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Detectability and structural stability of a liquid fiducial marker in fresh *ex vivo* pancreas cancer resection specimen on CT and 3T MRI

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Abstract

Objectives

The aim of this study was to test the visibility of a new liquid fiducial marker injected in *ex vivo* pancreas tissue on magnetic resonance imaging (MRI) and computed tomography (CT). Furthermore, its injection performance using different needle sizes was investigated as well as its structural stability after fixation in formaldehyde.

Material and Methods

Liquid fiducial markers with a volume of 20-100 µL were injected into the freshly resected pancreas of three patients (two males age 69 and 72, one female age 68) with suspected adenocarcinoma of the pancreatic head. Injection was performed under X-ray guidance using a high precision unit dose injector with different needle sizes (18G, 22G, 25G). While cooled on ice, the specimens were scanned on MRI and CT with routine clinical sequences. Signal threshold based segmentation was performed manually on CT. The marker volume visible on CT was compared to the actually injected volume as a measure of potential marker backflow. After rigid registration of the MR images to the CT data set, marker detectability was assessed by searching for the corresponding hypointense structure in the respective segmentation.

Results

Markers with a volume of ≥ 20 µL were easily detected as hyperintense structures on X-ray and CT. In clinically used T_1- and T_2-weighted 3T MRI sequences, all marker sizes ranging from 20µL – 100µL were visible as hypointensity. Since most markers were non-spherical however, MRI visibility was relatively poor and their differentiation from hypointensities caused by air cavities or surgical clips was challenging and only feasible with a reference CT. Marker backflow was observed when injected with an 18G needle, which was prevented by injection using a smaller 22G and 25G needle. The marker was stable after 24h fixation in formaldehyde where only small volume degradations were observed (6.6±13.0%) and with the exception of one instance no wash out occurred.

Conclusion

The liquid fiducial marker with injected volumes of 20µL – 100µL, injected in an *ex vivo* pancreatic cancer resection specimen, was visible as hyperintensity on kV X-ray, CT and hypointensity on MRI and stable over a period of 24 hours in formaldehyde. Since most injected markers were non-spherical, a marker size of

≥50µL is recommended for the clinically used MRI sequences. Most likely, *in vivo* marker injection will result in more spherical forms due to persisting metabolism, and this in turn will enhance MRI visibility in an hyper intense structure.

Key Words

Liquid fiducial marker, pancreatic adenocarcinoma, MRI visibility, ex vivo

1. Introduction

In radiation therapy (RT) accurate and precise delineation and localisation of the target volume is mandatory for high-quality dose delivery avoiding geometrical misses caused by setup errors and/or anatomical variations (e.g. organ motion, deformation and filling). In particular for mobile tumours, inter-fractional position verification is frequently performed based on X-ray radiographic imaging, termed image-guided radiotherapy (IGRT). IGRT of pancreatic ductal adenocarcinoma (PDAC) is, however, challenging since it is one of the abdominal soft tissue organs not visualized under X-ray guidance and hence requires anatomical surrogates, e.g. bony landmarks, intratumoral or adjacent stents or surgical clips, or solid fiducial markers [Ref: van der Horst_Int J Rad Onco_2014, Packard_Journ Med Imaging and Rad Onco, Chortogiannos_Journ Med Imaging Rad Sciences_2017). For several primary tumours, e.g., prostate cancer, solid gold fiducial markers implanted endoscopically have replaced bony landmarks as standard of care due to the low complication rate of the procedure and the small residual setup errors [Ref: VanderHorst_2013, Varadarajulu_2009]. By reducing setup margins, highly conformal radiation techniques, such as stereotactic ablative radiotherapy (SABR), have been pioneered and were reported to reduce treatment-related toxicity [REF: Gkika_2017, zu TOMO Chen_Journal of Thoracic Disease_2009, Drozds_Strahlenther Onkol_2016, van Baardijk Radiother Oncol 2012]. Conversely, solid gold markers not only deteriorate image quality in both computed tomography (CT) [Ref: Scherman Rydhög_2015] and magnetic resonance imaging (MRI) [Ref: Gurney-Champion_2015, Schneider_2017], but may additionally cause significant dose perturbations in particle therapy, a treatment modality currently assessed for PDAC [Ref: Giebeler_2010, Newhauser_2007].

