Validation of in vivo nodal staging of solid malignancies with USPIO-enhanced MRI: a workflow protocol

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Abstract

Background

In various cancer types, the first step towards extended metastatic disease is the presence of lymph node metastases. Imaging methods with sufficient diagnostic accuracy are required to personalize treatment. Lymph node metastases can be detected with ultrasmall superparamagnetic iron oxide (USPIO)-enhanced magnetic resonance imaging (MRI), but this method needs validation. Here, a workflow is presented which is designed to compare MRI-visible lymph nodes on a node-to-node basis with histopathology.

Methods

In patients with prostate, rectal, periampullary, esophageal, and head-and-neck cancer, *in vivo* USPIO-enhanced MRI was performed to detect lymph nodes suspicious for harboring metastases. After lymphadenectomy, but before histopathological assessment, a 7 Tesla (T) preclinical *ex vivo* MRI of the surgical specimen was performed, and *in vivo* MR-images were radiologically matched to *ex vivo* MR images. Lymph nodes were annotated on the *ex vivo* MRI for an MR-guided pathological examination of the specimens.

Results

Matching lymph nodes of *ex vivo* MRI to pathology was feasible in all cancer types. The annotated *ex vivo* MR images enabled a comparison between USPIO-enhanced *in vivo* MRI and histopathology which allowed for analyses on nodal, or at least on nodal station level.

Conclusions

A workflow was developed to validate *in vivo* USPIO-enhanced MRI with histopathology. Guiding the pathologist towards lymph nodes in the resection specimens during histopathological work-up allowed analysis at nodal level, or at least nodal station level of *in vivo* suspicious lymph nodes with corresponding histopathology, providing direct information for validation of *in vivo* USPIO-enhanced MRI detected lymph nodes.

Trial registration

VALINODE data is collected from patients undergoing USPIO-enhanced MRI in the clinical setting. Therefore, the trial has not been registered in an online trial register. 7TNANO1 is registered at Clinicaltrials.gov: NCT02751606, April 26 2016 (<https://clinicaltrials.gov/ct2/show/NCT02751606>). NANO-PANC is registered at Clinicaltrials.gov: NCT04311047, March 17 2020 (<https://www.clinicaltrials.gov/ct2/show/NCT04311047>). PRECIES is registered in the Dutch Trial Register: NTR6072, August 5 2016 (<https://www.trialregister.nl/trial/5797>). USPIO-NECK is registered at Clinicaltrials.gov: NCT03817307, January 25 2019 (<https://clinicaltrials.gov/ct2/show/NCT03817307>).

Background

Many different types of solid cancers have a high propensity to metastasize to locoregional and distant lymph nodes which is one of the most important prognostic factors for survival [1, 2]. Current clinical practice employs considerable overtreatment for precautionary reasons. If an accurate pre-treatment lymph node status is established, therapeutic strategies can be tailored to the individual patient. With continuously improving opportunities to selective treatment of individual metastatic deposits with surgery or radiotherapy, assessment and exact localization of lymph node metastases is crucial. Concurrently, increased certainty of the absence of lymph node metastases could safely minimize treatment. Therefore, highly sensitive detection of small lymph node metastases is urgently needed.

Compared to other imaging techniques, magnetic resonance imaging (MRI) provides superior soft-tissue contrast. This allows good visualization of small anatomical structures and can be used to characterize lymph nodes [3]. MRI in combination with a contrast agent of ultrasmall superparamagnetic particles of iron oxide (USPIO) might be a promising, generalized, diagnostic tool to detect lymph node metastases in various types of cancers [4]. In short, 24-36 hours after intravenous administration, normal lymph nodes accumulate USPIO nanoparticles in macrophages causing a strong attenuation of the MR signal, while suspicious lymph nodes without USPIO retain MR signal on T2* weighted sequences [5]. Although promising results were previously reported in different body regions [6], the clinical value of USPIO-enhanced MRI using state-of-the-art 3-dimensional (3D) sequences with high isotropic spatial resolution should be appropriately validated [7, 8]. This demands for an accurate validation of USPIO-enhanced MRI compared to histopathology on a nodal level.

