CORRELATING POST-OPERATIVE WHOLE MOUNT IMMUNOHISTOCHEMISTRY TO FUNCTIONAL MRI PARAMETERS IN PANCREATIC CANCER

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Target audience: Researchers and clinicians involved in (functional) MR imaging of pancreatic cancer.

Purpose: Despite advances in multimodality treatment options, pancreatic ductal adenocarcinoma still ranks 4th on the list of cancer-related deaths. Amongst other factors, therapy resistance is induced by the pancreatic tumor microenvironment which is characterized by an excess of stromal tissue, poor vascularization and consequently tumor hypoxia. Functional Magnetic Resonance Imaging (MRI) enables the non-invasive characterization of this tumor microenvironment. Using diffusion weighted imaging (DWI), water diffusivity can be used as measure for stromal deposition and dynamic contrast enhanced (DCE) MRI provides further insight on tumor vascularization. Although these sequences provide valuable information on macro scale, interpretation of the underlying biological processes is not unambiguous. Direct correlation of the MRI with immunohistochemical stainings for stromal deposition and vasculature would provide valuable insights how to interpret these data. However, tumor heterogeneity and scarcity of landmarks make correlation to small tissue biopsies challenging. In this study we therefore propose a method to directly project postoperative whole section tissue slices onto DWI and DCE MRI. We then use this method to correlate functional MRI parameters to local histology derived tissue characteristics.

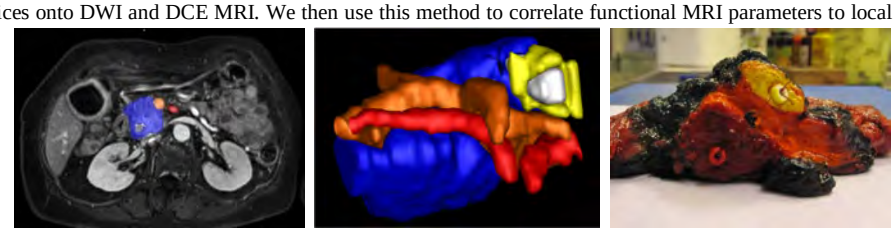

Figure 1 *Anatomical structures drawn on the MRI (left) showed good correlation with the tissue after resection (right) when rendered in 3D (middle). Blue: Pancreatic head, Yellow: pancreas dissection plane, Orange: mesenteric vein, Red: mesenteric artery, White: pancreatic duct.*

Methods: Four patients (3 male, 1 female) with (borderline) resectable pancreatic adenocarcinoma were prospectively recruited for MR imaging. Patients were imaged directly before pylorus-preserving pancreaticoduodenectomy. Imaging was performed on a Philips Ingenia 3T MR scanner. DW-images were obtained under respiratory triggering using a single-shot echo-planar sequence: voxel size 3x3x3.7mm, 0.3mm slice gap, FOV 432x108x72mm, TE/TR 44/2300ms, BW 62.5Hz/voxel. We obtained 12 b-values (0, 10, 20, 30, 40, 50, 75, 100, 150, 250, 400 and 600 s/mm^2). Data was fitted with the intra voxel incoherent motion (IVIM) model to obtain *f* (perfusion fraction), *D* (diffusion coefficient) assuming a *D** (pseudo diffusion coefficient) of 0.0453mm^2/s.[1] The DCE protocol comprised a dynamic series consisting of a 3D FFE sequence with 30 slices; slice thickness 2.5mm (5mm non-interpolated), FOV 400x400mm, matrix size 160x160, TR/TE/FA 3.2/2.0ms/20° and a temporal resolution of 1.75s. 0.1mmol/kg of 1.0mmol/ml Gadovist® (Bayer) was administered 15s after start of the dynamic series at a rate of 5ml/s followed by a 15ml saline flush. Scanning was continued for 4.5min. Baseline T1 was measured before contrast administration by use of a Look Locker sequence. The arterial input function was automatically selected from the abdominal aorta and tissue concentration curves were fitted according to the extended Tofts model to retrieve K^{trans}-maps.[2] To minimize peristaltic movement during acquisition, 1ml of 20mg/ml Buscopan® (Boehringer Ingelheim) was administered twice; before start of the DWI and DCE acquisition. For anatomical correlation a mDIXON sequence was performed 35 seconds after injection of a second equivalent contrast bolus. All functional MRI data was projected on the mDIXON image by automated non-rigid image registration with the Elastix package, using mutual information.[3]