A new biocompatible liquid marker, BioXmark, has been developed, which forms a semisolid gel after injection into soft tissue, consists of low *Z*-elemental (non-ferrous and non-magnetic) composition and causes minimal proton dose perturbations [Ref: Scherman Rydhög_2017; Jølck_2014]. Moreover, the marker remains chemically stable during normo-fractionated and high-dose single-fraction irradiation schemes [Ref: Troost_2017]. Finally, a recent clinical study confirmed its applicability for use in patients

with locally advanced lung cancer and no significant positional and structural degradation was observed over a 7-weeks course of radiation treatment with photons [Ref: Scherman Rydhög_2016].

Patients with PDAC scheduled for proton therapy might also benefit from this marker, since it can be endoscopically implanted using very thin (≤25 G) needles, allowing for minimal invasive injection, and its size and visibility on MRI, kV X-ray and CT images can be accurately adjusted by altering the injected volume. A recent phantom study that quantitatively investigated the marker's MRI characteristics in terms of visibility and artefacts has shown that this marker is visible on MRI as a strong signal void in both T1 and T2 weighted images at 3T and, in contrast to solid markers, its degree of visibility does not correlate to the degree of artefacts [Ref: Schneider_2017]. In part contradictory, in a recent study the BioXmark marker was found to be iso-intense compared to prostatic tissue in T2 weighted images at 1.5T [Ref: De Roover 2018]. Since in MR guided radiotherapy target and organ at risk delineation is often performed on or supported by T2 weighted images, the visibility of fiducial markers in these sequences is particularly crucial.

Therefore, the aim of the present study was to assess the marker's visibility in real pancreatic tissue on CT and T1 and T2 weighted MRI, and to examine the marker's structural stability after injection using different needle sizes. However, at present this marker is still lacking CE-marking and has thus not been approved for *in vivo* clinical use in patients with PDAC. In order to prepare the clinical application of the injectable fiducial marker, the study was hence performed by injecting the marker into fresh *ex vivo* pancreas resection specimens.

2. Materials and Methods

2.1. Pancreatic resection specimen

Three patients (two males age 69 and 72, one female age 68) suspected to have an adenocarcinoma of the pancreatic head scheduled for primary pancreaticoduodenectomy provided informed written consent for *ex vivo* injection of the liquid fiducial marker into the obtained resection specimen and for subsequent X-ray, CT and MR imaging thereof. Immediately after resection, the resection specimen was stored in a container with ice at approximately 4°C to prevent enzymatic degradation, and was transported to the Department of Pathology for immediate biopsy selection for frozen section analysis. Thereafter, the injection of the markers and imaging of the resection specimen were performed within the next 2 hours, while being transported on

ice. Surgical clips or sutures placed into the resection specimen during the surgical resection were not removed and thus still present during marker injection and subsequent imaging. The study was approved by the local Ethics committee of the Faculty of Medicine and University Hospital Carl Gustav Carus of the Technische Universität Dresden (EK-534122015).

2.2. Fiducial marker injection

The liquid marker (BioXmark®, Nanovi Radiotherapy A/S) is a three-component fully biocompatible liquid soft-tissue marker. After injection into soft tissue, ethanol diffuses out of the marker, causing an increase of marker viscosity resulting in a gel-like structure within 30 min after injection. Several liquid markers (5-6) of various volumes (range 20–100 µL) were injected at a depth of 1-2cm directly adjacent to the tumour boundaries (~30 min after surgical resection). Moreover, three different needle sizes (18G, 22G, 25G) were used during the injection into the three specimens respectively. For this, the needles were attached to a unit dose injector (MicroDose™, Vlow Medical B.V., Eindhoven, The Netherlands) for accurate and reproducible injection. The injection was performed under kV-X-ray guidance to facilitate the subsequent differentiation of injected markers and surgical clips or sutures.

2.3. MR and CT image acquisition

Immediately after marker injection, the specimens were scanned with clinically used scan protocols for pancreatic cancer patients (see table 1) on CT (*Siemens SOMATOM Definition AS, Siemens Healthineers, Erlangen, Germany*) and MRI (3.0 T *Philips Ingenuity TF PET/MR scanner, Philips Healthcare, Eindhoven, The Netherlands*). MR images were acquired with a 32-channel SENSE Torso/Cardiac coil performing a T_1-weighted spoiled gradient echo sequence (THRIVE) and a T_2-weighted turbo spin echo sequence. The scan was performed with the specimen still placed on ice (see figure 1). To test the stability of the marker after fixation in formaldehyde for 24 hours, the complete scan protocol was repeated for one of the three resection specimens.

