [2]Costello&Franklin. Mol Cancer 2006 [3]Bertilsson et al. Clin Cancer Res 2012 [4]Langer et al. Radiology 2010