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Translational Science

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Susceptibility Perturbation MRI Maps Tumor Infiltration into Mesorectal Lymph Nodes

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Abstract

Noninvasive characterization of lymph node involvement in cancer is an enduring onerous challenge. In rectal cancer, pathologic lymph node status constitutes the most important determinant of local recurrence and overall survival, and patients with involved lymph nodes may benefit from preoperative chemo and/or radiotherapy. However, knowledge of lymph node status before surgery is currently hampered by limited imaging accuracy. Here, we introduce Susceptibility-Perturbation MRI (SPI) as a novel source of contrast to map malignant infiltration into mesorectal lymph nodes. SPI involves multigradient echo (MGE) signal decays presenting a nonmonoexponential nature, which we show is sensitive to the underlying microstructure via susceptibility perturbations. Using numerical simulations, we predicted that the large cell morphology and the high cellularity of tumor within affected mesorectal lymph nodes would induce signature SPI decays. We validated this prediction in mesorectal lymph nodes excised from total mesorectal excision specimens of patients with rectal cancer using ultrahigh field (16.4 T) MRI. SPI signals distinguished benign from malignant nodal tissue, both qualitatively and quantitatively, and our histologic analyses confirmed cellularity and cell size were the likely underlying sources for the differences observed. SPI was then adapted to a clinical 1.5 T scanner, added to patients' staging protocol, and compared with conventional assessment by two expert radiologists. Nonmonoexponential decays, similar to those observed in the *ex vivo* study, were demonstrated, and SPI classified lymph nodes more accurately than standard high-resolution T_2-weighted imaging assessment. These findings suggest this simple, yet highly informative, method can improve rectal cancer patient selection for neoadjuvant therapy.

Significance: These findings introduce an MRI methodology tailored to detect magnetic susceptibility perturbations induced by subtle alterations in tissue microstructure.

Introduction

In rectal cancer, pathologic lymph node status of the mesorectum constitutes the most important determinant of local recurrence and overall survival (1). Standard assessment is based on hematoxylin and eosin (H&E)-stained slides (1), which can identify malignancy based on the contrast between small, roughly round, and loosely-packed leucocytes and much larger, goblet-shaped, tightly packed malignant epithelial cells. This information becomes available only with radical surgery; however, preoperative neoadjuvant chemoradiation therapy is associated with lower incidences of local relapse in patients with high-risk features, including lymph node positivity, when compared with postoperative regimens (6% vs. 13% at 5 years; ref. 2). This justifies the need for accurate preoperative noninvasive imaging-based lymph node characterization.

MRI is safe, noninvasive, and benefits from extremely versatile physics, giving rise to a multitude of contrast mechanisms, which, in turn, can highlight different aspects of biological tissue. One of the most important sources of MRI contrast is dephasing in the transverse plane, typically characterized by a time constant T_2–the spin–spin relaxation. T_2 is affected by alkali cation composition and tissue microstructure and has been shown to be capable of differentiating between normal tissue and malignant tumors since the very beginning of magnetic resonance experiments in tissues (3). Indeed, T_2-weighted MRI (T2-WI) remains the gold standard for pelvic staging in rectal cancer, but it is unfortunately very limited with respect to lymph node involvement (4, 5). However, more generally, the MRI signal can be sensitized to transverse dephasing induced by local field variations arising from susceptibility distributions. In this case, the transverse relaxation constant is described by $1/T_2^* = 1/T_2 + 1/T_2'$, where T_2' characterizes the additional source of dephasing (6). Divalent calcium or trivalent iron cations, as well as tissue oxygenation, are important

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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sources of susceptibility-induced local field variations and their characterization is routinely performed using T_2^*-sensitive sequences such as gradient-echo MRI. Interestingly, gradient echo MRI has been also performed in the context of lymph node characterization. A study on dissected axillary lymph nodes at 7T demonstrated that the T_2^* of metastatic lymph nodes was distinct from that of benign nodes (7). A subsequent similar study performed *in vivo* at 3T reached similar conclusions (8), although the mechanism underlying these T_2^* variations remains to be elucidated.