Few attempts have been undertaken to match individual lymph nodes on MRI with the corresponding lymph node on histopathology after lymphadenectomy [9, 10]. Although results were promising, correlation of lymph nodes on a node-to-node level remains difficult and small lymph nodes (< 3mm) often cannot be correlated with pathology. A seven Tesla (T) *ex vivo* MRI might enable to visualize the detailed anatomy of the resected specimen with the locations of dissected lymph nodes. In this study, we developed a protocol to correlate *in vivo* visible lymph nodes with histopathology data on a node-to-node level using 7T *ex vivo* MRI of lymphadenectomy specimens as an intermediate step. This is a way to validate *in vivo* MRI with histopathology on a nodal level to improve nodal staging.

Materials And Methods

Subjects

The data is collected from one trial investigating *ex vivo* MR scanning of the resection specimen in prostate cancer patients who underwent USPIO-enhanced MRI in the clinical setting and from four trials that investigate the feasibility of USPIO-enhanced MRI for detecting suspicious lymph nodes (rectal cancer NCT02751606, pancreatic and periampullary cancer NCT04311047, esophageal cancer NTR6072, and head-and-neck cancer NCT03817307). To illustrate the feasibility of the proposed workflow, one patient for each of the five types of solid malignancies is presented. Characteristics of all patients are described in table 1. All studies were approved by the central ethics committee on research involving human subjects and the local ethics committee Arnhem-Nijmegen. Signed informed consent was obtained before patient inclusion according to each corresponding research protocol. In all patients, 2.6 mg/kg body weight of USPIO (Ferumoxtran-10, Ferrotran® SPL Medical B.V. Nijmegen, the Netherlands) mixed with 0.9% saline solution was administered intravenously with a slow-drip infusion of 30-45 minutes under continuous clinical supervision. 24-36 hours later an MRI examination was performed using a 3T MR system (Magnetom Skyra or Prisma, Siemens Healthcare, Erlangen, Germany). Sequences were focused on detecting USPIO in lymph nodes and consisted of a 3D T1-weighted volumetric interpolated breath-hold examination (VIBE) Dixon pulse sequence and 3D multi gradient echo (MGRE) sequence [11]. The MRI pulse sequence parameters are described in tables 2 and 3. All patients, except for the head-and-neck cancer patient, received intramuscular smooth muscle and bowel relaxant (Scopolamine Butyl Bromide, Sanofi-aventis Netherlands B.V.) to reduce peristaltic motion. The periampullary cancer patients also received glucagon to reduce peristaltic motion,

Table 1: Patient characteristics.

Patient	Sex	Age	Cancer type	TNM-stage	Therapy	USPIO-surgery interval	Study registration number
1.	Male	67	Prostate	cT3N1M0	Pelvic lymphadenectomy followed by post-operative radiotherapy	1 day	None
2.	Male	76	Rectum	cT2N0	Total mesorectal excision	9 days	NCT02751606
3.	Male	58	Periampullary	pT2N2M1*	Pancreaticoduodenectomy with lymphadenectomy followed by postoperative chemotherapy	8 days	NCT04311047
4.	Female	65	Esophagus	cT2-3N1	Neoadjuvant chemoradiotherapy followed by esophagectomy	1 day	NTR6072
5.	Male	51	Head-and-neck	cT3N0M0	Primary tumor resection and cervical lymphadenectomy followed by postoperative radiotherapy	5 days	NCT03817307
Abbreviations: c = clinical, p = pathological, TNM = tumor node metastasis, USPIO = ultrasmall superparamagnetic iron oxide.							
* This patient had metastatic distal lymph nodes, which are considered as M1 according to the TNM 8 th edition							

Table 2: Overview of parameters regarding the *in vivo* T1-weighted MR sequences.