Table 1: Scan protocols used on MRI and CT for imaging of the pancreatic resection specimens after marker injection.

Imaging modality	In-plane resolution [mm²]	Slice thickness [mm]	FOV [mm³]	TE/TR [ms]	BW [Hz]	FA [°]	SENSE Factor	X-Ray exposure [mAs]	X-Ray voltage [kVp]
MRI – T1w	1.51×1.51	3	258×258×120	1.4/3.2	721	10	2	-	-
MRI – T2w	0.76×0.76	3	254×254×79	80/800	290	90	2	-	-
CT	0.98×0.98	2.0	500×500×242	-	-	-	-	12	120

Abbreviations: FOV = field of view; TE = echo time; TR = repetition time; BW = bandwidth; FA = flip angle.

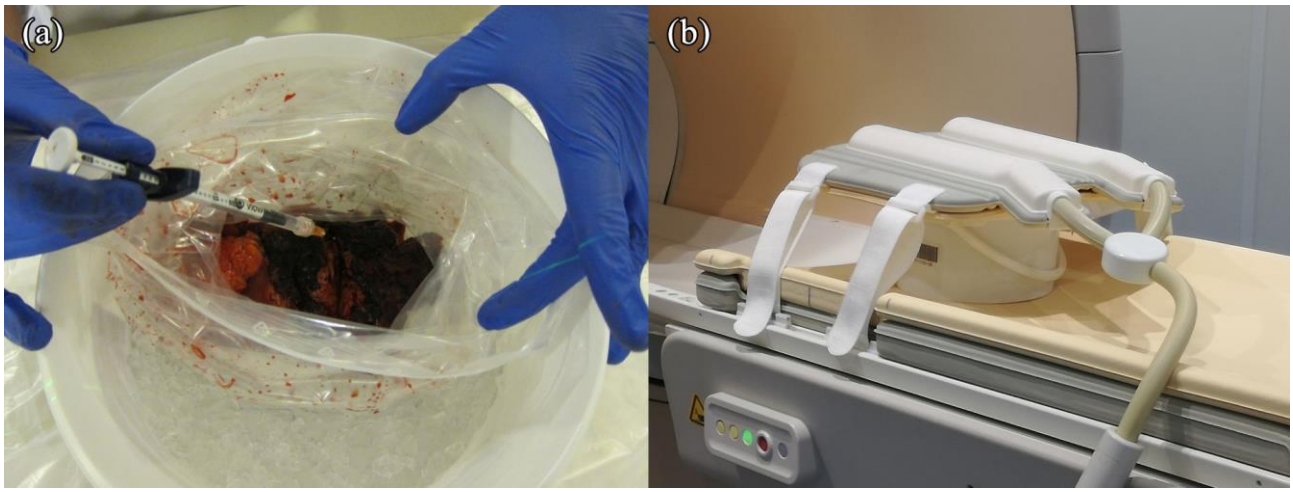

Figure 1. (a) Container with resection specimen on ice during marker injection. (b) Scan setup on the MR scanner with the anterior and posterior parts of 32-channel SENSE Torso/Cardiac coil encapsulating the ice-filled container with the resection specimen.

2.4. Analysis

The markers were first segmented as hyperintense structures in the acquired CT images with the open source software 3D Slicer [Ref:Pieper 2004] by threshold segmentation such that the lower threshold was selected to be three standard deviations higher than the mean signal intensity of the pancreatic tissue (i.e. 129.3 ± 4.1 HU) to ensure that with 99.7% confidence no tissue voxels were falsely segmented. In order to correctly assign the respective markers and to prevent erroneously segmenting surgical clips or sutures instead of liquid fiducial markers, the X-ray images taken during injection were used as a visual aid. The CT images were then manually registered onto the MR images taking into account the specimen contour (Figure 2).

Within the pancreatic resection specimen defined by the CT segmentation, markers were expected to appear hypointense on the MR images [Ref: Schneider et al]. Marker visibility was thus approved when a hypointense structure was present in the respective segmentation.

In order to try to quantify a backflow of marker material during injection, the ratio of marker volume segmented on CT to the actually injected volume was assessed. While due to a marker size depending partial volume effect, the segmented volume was expected to be larger than the injected volume, the multiple of the respective marker size was expected to be smaller in case of marker backflow.