T_2^* is a "generic" parameter, which can only be accurately estimated in the (approximately) linear, low echo-time regime of a multigradient echo (MGE) signal decay. This generic linear decay with the log of the signal decay is quite "featureless"; rather dramatic changes in susceptibility would be required to induce a significant variation in the T_2^*. However, the underlying physics (9–11) suggests that in most realistic scenarios, magnetic fields would be distributed according to susceptibility-driven perturbations leading to nonexponential (9) and even nonmonotonic (10) signals, which potentially reflect microstructural tissue properties. Curiously, the vast majority of studies assumed that it is sufficient to measure the "linear" component at short echo times, and only very few studies ventured toward measuring the full MGE decay up to longer echo times (TE), which could reflect the underlying susceptibility perturbations much better. Indeed, the few studies that have measured the full MGE decay have discovered that much more detailed information could be potentially extracted. For example, Chen and colleagues reconstructed the shape and orientation of white matter substructures and their arrangement relative to one another in humans at 3T from multiexponential MGE decays measured up to long TEs (9). Nunes and colleagues probed *ex vivo* rat spinal cords at 16.4 T and found nonexponential, and nonmonotonic MGE decays, which were simply modeled by two compartments (axons + extraaxonal space), leading to axon density quantification (10). Qian and colleagues scanned tibial cartilage explants at 3T and classified different types of MGE decays, where the short T_2^* component differentiated diseased from healthy cartilage successfully, while the monoexponential T_2^* model did not (11).

Normal lymph nodes are composed of a relatively uniform and loose distribution of small lymphocytes. In contrast, malignancy is characterized by the presence of tightly-packed large malignant epithelial cells. Because susceptibility-driven magnetic field distributions depend on the underlying compartment sizes, we hypothesized that MGE signals could reflect these intrinsic susceptibility disturbances and distinguish tumor-infiltrated from normal nodal tissue. We term this long-echo MGE approach Susceptibility Perturbation MR Imaging (SPI) because it probes susceptibility perturbations due to microstructure. We tested SPI using numerical simulations and ultrahigh magnetic field MRI experiments (16.4 T) in mesorectal lymph nodes extracted from the surgical specimens of patients with rectal cancer. We elucidated the mechanisms underlying the emerging SPI contrasts using quantitative histologic analysis. Finally, we translated the experiment to an *in vivo* 1.5 T scanner, upon patient staging, for clinical applicability assessment, in which the SPI contrast was again observed. Our findings indicate that SPI is superior to the current standard methodology for lymph node involvement characterization *in vivo*.

Materials and Methods

Simulations

Numerical simulations were performed to predict whether changes in cellularity could affect MGE decays. To this end, we simulated susceptibility-driven magnetic field distribution maps arising from randomly packed spheres with different distributions of cell sizes and intracellular volume fractions. The magnetic field distribution for an individual sphere of radius R located at position (x_0,y_0,z_0) in a 3D mesh (x,y,z) is given in Eq. A (12, 13):

$$\Delta B^{sph}(x,y,z) = B_0 \begin{cases} \frac{1}{3}\chi R^3 \frac{(2(z-z_0)^2-(x-x_0)^2-(y-y_0)^2)}{((z-z_0)^2+(x-x_0)^2+(y-y_0)^2)^{5/2}} & \text{outside the sphere} \\ 0 & \text{inside the sphere} \end{cases} \quad \text{(A)}$$

Following others (14), in this study we used a susceptibility difference of $\chi = 6 \times 10^{-7}$ (14) in all simulations.