	Head-and-neck cancer	Esophageal cancer	Periampullary cancer	Rectal cancer	Prostate cancer
Sequence	VIBE Dixon	VIBE Dixon	VIBE Dixon	VIBE Dixon	VIBE
Voxel size (mm ³)	0.8 x 0.8 x 0.8	1.4 x 1.4 x 1.4	1.4 x 1.4 x 1.7	0.9 x 0.9 x 0.9	0.73 x 0.89 x 0.91
FOV (mm ³)	260 x 260 x 154	450 x 394 x 168	394 x 450 x 177	350 x 350 x 173	328 x 328 x 175
Acquisition mode	3D	Breath hold 3D	Breath hold 3D	3D	3D
TE (ms)	2.57, 3.8	1.23, 2.46	1.23, 2.46	1.2, 2.5	2.5
TR (ms)	6.02	4.21	3.86	5.8	6.5
Bandwidth (Hz)	300	20	20	305	510
Acquisition time (min)	4:53	18 sec for each breath hold	12 sec for each breath hold	4:56	9:13
Flip angle (°)	10	9	9	10	10
Abbreviations: 3D = 3-dimensional, FOV = field of view, Hz = Hertz, min = minutes, ms = milliseconds, TE = echo time, TR = repetition time, VIBE = volumetric interpolated breath-hold examination.					

Table 3: Overview of parameters regarding the *in vivo* T2*-weighted iron-sensitive MR sequences.

	Head-and-neck cancer	Esophageal cancer	Periampullary cancer	Rectal cancer	Prostate cancer
Sequence	mGRE	mGRE	mGRE	mGRE	MEDIC
Voxel size (mm ³)	0.8 x 0.8 x 0.7	1.5 x 1.5 x 1.5	1.3 x 1.3 x 1.3	0.85 x 0.85 x 0.85	0.73 x 0.73 x 0.73
FOV (mm ³)	260 x 260 x 154	336 x 279 x 30	291 x 291 x 211	328 x 328 x 190	328 x 328 x 164
Acquisition mode	3D	Breathhold 3D	Breathhold 3D	3D	3D
Acquired TEs (ms)	2.5 - 27.1	2.7 - 16.7	2.7 - 16.7	3.6 – 17.2	12
Reconstructed TEs (ms)	12	12	12	12	n.a.
TR (ms)	31	20	20	590	21
Bandwidth (Hz)	360	380	380	3x1	172
Acquisition time (min)	11:08	19 seconds each breathhold	20 seconds each breathhold	11:28	12:27
Flip angle (°)	10	10	10	10	10
Abbreviations: 3D = 3-dimensional, FOV = field of view, Hz = Hertz, MEDIC = multiple echo data image combination, mGRE = multi-gradient echo sequence, min = minutes, ms = milliseconds, n.a. = not applicable, TE = echo time, TR = repetition time.					

Surgery and *ex vivo* MR examination

Resection of the primary tumor and lymphadenectomy was performed according to routine clinical procedure. Immediately after resection, the specimen was transported at room temperature from the operating room to the Department of Pathology. The surgical specimen was, according to each different study protocol, either fixated with formalin before (prostate, rectal, esophageal, and head-and-neck cancer)) or after *ex vivo* MRI (periampullary cancer). This *ex vivo* MRI was executed on a 7T preclinical MR system using a volume radiofrequency coil (ClinScan, Bruker® BioSpin, Ettlingen, Germany). The specimen was placed in a proximal-distal position in the preclinical MR system (figure 8). The MR protocol consisted of a T1-weighted 3-dimensional GRE sequence with frequency-selective lipid excitation and a 3D multi-GRE pulse sequence with five acquired echoes and frequency selective water excitation. The *ex vivo* MR sequence parameters are described in table 4. All surgical specimens with a size exceeding 10 cm were scanned in multiple contiguous sections and were eventually merged to one composed MR image dataset. MeVisLab software (MeVis Medical Solutions, Fraunhofer MEVIS) was used to view and annotate the 3D *ex vivo* composed lipid and water-selective images simultaneously. All structures that were spherical and bordered in three dimensions were identified as lymph nodes.