In order to assess the stability of the fiducial marker after fixation of the resection specimen in formaldehyde for 24 hours, for one resection specimen the segmentation procedure was repeated on the CT scan obtained after fixation. The segmented volumes of the respective markers on CT before and after fixation were finally compared to deduce a possible degradation.

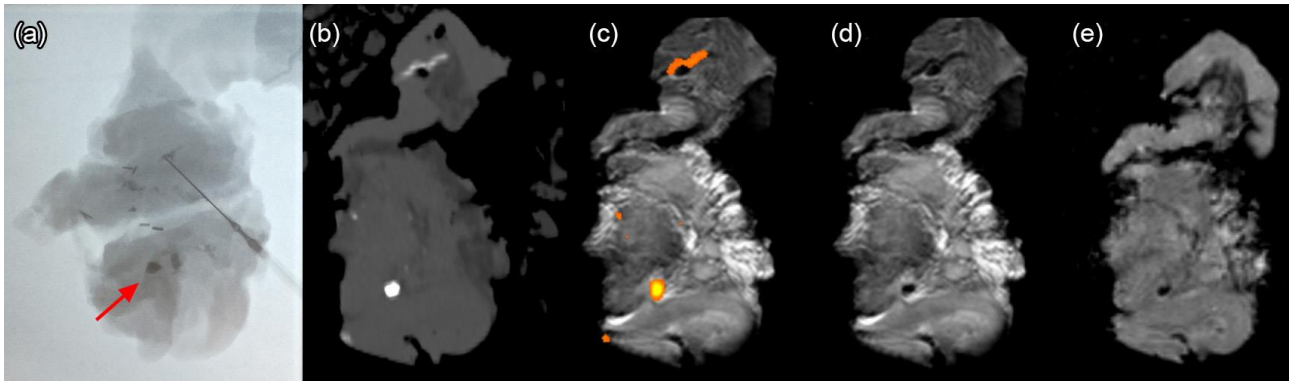

Figure 2. (a) kV X-ray of a resection specimen during marker injection in which the needle is visible on the right, as well as several surgical clips close to the marker injections of which a 100µL marker is marked with a red arrow. Coronal slice where this 100µL marker is visible on (a) CT and (b) T_2-weighted Turbo Spin Echo (TSE) MRI superimposed with the threshold based orange-yellow CT segmentation (c) Native T_2-weighted TSE MRI and (d) T_1-weighted Gradient Echo (GRE) MRI. The injected 100µL fiducial marker is depicted as hyperintensity on CT and as signal void on T_2- (c) and T_1-weighted (d) MRI.

3. Results

While in two resection specimen 6 markers were injected, in one specimen only five markers were injected due to a relatively small size of the specimen. Furthermore, for two specimens a unit dose injector was used

which allowed smallest injections of 25µL steps while for the last specimen only a unit dose injector was available which allowed smallest injections in steps of 20µL. This lead to a slightly different size and number of injected markers throughout the used resection specimens.