Guided by our histology assessment, we designed two tissue configurations corresponding to the microstructure of benign and malignant tissue, respectively. The benign tissue configuration employed a distribution of spherical radii corresponding to a lognormal distribution of areas with a mean of 12.2 μm^2 and SD of 6.1 μm^2 packed in a $50 \times 50 \times 50$ μm^3 cube with intracellular volume fraction of 24%. For the malignant tissue configuration, we used a similar lognormal distribution, but now with a mean of 92.7 μm^2 and SD of 49.8 μm^2, packed in a $50 \times 50 \times 50$ μm^3 volume with 61% intracellular volume fraction. Once the configurations were set, the field map $\Delta B(x,y,z)$ was calculated as the sum over the contributions of all individual spheres within each substrate.

Once the field maps were produced for every substrate, the MGE signal in the simulated "voxel" could be calculated by simply summing across the entire "voxel", as represented in Eq. B:

$$S_{MGE}(TE) = \iiint_{x,y,z} \exp(i\gamma \Delta B(x,y,z) \cdot TE) \quad \text{(B)}$$

Ultrahigh field SPI of *ex vivo* lymph nodes

Institutional setting and lymph node harvesting. This study was approved by the institutional ethics committee and written informed consent was obtained from twenty-five consecutive patients with rectal cancer that agreed to participate in the study. Six of these patients were excluded because they underwent neoadjuvant therapy and another three were excluded because they chose to be operated in a different institution. Sixteen patients underwent surgery without neoadjuvant therapy and their total mesorectal excision specimens were immersed in a 4% formaldehyde solution for 72 hours. During macroscopic specimen processing, the otherwise discarded halves of lymph nodes present in more than 1 cut slice, approximately 5 mm in thickness, were collected and labeled to match the halves sent for pathologic staging, which was performed by a gastrointestinal pathologist (8 years of experience), according to the cancer protocol defined by the College of American Pathologists (https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates). Pathologic staging resulted in the exclusion of an additional 5 patients with node-negative disease. A total of 11 patients were included (mean age 61.6 years, 5 males).

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Acquisition protocol at 16.4T

Retrieved lymph node "halves" from each patient were grouped in benign/malignant pairs based on the information from pathologic staging, preferably originating from the same histopathologic block and with a similar size. Prior to scanning, the nodes were washed with a 1% PBS solution for 24 hours and then immersed in Flourinert within a 10 mm NMR tube.

The preclinical MRI images were acquired at 37°C on a 16.4T Bruker Aeon Ascend Scanner (Bruker Biospin) using a Micro5 probe with a gradient system capable of producing up to 3,000 mT/m in all directions. An MGE acquisition was performed with the following parameters: 50 echo times starting at 1.6 ms with 1.4 ms interval, repetition time of 1,500 ms, flip angle of 50°, slice thickness of 0.3 mm, a field-of-view of 12×12 mm^2, and matrix size of 120×120, leading to an in-plane resolution of 0.1×0.1 mm^2. The acquisition bandwidth was set to 125 kHz and 25 signal averages were acquired, leading to a total scan time of 1 hour 12 minutes per node.

Histopathologic analysis for validation of the MRI findings

The scanned "halves" of lymph nodes were embedded in paraffin and 6 consecutive 4 μm slices were cut every 50 μm using a Leica RM2245 microtome (Leica Biosystems) in a plane parallel to the cut surface of the node, similarly to the MRI acquisition. One slice per interval was stained with H&E and analyzed by the gastrointestinal pathologist (8 years of experience) using a Zeiss Axio Lab A1, (Carl Zeiss Microscopy GmbH) with a 40× amplification. The analysis of the scanned "halves" resulted in reclassification of 3 previously defined benign nodes as malignant. Given they all originated from patients already classified as N+, this did not impact patient management. In total, we scanned and analyzed 29 benign and 35 malignant lymph nodes.

In four representative lymph nodes, the remaining 4-μm slices were used for additional characterization: Pearls coloration (Iron Staining Kit, Ventana Medical Systems, Inc.) was used to quantify iron-containing MR-field–disturbing particles; an antibody against CD45 (CD45, QBEnd/10, Leica Biosystems) was used to mark leucocytes; and multi-cytokeratin AE1/AE3 (Leica Biosystems) was used to mark adenocarcinoma cells.