Table 4: Overview of *ex vivo* MR parameters (7 Tesla, Bruker ClinScan)

<i>Ex vivo</i> MRI	3D lipid excitation	3D water excitation
Acquisition time (min)	13	20
TR (ms)	15	30
TE (ms)	3.0	6.2
Resolution (mm ³)	0.29 x 0.29 x 0.29	0.29 x 0.29 x 0.29
Flip angle (°)	10	14
Abbreviations: 3D = 3-dimensional, min = minutes, MRI = magnetic resonance imaging, ms = milliseconds, TE = echo time, TR = repetition time.		

The interval between USPIO administration and surgery varied between 1-9 days (table 1). In our experience, USPIO remains visible on MR-images until 2-3 days after infusion. The effect of iron particle accumulation in healthy (parts of) lymph nodes is only visible when the *ex vivo* MRI is performed within this timeframe.

MRI guided pathology and node-to-node correlation

In vivo MR-images were evaluated by one or two radiologist(s) and the researcher coordinating the study. Based on lymph node size and appearance, and their location in relation to anatomical landmarks, these *in vivo* nodes were correlated to the *ex vivo* MRI images. Subsequently, the lymphadenectomy specimen was dissected at the Department of Pathology with the 3D *ex vivo* MR images presented on-screen to guide the pathologist [12]. The matched lymph nodes were located in the specimen, taken out, and separately enclosed in a tissue cassette. The lymph nodes were numbered accordingly on *in vivo* MRI, *ex vivo* MRI and the corresponding tissue cassette. All lymph nodes were, when larger than 5.0 mm, sectioned multiple times in 2.0-3.0 mm thick sections for paraffin embedding. All nodes were examined with a hematoxylin and eosin (H&E) stain on 4 µm sections. Relating the histopathological slide to the corresponding *ex vivo* assigned lymph node and subsequently to the corresponding *in vivo* identified lymph node provided the complete node-to-node correlation.

Results

In vivo USPIO-enhanced MRI and *ex vivo* MRI of resected specimens were feasible in all five patients. The general workflow to improve nodal staging for solid malignancies of different anatomical origins is schematically visualized in figure 1. Due to different clinical routines in pathological work-up for each primary tumor localization, each tumor-specific workflow currently developed has differences in approach. These specifics steps are outlined below per primary tumor localization.

Prostate cancer

A bilateral extended pelvic lymph node dissection (PLND) was performed in the patient with prostate cancer for diagnostic purposes before treatment with radiotherapy. Each anatomical nodal station is separately dissected and pinned onto the corresponding region of a schematic anatomical plate (figure 3). In order to preserve anatomical orientation, each nodal station is colored differently (figure 3) and left- and right specimens are separately vacuum sealed using the TissueSAFE plus device (Milestone, Sorisole, Italy, figure 9). Vacuum sealing the specimens offers several advantages: the separate specimens of each nodal station are scanned in their original confirmation, scanning the separate specimens together ensures that enough MR signal is generated, and their orientation is preserved hence routine clinical practice is not disturbed. An *ex vivo* MRI of the vacuumed specimens is performed. The specimens are subsequently processed routinely at the dissection room; *ex vivo* MR-images are not present during this process. The

results of both the *in-* and *ex vivo* MRI are correlated to the final histopathological results on a level-to-level basis. In case *ex vivo* MRI yields a higher number of lymph nodes than histopathological analysis, additional dissection of the specimen is performed to determine if the number of harvested evaluated lymph nodes can be increased.

Rectal cancer

In the rectal cancer patient, surgical resection was performed according to the principle of a total mesorectal excision (TME) [13]. Lymph nodes that are likely to be involved in rectal cancer are located in the mesorectum, which is the fat tissue surrounding the bordered on the outside by the mesorectal fascia. This fascia forms an important anatomical barrier for tumor spread and is the intended surgical boundary of the TME procedure. Matching the *ex vivo* specimen to the *in vivo* appearance of the mesorectum after a well-performed surgical procedure is therefore relatively easy. Pathological assessment of the resected specimen is performed according to the method described by Quirke et al [14]. The specimen is evaluated from distal to proximal by slicing serial transverse tissue lamina with a 5 mm interval. All nodes detected on pathology were correlated to the *ex vivo* MR-images which could be correlated to the *in vivo* MR-images for a node-to-node analysis (figure 4).