3.1. Marker visibility on T1- and T2-weighted MRI

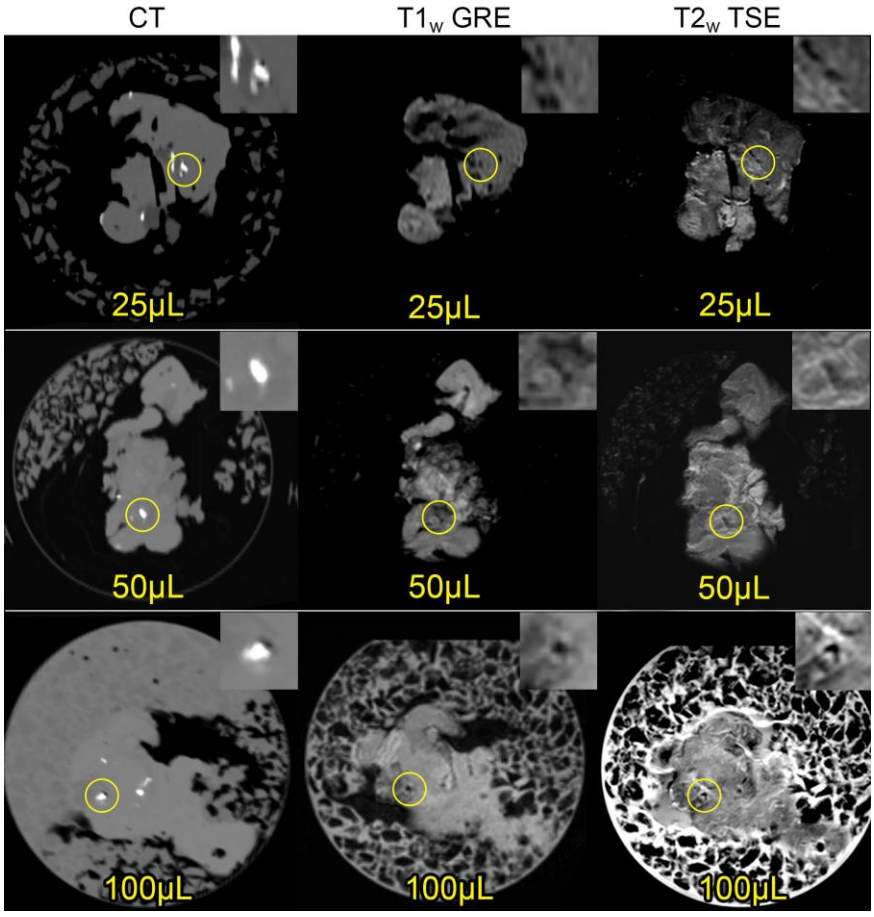

Figure 3. Coronal view of all three resection specimens on CT [left], T_{1w} gradient echo (GRE) MRI [middle] and T_{2w} turbo spin echo (TSE) MRI [right]. Markers of different sizes (25-100 µL) are visible as hyperintensity on CT and hypointensity on both $T1_w$ and $T2_w$ MRI. On the top right of each segment a close up of the yellow circled region of interest is shown for clear visualization of the marker.

Figure 3 presents imaging slices of the resection specimen on CT, T_1- and T_2-weighted MRI from all three patients included in this study. As can be appreciated from Figure 3, the liquid fiducial marker is detectable on CT as hyperintense structure and on both T_1- and T_2-weighted MRI as hypointense structure, respectively. Marker of all sizes (20-100µL) tested in this study could be detected with the clinically used sequences. Noteworthy, on CT it was detected that injection generally resulted in a non-spherical marker, making MR

visibility strongly dependent on slice orientation and voxel size. Moreover, a diffuse injection or injection too close to the surface hampered MR visibility leading to 4 of 17 markers which where non-detectable on MRI. Images of all 17 markers visible or non-visible on CT and MRI can be found in the supplementary.

3.2. Fiducial marker injection performance as a function of needle size

The injection was performed with needle sizes of 18G, 22G and 25G. In the case of 18G needles, marker backflow out of the injection site was observed. This was prevented by the use of smaller needle sizes of 22G or 25G. However no correlation was found between the needle size and the ratio of segmented volume to injected volume as a measure of marker backflow due to the larger variance within the respective specimens and marker sizes (Figure 4).