MR image analysis

Multigradient echo datasets were denoised in Matlab (Mathworks) using Marchenko–Pastur principle component analysis (15) with a window size of 7×7. Benign and malignant histology-matched regions-of-interest (ROI) were defined by a dedicated radiologist (10 years of experience). Three ROIs were placed per node, in the most representative slice; in malignant nodes, malignant areas were selected on the basis of MR-histology coregistration (see subtopic below). The mean signal value per node was computed for each TE. Although SPI in principle leads to a field distribution, we here simplified the complex expressions by assuming that one, two, or three components would be sufficient to describe the signal decay. In the multiexponential models, one compartment was arbitrarily assumed to be on resonance while the others exhibited a frequency shift. The signal expressions for 1-compartment, 2-compartment, and 3-compartment models are given in Eqs. C, D, and E, respectively.

$$S_{1c} = S_0 \exp\left(-TE/T_2^*\right) \tag{C}$$

$$S_{2c} = S_0 \left| f^a \exp\left(-\frac{TE}{T_2^{*a}}\right) + (1-f^a)\exp\left(-TE\left(\frac{1}{T_2^{*b}} + i\Delta\omega^b\right)\right)\right| \tag{D}$$

$$S_{3c} = S_0 \left| f^a \exp\left(-\frac{TE}{T_2^{*a}}\right) + f^b \exp\left(-TE\left(\frac{1}{T_2^{*b}} + i\Delta\omega^b\right)\right) + \left(1-f^a-f^b\right)\exp\left(-TE\left(\frac{1}{T_2^{*c}} + i\Delta\omega^c\right)\right)\right| \tag{E}$$

where TE is the echo time, S_0 is the signal at TE = 0, and T_2^{*i} is the relaxation time of compartment i, which has a volume fraction f^i and frequency shift $\Delta\omega^i$.

Voxel-by-voxel fitting of the signal models above to the experimental data were performed for the whole dataset.

MR-pathology coregistration

Histology H&E slides were scanned at a Philips Ultrafast Scanner 1.6 (Philips Healthcare), stacked into a volume, and registered to MGE images using SimpleElastix (16). For the stacking step, a 2D-rigid pairwise registration was performed, in which a set of 3 to 6 corresponding control points were defined on each pair of histology images and the translations and rotations were estimated using adaptive stochastic gradient descent, minimizing both the advanced Mattes mutual information and the corresponding points Euclidean distance metric. The registration of a given stack to the MGE images utilized a 3D-rigid registration scheme with 6 to 12 corresponding control points defined in the two volumes and optimized using adaptive stochastic gradient descent that minimized both the advanced mattes mutual information and the corresponding points Euclidean distance metric.

Histology quantification

With the purpose of understanding the parametric differences encountered between benign and malignant lymph node tissue, we analyzed histology H&E slides and obtained measurements of cellularity and cell size. To ensure histology measurements were as representative as possible, we first selected the single H&E slide from the volume stack that best matched the MGE image in which the ROIs were placed. The selection was based on specific contour and inner landmarks that could be found in both MGE and scanned H&E images at low magnification (×0.03). Then, from each MGE-ROI–matching histology area and at higher magnification (×0.42), 3 random 50×50 μm fields were selected and a Cell Detection Tool (QuPath) was employed to quantify cell number, nucleus area, and cell area.

Statistical analysis

Signal decay models were compared on the basis of Bayesian Information Criterion (BIC), which describes the goodness of fit, penalizing for increasing number in model parameters, as

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represented in Eq. F.

$$BIC = \ln(N)k - 2\ln(\hat{L}) \quad \text{(F)}$$

where N is the number of data points, k is the number of model parameters (1 for monoexponential decay, 4 for biexponential decay, and 7 for triexponential decay), and $\ln(\hat{L})$ is the maximum value of the log likelihood function of the model. Specifically, $\ln(\hat{L})$ is the negative of the sum of squared differences between the model prediction and the measured data, which was also used as objective function for nonlinear fitting of the models.