Periampullary cancer

In the patient with periampullary cancer a pancreaticoduodenectomy with lymphadenectomy was performed. Most lymph nodes were *en-bloc* resected with the tumor, additionally several lymph nodes were resected separately. After resection, the surgeon pinned the specimen onto an anatomical drawing displaying the pancreatic region including its lymph node stations of which a digital photograph was taken (figure 5). At the department of Pathology, the resection planes are inked, the primary tumor is incised and the entire specimen is fixated with formalin [15]. The fixated specimen is processed routinely according to the axial slicing technique (figure 5) [15]. Nodal correlation is performed on a level-to-level basis and, where possible, on a node-to-node basis.

Esophageal cancer

For the esophageal cancer patient, esophagectomy with two field lymphadenectomy (thoracic and abdominal lymph node stations) was performed after neoadjuvant chemoradiotherapy. During surgery, the esophagus including locoregional lymph nodes were *en-bloc* dissected. The surgeon labelled all the lymph node stations within the surgical specimen with colored plastic beads, according to the TIGER study protocol [16]. Differences in configuration of the *ex vivo* specimen as compared to its original anatomical location makes it difficult to correlate the lymph node stations using anatomical landmarks. However, using the labelled location of the lymph node stations, lymph nodes on *in vivo* MRI are matched to *ex vivo* MRI and histopathology on a nodal, or at least a nodal station, level (figure 6).

Head-and-neck cancer

In the head-and-neck cancer patient, the neck dissection specimen was taken out *en-bloc*. The primary tumor was resected separately. After resection, the specimen was pinned to a grid with a reference drawing of the neck including its anatomical nodal levels and fixated with formalin (figure 7). Next, the different neck levels are separated. In order to maintain orientation, these pieces are vacuum sealed in their original configuration with a TissueSAFE plus system (figure 9) and scanned *ex vivo* in the 7T preclinical MR system. The cuts, or the boundaries between the neck levels, are visible on *ex vivo* MRI. All suspicious nodes were individually matched on *in vivo* MRI, *ex vivo* MRI and histopathology. These nodes are cut into multiple sections and stained with immunohistochemistry in addition to standard hematoxylin and eosin staining. All non-suspicious nodes are routinely dissected and processed per level. Therefore, this workflow enabled node-to-node correlation for suspicious nodes and level-to-level correlation for non-suspicious nodes.

Discussion

We developed a method which enabled correlation of *in vivo* detected lymph nodes from different primary solid malignancies with pathology. The workflow was technically feasible in prostate, rectal, periampullary, and head-and-neck cancer patients. The intermediate step of the 7T *ex vivo* MRI scan of resected specimens allowed in principle correlation on a nodal level, but at least on a nodal station level between the *in vivo* MRI images and histology. For each tumor location, different approaches were required to maintain anatomical orientation of the resected specimen and correctly match the results to the pathological evaluation. In esophageal, periampullary, and rectal cancer, lymph nodes were dissected *en-bloc* with the primary tumor. Therefore, lymph nodes could be matched using corresponding anatomical landmarks to the *ex vivo* MRI. In head-and-neck and prostate cancer, lymphadenectomy and primary tumor dissection were performed separately. Hence the lymphadenectomy specimens were vacuum sealed in plastic bags in order to maintain orientation and easy positioning of the specimen within the scanner.