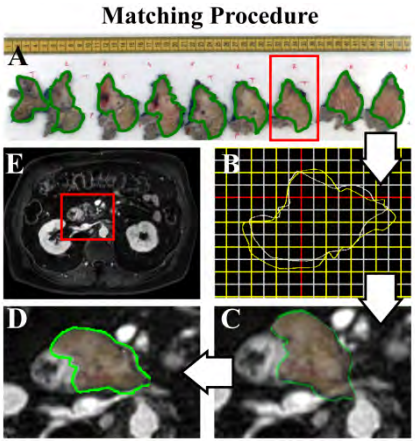

Figure 2 *Matching procedure: A. Tissue slices with drawn contours; B. Orientation of adjacent contours; C. Rotation and translation of tissue slice projected on MRI; D. Final match of tissue slice to MRI; E. Reference anatomical MRI*

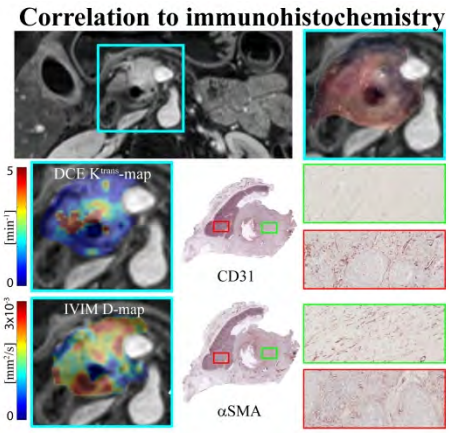

Figure 3 *Correlation of functional MRI to immunohistochemical stainings. Upper row: MRI with overlying tissue slice. Middle row: The area with high K^{trans} values shows an area with higher vessel density on CD31 staining (upper red box). Lower row: Low D-values are shown in an area with high stromal content on αSMA staining (lower green box).*

Pathology matching: After resection, the surgeon sutured colored beads to relevant anatomical structures (mesenteric vein and artery margins, bile and pancreatic duct) on the tissue specimen. Hereafter, the tissue was directly transferred to the pathology department where resection and dissection planes as well as the anatomical structures were inked by the pathologist (Figure 1). After 24 hours of fixation in formaldehyde, the tissue was sliced in the axial plane to retrieve ~5mm thick tissue slices. These slices were then numbered and photographed from both sides. One slice with evident tumor was selected for whole mount processing, embedding the complete tissue slice. Other tumor comprising slices were divided into standard pathology processing cubes (~2x2 cm). In this phase of the study all whole mount tissue slices were stained for stromal deposition (Smooth Muscle Actin (αSMA)) and vessels (CD31). On the photographed tissue slices the contour of the pancreas was manually outlined (Figure 2A). Next, contours of adjacent slices were aligned, so slight deviations between the orientations of the slices were corrected for (Figure 2B). The resulting outlined slices were stacked to form a three dimensional volume of the pancreas tissue specimen. This volume was then projected onto the anatomical MR-image (Figure 2C). Finally, the tissue volume was manually rotated and translated so anatomical structures matched corresponding features on the MRI (Figure 2D). Stained coupes were aligned with the corresponding tissue photograph and transposed to the MRI accordingly.

Results: Tissue specimens after resection showed good visual agreement when anatomical features were drawn on MRI (Figure 1). Based on corresponding anatomical structures, the post-operative tissue volume could be matched to the MRI in all 4 patients. Figure 3 shows a typical example of combined functional MRI parameters with tissue stainings. In this case, higher values on the K^{trans}-map corresponded with higher vessel density on the CD31 staining in a healthy part of the pancreas. Lower D-values were found in an area with higher stromal content on αSMA staining, corresponding with the tumor area.

Discussion & Conclusion: In this pilot study we showed the feasibility of matching post-operative axial tissue slices to in vivo MRI of the pancreas. Matching immunohistochemically stained whole mount tissue slices provides direct information on localization and local tumor heterogeneity for validation of functional MRI parameters. Expanding this work in a larger patient group will provide valuable insights on underlying biological processes of functional MRI parameters in pancreatic cancer.

References: 1. Le Bihan D et al., *Radiology*. 1988;168(2):497-505. 2. Tofts PS et al. *J. Magn. Reson Imaging*. 1999;10(3):223–32. 3. Klein S et al., *IEEE Trans. Med. Imaging* 2010;29(1):196–205.