For all parameters extracted from the models, comparison between benign and malignant was based on the Mann–Whitney *U* test, given data were not normally distributed. An $\alpha < 0.05$ was considered as the statistical significance threshold.

Spearman rank correlation coefficient was the test used to establish the statistical dependence between the rankings of model parameters and histology metrics. Bonferroni correction was employed to account for multiple comparisons.

Translation of SPI to the clinic, *in vivo* at 1.5 T

This study was also approved by the institution's ethics committee and written informed consent was obtained. In 8 participating patients, butylscopolamine 20 mg was administered intravenously to minimize bowel movement and a stereotactic body radiotherapy pressure belt with manual pump insufflated to 20–40 mmHg (Orfit Industries) was used to minimize respiratory movement artifacts. A MGE sequence with the following parameters was added to the staging pelvic MRI, which was performed on a 1.5 T Clinical Scanner (Ingenia, Philips Healthcare): 32 TEs were acquired starting at 2.37 ms with a 2.37 ms interval; repetition time of 1971 ms; flip angle of 55°; slice thickness of 4 mm; field of view of 20×20 cm^2, matrix size of 480×480, bandwidth of 431 Hz, and 2 signal averages, leading to an in-plane resolution of 0.42×0.42 mm^2 and a total acquisition time of 11 minutes 19 seconds.

Whole-node ROIs were defined for all visible lymph nodes on the single slice with the largest surface by a general radiologist (11 years of experience). Mapping during specimen processing allowed 36 benign and 27 malignant lymph nodes originating from the 6 patients subsequently selected for surgery without preoperative therapy at multidisciplinary team meeting (mean age, 61.7 years; 4 males) to be matched to MGE images (2 patients were excluded because they underwent neoadjuvant therapy). Models were fitted to the median magnitude signal of each lymph node, given the reduced number of voxels per node and deviation from normality of the distribution of signal intensities. Two radiologists dedicated to gastrointestinal and abdominal imaging (10 and 13 years of experience), blinded to pathology results, independently performed a node-by-node classification in the corresponding axial, coronal, and sagittal high-resolution T_2-WI in accordance with the ESGAR 2016 recommendations for nodal staging (17). Reliability of the T_2-WI–based analysis was estimated using the intraclass correlation coefficient (<0.40 = poor; 0.40 to 0.59 = Fair; 0.60 to 0.74 = good; 0.75 to 1.00 = excellent). The SPI and T_2-WI analysis and their combination were compared using logistic regression. The statistical significance of the differences between the corresponding areas under the ROC curves (AuROC) were tested using the De Long Test.

Results

Simulations

Our first goal was to simulate whether microstructural changes induced by the infiltration of malignant cells into lymph nodes would produce significant perturbations to magnetic field distributions. Figure 1A and B simulate the spatial distribution of magnetic fields in the "benign" substrate (small spheres, low volume fraction) and "malignant" substrate (infiltration of large spheres, with large volume fraction), respectively. Importantly,