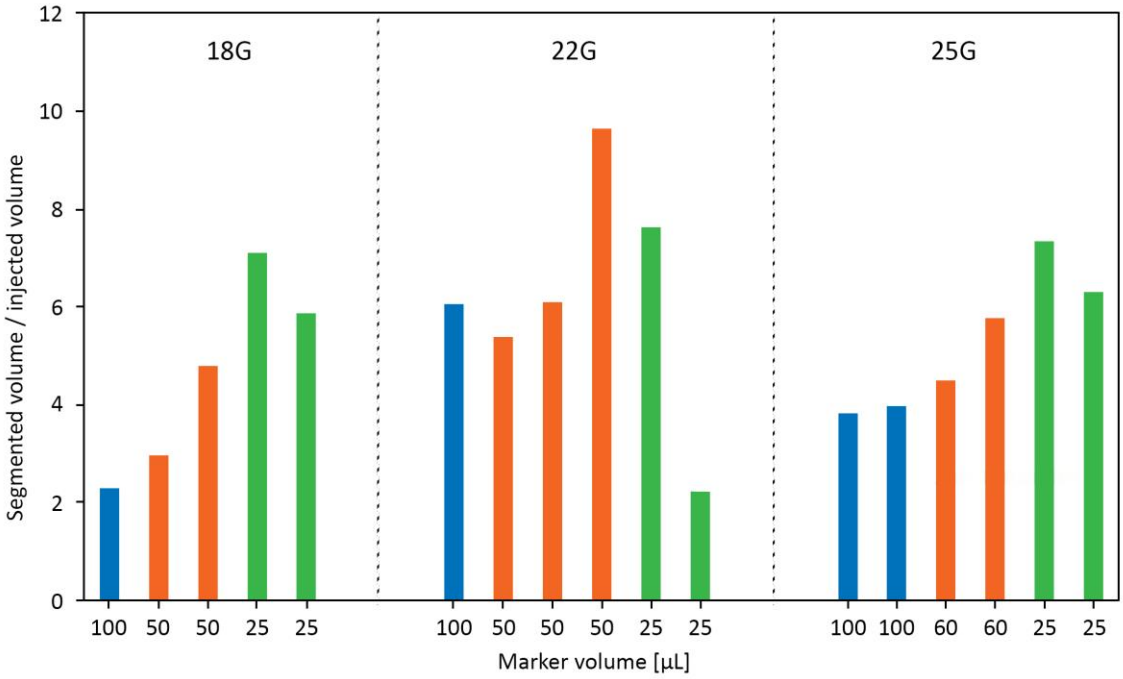

Figure 4. Ratio of fiducial marker's segmented volume to the injected volume. The 17 markers with volumes between 20-100µL were injected into the three resection specimens using different needle sizes (18G-25G).

3.3. Fiducial marker stability after 24h fixation in formaldehyde

After 24h fixation in formaldehyde all markers in the investigated resection specimen were still visible on CT (see Figure 5 for a coronal maximum intensity projection) and MRI (Figure 6), and geometrically stable.

Overall, the volume degradation was 6.6±13.0%. One 60µL marker was partially washed out of the specimen as was detected on the CT images.

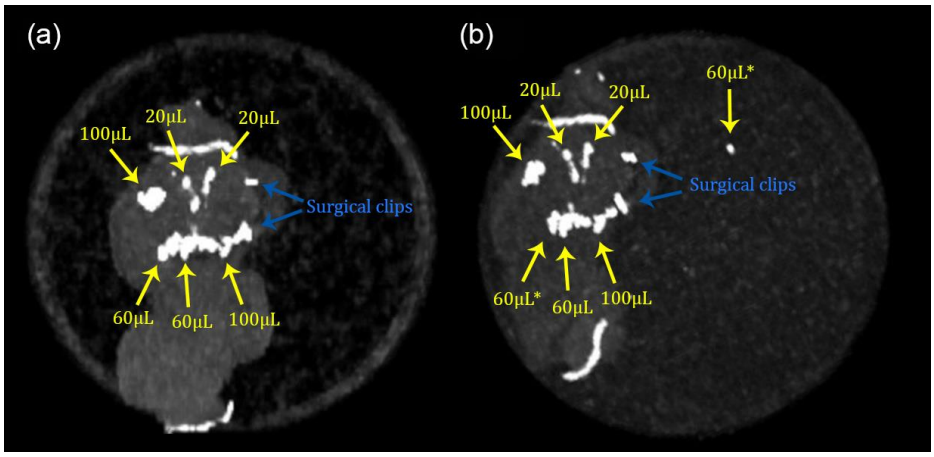

Figure 5. Maximum intensity projection of the CT from the resection specimen before (a) and after (b) fixation in formaldehyde. All 6 injected markers were stable in their size and position. It can be appreciated that one hyperintense structure, i.e. the partially washed out 60 µL marker (markerd with an asterix), was found in the top right corner of the container filled with formaldehyde.