Comparison with literature

Few attempts have been made to match individual lymph nodes visible on MRI with their precise histologic matching after lymphadenectomy. For rectal cancer, Park et al proposed a method where *ex vivo* ultrasound was used to match *in vivo* MRI with histopathology [9]. The use of identical scanning parameters for both *in vivo* MRI and *ex vivo* ultrasound enabled successful matching on a nodal level in 91% of the lymph nodes. However, only lymph nodes >3 mm were depicted with MRI and matched with histopathology. A recent study applied ultrahigh field *ex vivo* MRI of TME specimens in 22 rectal cancer patients and demonstrated that the mean size of the lymph nodes found on *ex vivo* MRI was below 3 mm [12]. Luciani et al. [10] studied 1.5T *ex vivo* MRI for precise node-to-node correlation in female breast cancer patients, but 20% of nodes found with pathology were not depicted with *ex vivo* MRI. A likely explanation is that the used magnetic field strength was only 1.5T, MR sequences were 2-dimensional and slice thickness was 2-3 mm, easily missing the smaller sized nodes. By comparison in the present study, *ex vivo* MRI was performed with 3D sequences and a partition/slice thickness of 0.29 mm at a field strength of 7T, most likely improving nodal yield on MRI. Korteweg et al examined healthy axillary lymphoid tissue of two deceased females on a clinical 7T *ex vivo* MRI system and detected lymph nodes <1 mm, corroborating our results [3]. Both studies [10, 3] proposed a framework for exact matching of radio-pathological findings by pinning the dissected specimen to an MR compatible grid containing an explanatory absciss and ordinate [10] or vertical and horizontal reference lines [3]. For each lymph node, the in-plane position was assessed and correlated to the MR images. Matching was successful in 80% [10] and 88% [3] of the lymph nodes, although this percentage strongly depends on the total amount of identified lymph nodes.

Strengths and limitations

We developed a workflow to match individual lymph nodes detected on *in vivo* MRI to the resection specimen by incorporating 7T *ex vivo* MRI to the protocol. This method can be used in studies validating MRI, such as USPIO-enhanced MRI, for detection of lymph node metastases on a nodal level with histopathology as gold standard. The method was illustrated in prostate, rectal, periampullary, esophageal, and head-and-neck cancer. The results demonstrate that various approaches enabled maintenance of orientation in both large, small and anatomically complex resection specimens. Therefore, the presented workflow can easily be adapted to other organs. High resolution *ex vivo* MRI images provide direct information on the location of lymph nodes in resection specimens and is of immediate help to harvest nodes in general, or suspicious nodes in particular. In case of a short time interval between USPIO-enhanced MRI and surgery, maintenance of signal intensity on the *ex vivo* MR-images represents the absence of USPIO deposits and thus a lymph node suspicious for harboring a metastasis. This functional information can be beneficial for node-to-node correlation. Radiologic differentiation between a lymph node and a blood vessel was

formerly potentially difficult. However, using 3D high-resolution MR sequences enabling visualization of nodal structures in the transverse, sagittal and coronal plane, this is no longer an issue.

Some potential limitations should also be mentioned. Node-to-node correlation for all lymph nodes is challenging. Differences in spatial resolution between *in* and *ex vivo* exams can lead to differences in detection of lymph nodes, particularly the small ones. Additionally, there could be a difference between the resected volume and a volume defined as resected on *in vivo* images, which also can lead to differences in the amount of evaluated lymph nodes. Also, the fixation process needed for the pathology processing causes volume change of the specimen, which leads to a change of configuration of the specimen and landmarks. Neck dissection and PLND specimens, for example, are relatively small but harbor many lymph nodes and generally contain only a few anatomical landmarks. Therefore, it is of paramount importance that the anatomical orientation of the specimen is well documented during dissection. In this way a reliable node-to-node correlation can be achieved or in case of clusters of small lymph nodes a per nodal station correlation can be performed. In addition, the *ex vivo* MRI requires a considerable investment of time which is not always desirable in clinical practice. Likewise, not every institution has an 7T preclinical MR system available for *ex vivo* measurements. Therefore, the method we developed is particularly suitable for studies validating imaging techniques for nodal assessment.