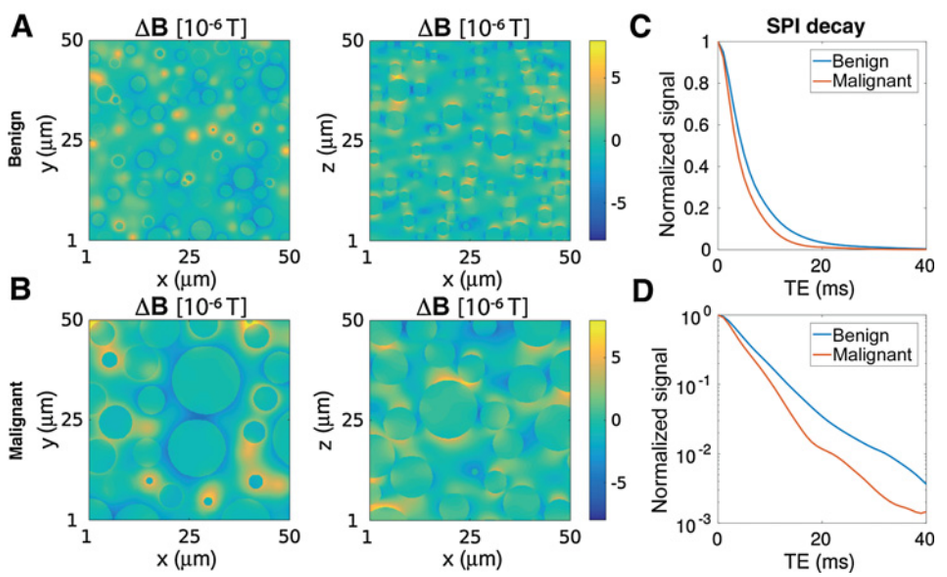

Figure 1.
Spatial distribution of the magnetic field induced by a distribution of spherical cells corresponding to benign tissue over a 50×50 μm^2 field of view in the XY and XZ planes (**A**) and malignant tissue over a 50×50 μm^2 field of view in the XY and XZ planes (**B**). **C,** Normalized signal decay as a function of TE for the two substrates corresponding to benign and malignant tissue. **D,** Normalized log signal decay as a function of TE for the same two substrates.

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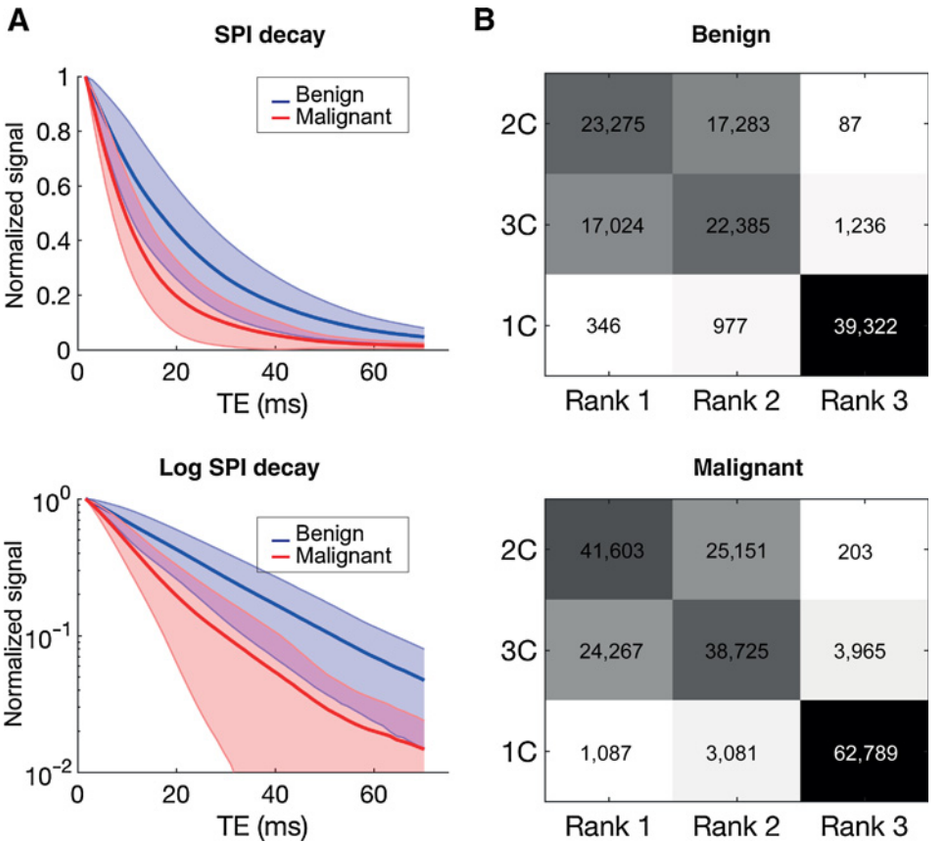

Figure 2.