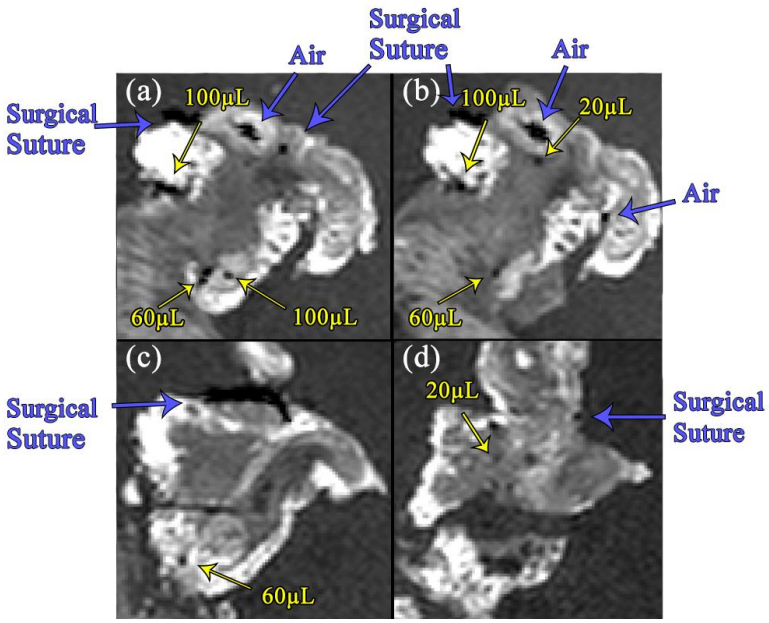

Figure 6. Four representative T1w-MRI slices of a pancreatic cancer resection specimen fixated in formaldehyde showing all six injected markers. Remaining hypointensities caused by a surgical suture and air cavities are also visible.

4. Discussion

In this study the liquid fiducial marker BioXmark® was tested for the first time *ex vivo* in freshly resected pancreatic cancer specimen. Marker detectability on CT was satisfactory with markers clearly depicted as hyperintense structures. On MRI marker detectability was difficult due to signal voids from tissue heterogeneity, air cavities, surgical clips or sutures resembling the fiducial markers in MRI. To distinguish fiducial marker induced signal voids from the latter, a prior registration with the CT images was necessary. Moreover, marker backflow was reduced using smaller needle diameters, and the marker was found stable after 24h fixation in formaldehyde.

The detectability on MRI, at least *ex vivo*, may improve using a marker composition with gadolinium leading to a hyperintense marker. However, in the *in-vivo* situation, the marker performance is thought to improve since less or no hypointensities caused by air cavities and/or surgical clips are present. Conversely, since fiducial marker injection in case of PDAC is often performed into the adipose tissue in direct proximity of the tumour, marker visibility on fat-saturated MRI sequences will be hampered, as also found in clinical practice with fiducial gold markers in our institute (data not shown).

After registration with the CT images of the specimen, many markers were still only poorly visible or even not visible at all. When comparing these markers to the CT images, it appears that in these cases the injection was not sphere-like. Hence detectability was strongly depending on the injected shape and the distance to the specimen surface. Nevertheless, in particular larger markers ≥50µL, which formed sphere-like volumes, were visible as strong signal void on both T_1- and T_2-weighted MRI. This is concordant to the results found in [Ref: Schneider2018], where the marker was found to be causing signal voids at 3T independent of the contrast mechanism due to an apparent lack of water protons. In [De Roover 2018] the marker was found to be isointense to prostatic tissue on T_2w-MR images at 1.5T. These different findings may resulted from the lower field strength used in their study. More likely however this effect could have resulted from a signal contamination caused by the phantom material in which the marker was injected into. Since the ellipsoid shaped 300µL marker analysed in the study by De Roover et al. [De Roover 2018] showed a size in slice encoding dimension which was close to the slice thickness acquired in MRI (4 mm), a partial volume effect cannot be ruled out.

When introduced into clinical practice after successful CE marking, it is expected that the *in vivo* use will lead to more spherical and hence better detectable markers on MRI. This is caused by the persisting metabolism and fast efflux of ethanol after injection. Alongside, in the *in vivo* situation motion blurring will impede on the MR quality and will most likely require larger marker volumes of 50µL – 100µL to still be visible on MRI. These volumes should preferably be injected with a thin needle preventing marker backflow.

The marker's visibility on different anatomical imaging modalities is of great value to the community of radiation oncology. Besides its minimal proton dose perturbations [Ref: Scherman Rydhög_2017; Jølck_2014], the stability during a prolonged course of fractionated, high-dose radiotherapy [Ref: Troost_2017] and also after fixation in formaldehyde makes the marker promising for neoadjuvant and definitive treatment concepts involving photon or proton beam therapy.

In conclusion, the liquid fiducial marker with injected volumes of 20µL – 100µL, injected in an *ex vivo* pancreatic cancer resection specimen, was visible on kV X-ray, CT and MRI, and stable over a period of 24 hours in formaldehyde. Since most injected markers were non-spherical, a marker size of ≥50µL is recommended for the clinically used MRI sequences. Most likely, *in vivo* marker injection will result in more spherical forms due to persisting metabolism, and this in turn will enhance MRI visibility.

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Disclosure of Conflicts of Interest

None.

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