Clinical implications

In various types of cancer, detection of small lymph node metastases is urgently needed. Both over- and undertreatment in oncological therapeutic regimens result in unnecessary morbidity and mortality. Treatment needs to be tailored on a patient level to overcome this problem. Thus, knowledge regarding merely the presence (N+) or absence (N0) of nodal metastases is not sufficient anymore. Clinicians need to be informed about the number, size and exact location of metastatic deposits which is a prerequisite to target therapies. Individual treatment strategies consist of local tumor excision or ablation without lymphadenectomy in N0 patients, reducing unnecessary morbidity in this group of patients. In N+ cancer patients the exact N-staging can lead to additional treatment options for local lymph node metastases such as surgical resection, ablation, or image guided radiotherapy. Currently, a variety of promising non-invasive diagnostic imaging tests are developed to detect lymph node metastases, e.g. hybrid PET-MRI, USPIO-enhanced MRI and targeted fluorescence imaging [17, 4]. Using our described workflow with 7T *ex vivo* MRI as an intermediate step to guide histopathological work-up, new techniques can be validated on a node-to-node level with histopathology. The detailed 7T *ex vivo* MRI of the resection specimen aids in finding the smallest lymph nodes that otherwise might be missed with routine pathological evaluation [12], supporting the validation of *in vivo* imaging techniques.

Conclusion

A technically feasible method was developed to correlate lymph nodes identified on USPIO-enhanced MRI for the detection of lymph nodes metastases with histopathology of the same nodes as gold standard. This workflow was suitable in various anatomical regions. *Ex vivo* MRI-guided pathological dissection enabled lymph node correlation on a nodal or station level between *in vivo* MR-images and histopathology. This approach allows a validation of *in vivo* USPIO-enhanced MRI for evaluation of loco-regional lymph nodes which is essential for a subsequent diagnostic accuracy assessment.

Abbreviations

3D 3-dimensional

B.V. private company (Dutch: besloten vennootschap)

FOV field of view

Hz hertz

Kg kilogram

MEDIC multiple echo data image combination

Mg milligram

mGRE multi-gradient echo

Min minutes

MRI magnetic resonance imaging

Ms milliseconds

n.a. not applicable

N+ lymph node positive for harboring metastases

N0 lymph node negative for harboring metastases

PLND pelvic lymph node dissection

SPL savings patients' lives

T tesla

TE echo time

TME total mesorectal excision

TNM tumor node metastases

TR repetition time

USPIO ultrasmall superparamagnetic iron oxide

VIB volumetric interpolated breath-hold examination

Declarations

Ethics approval – the data was selected and derived from five scientific trials (VALINODE, 7TNANO1, NANO-PANC, PRECIES, USPIO-NECK) which all obtained ethical approval from the local ethics committee Arnhem-Nijmegen.

Consent to participate – all patients included provided informed consent for study participation.

Consent for publication – all patients included provided informed consent for their data to be published anonymously.

Availability of data and material – The data that support the findings of this study are available upon reasonable request from the corresponding author DD. The data are not publicly available due to them containing information that could compromise research participant privacy.

Competing interests – Patrik Zámecnik declares to be a “Scientific Advisor to SPL Medical B.V.” and owner of options of SPL Medical B.V. The other authors declare that they have no conflicts of interest with the content of this article.

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Author's contributions – All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Daphne Driessen, Didi de Gouw, Rutger Stijns, Geke Litjens, Bas Israël, John Hermans, Tim Dijkema, Bastiaan Klarenbeek, Rachel van der Post, Iris Nagtegaal, Adriana van Engen-van Grunsven, Lodewijk Brosens, Patrik Zámecnik and Tom Scheenen. Andor Veltien and Bart Philips developed the *ex vivo* MR protocol. Tom Scheenen contributed significantly to discussion of the content and revision of the manuscript and is guarantor of the entire study. The first draft of the manuscript was written by Daphne Driessen and Didi de Gouw and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Schematic overview of the workflow

Figure 2

Example of tissue fixation in head-and-neck cancer. A grid containing a reference drawing of the neck including the levels (a) is present at the operating room. After resection, the fresh specimen is pinned to this grid (b) and is subsequently fixated with formalin at the department of Pathology (c)