A, Normalized average signal and log average signal with increasing TE of benign ($n = 29$) and malignant ($n = 35$) lymph node datasets. Shaded areas represent the SD limits. **B,** Number of entries per rank of voxel-by-voxel analysis using BIC for benign and malignant lymph nodes. Notice 2-compartment model ranked first and 1-compartment model ranked last in most instances for both benign and malignant datasets.

these field distributions give rise to a nonmonoexponential MGE signal decay (Fig. 1C, note the signal oscillation), which is more evident perhaps in the logarithmic plot (Fig. 1D). Perhaps even more importantly, these simulations predict a slower decay for small spheres with low volume fraction (benign tissue) and a faster decay for larger spheres with high volume fraction (malignant tissue), suggesting that MGE measurements could inform about tissue microstructure.

Ultrahigh field SPI of *ex vivo* lymph nodes

To test the validity of the above predictions and investigate whether SPI could distinguish between normal nodal tissue and nodal tissue infiltrated by malignancy, we performed ultrahigh field *ex vivo* experiments with very high spatial and TE resolution on 29 benign and 35 malignant lymph nodes (64 lymph nodes in total). Figure 2A shows that the normalized MGE signal decay for both benign and malignant tissue is indeed nonmonoexponential. TE-dependent differences between the signal decay of the different groups are already apparent from the raw data (Fig. 2A). A BIC analysis for model selection (e.g., between Eqs. A, B, and C) reveals that the 2-compartment model represents the data better than a 1- or 3-compartment model in the vast majority of voxels (Fig. 2B).

Figure 2A also reveals clear and dramatically different SPI decays for benign and malignant tissue: while the former decays slowly, the latter decays much more rapidly.

Given that the 2-compartment model was selected from the BIC values, further analysis was focused on this model. The median values of T_2^*a were significantly shorter in malignant tissue, by a factor of approximately 2 (malignant = 15.05 ms; benign = 29.59 ms; $P = 1 \times 10^{-4}$), as were the median values of T_2^*b, by a factor of approximately 2.3 (malignant = 10.71 ms; benign = 24.64 ms; $P = 1 \times 10^{-4}$). In addition, in absolute value, the median frequency shift $\Delta\Omega$ was significantly larger in malignant tissue, by a factor of approximately 1.6 (malignant = −0.08 rad; benign = −0.05 rad; $P = 8 \times 10^{-4}$). The fraction of the first component did not exhibit a significant difference between tissues (malignant = 0.62; benign = 0.66; $P = 0.097$). Figure 3 depicts these measurement differences as boxplots.

We next turn to the histologic analyses, where striking microstructural differences were found. In benign nodal tissue, the predominant cell type—lymphocyte—is small, round, and practically devoid of visible cytoplasm. Adenocarcinoma cells, on the other hand, are cytoplasm-rich and much larger. When quantified, we found that cell size, both in terms of nuclear area (benign: $\bar{x} = 12.36$ and $\sigma_{\bar{x}} = 0.99$ μm^2; malignant: $\bar{x} = 23.77$ and $\sigma_{\bar{x}} = 4.16$ μm^2; $P = 5.99 \times 10^{-7}$) and total cell area (benign: $\bar{x} = 12.36$ and $\sigma_{\bar{x}} = 0.99$ μm^2; malignant: $\bar{x} = 104.34$ and $\sigma_{\bar{x}} = 23.53$ μm^2; $P = 2.23 \times 10^{-8}$), was higher in malignant tissue while the mean number of cells per surface area was much lower (benign: $\bar{x} = 19.91 \times 10^{-3}$ and $\sigma_{\bar{x}} = 2.69 \times 10^{-3}$ cells/μm^2; malignant: $\bar{x} = 6.62 \times 10^{-3}$ and $\sigma_{\bar{x}} = 3.73 \times 10^{-3}$ cells/μm^2; $P = 5 \times 10^{-3}$). We also directly correlated SPI parameters with the cellularity and cell size by registering MR images with the histologic slices (Fig. 4A). We found significant correlations between T_2^*b, our best-performing parameter, and both number of cells