Figure 3

Example of the workflow in prostate cancer. In vivo MRI with a suspicious lymph node in the right obturator fossa (a, white dashed lined box). Each nodal station was colored and pinned to an anatomical reference drawing for preservation of orientation (b). The surgical specimen was vacuum sealed to fixate the specimen for the ex vivo MRI (c). Due to a short USPIO-MRI interval of 1 day, USPIO particles were still present in the resection specimen. Since there was no loss of signal intensity in this lymph node, it was regarded suspicious (d, white dashed lined box). Pathology assessment with hematoxylin and eosin staining showed that this lymph node was metastatic (e)

Figure 4

Example of the workflow in rectal cancer. A coronal view of the in vivo MRI showing the rectum within the white dashed lined box with a non-suspicious lymph node located right pararectally close to the rectal wall (a). After TME surgery the specimen was fixated in formalin (b) of which an ex vivo MRI scan was performed. After the ex vivo scan, the specimen is sliced in 5 mm thick lamina slices (c) which can be compared to the transverse images of the ex vivo MRI (d). The lymph node was detected on ex vivo MRI as well as on pathology. Since the USPIO-surgery interval was 9 days, and

USPIO was no longer present in the resection specimen, there were no changes in signal intensity. For this reason, the lymph node has maintained signal on the water-selective ex vivo MR-image (d). Histopathological assessment with hematoxylin and eosin staining revealed a non-metastatic lymph node (e)

Figure 5

Example of the workflow in periampullary cancer. A transversal view of the T1 VIBE Dixon in vivo MRI showing the pancreas with a suspicious lymph node located dorsal from the pancreatic head in the lined box (a). After pancreaticoduodenectomy the fresh resection specimen was pinned on an anatomical drawing (b) and an ex vivo MRI scan was performed on which the same lymph node could be identified on the lipid selective image (c) and the water selective image (d). The USPIO-surgery interval was 8 days, thus USPIO was no longer present in the resection specimen and therefore did not elicit any signal intensity changes. After the ex vivo MRI the specimen was fixated and sliced into 5mm thick slices (e). Histopathologic examination with hematoxylin and eosin staining revealed a metastasis within the lymph node (e)

Figure 6

Example of the workflow in esophageal cancer. Lymph nodes were identified on the in vivo MRI after neoadjuvant chemoradiotherapy (a). Nodes could be matched using corresponding anatomical landmarks to the surgical specimen (b) and ex vivo MRI (c, d). The lipid-selective (c) and water-selective (d) ex vivo MRI guided the pathologist towards the lymph nodes for histopathological analysis of each node with hematoxylin and eosin staining (e). The white dashed lined box on the ex vivo MRI (c, d) and pathology (e) contains a healthy lymph node with USPIO contrast (the USPIO-surgery was 1 day). Therefore, the lymph node has lost its signal intensity on the water selective image

Figure 7

Example of the workflow in head-and-neck cancer. A lymph node was located on the in vivo MR image (a, white dashed lined box). After lymphadenectomy, the fresh specimen was pinned to a grid (b) and fixated with formalin (c). Of the fixated specimen, an ex vivo MRI was performed. The lymph node of interest identified on the in vivo MR image was correlated to the ex vivo MR image (d). Since the USPIO-surgery interval was 5 days, and USPIO was no longer present in the resection specimen, there were no changes in signal intensity. For this reason, the lymph node has maintained signal on the water-selective ex vivo MR-image (d). The pathologist was guided by the ex vivo MRI to dissect this node and enclose it separately. Histopathologic examination with hematoxylin and eosin staining revealed a healthy lymph node (e)

Figure 8

Example of the set-up for ex vivo MRI using the 7T preclinical MR system. From left to right: a formalin fixated surgical rectal cancer specimen is placed on a paper sheet. Next it is rolled and placed in a plastic tube and positioned within the MR system

Figure 9

Example of a cervical lymphadenectomy specimen vacuum sealed with the TissueSAFE plus device (Milestone, Sorisole, Italy)