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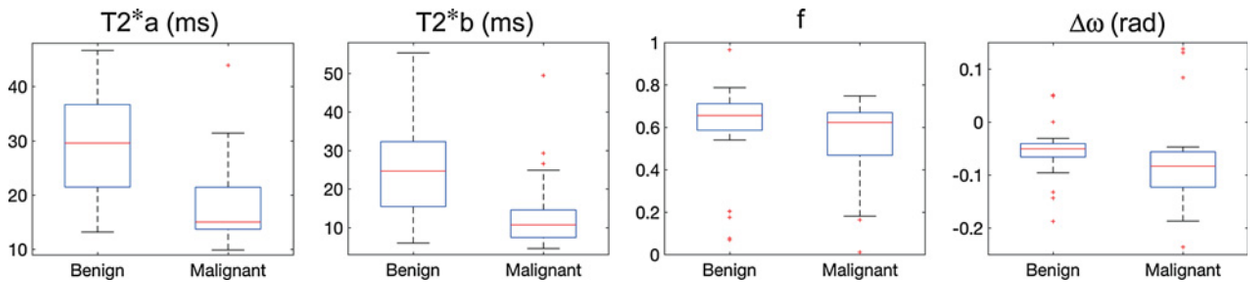

Figure 3.
Boxplots depicting the measurement differences for the 2-compartment model-derived parameters. Significant differences between benign and malignant lymph nodes were found for T_2^*a, T_2^*b, and $\Delta\Omega$, but not for f.

per surface area ($\rho = 0.52$, considered moderate; $P = 9.18 \times 10^{-6}$) and mean nuclear area ($\rho = -0.56$, considered moderate; $P = 1.14 \times 10^{-6}$; Fig. 4B).

We also noted a variety of infiltration patterns in malignant lymph nodes, namely cellular areas (those constituted by tightly packed malignant cells), areas of necrosis and areas of tumor-induced desmoplasia. These patterns tend to coexist in malignant lymph nodes in varying proportions but when selective ROIs were placed in relatively "pure" areas, we discovered that areas with tightly packed malignant cells tended to produce a slower MGE signal decay compared with areas characterized by desmoplesia or necrosis but still, the decay occurred much faster than in benign nodal tissue (Fig. 5). To investigate the mechanism underlying the SPI decay differences, we performed staining for different markers (Fig. 5). Importantly, no iron particle accumulation was found in the selected fields neither in benign nor in malignant nodes, suggesting iron is not involved in the contrasts obtained in this study.

Translation of SPI to the clinic, *in vivo* at 1.5 T

We next aimed to assess the clinical applicability of SPI for mesorectal lymph node characterization prospectively, upon rectal cancer staging. The nonmonoexponential nature of the MGE signal decay was evidenced for both benign ($n = 36$) and malignant ($n = 27$) lymph node datasets (63 lymph nodes in total), and again revealed radically different decay characteristics: the benign nodes had a much slower decay compared with the malignant. Figure 6 shows SPI decays from a benign and a malignant node and in from Supplementary Fig. S1, the corresponding full extent of TEs is displayed.

The BIC analysis ranked the biexponential model first and the monoexponential model last in most instances. Differences in the parameter values derived from the biexponential model are presented in Figure 7A. T_2^*a carried differences between benign and malignant lymph nodes with statistical significance (malignant: median = 50.86 ms; iqr = 20.84 ms; benign: median = 58.87 ms; iqr = 29.50 ms; $P = 0.02$), as did T_2^*b (malignant: median = 2.96 ms; iqr = 0.73 ms; benign:

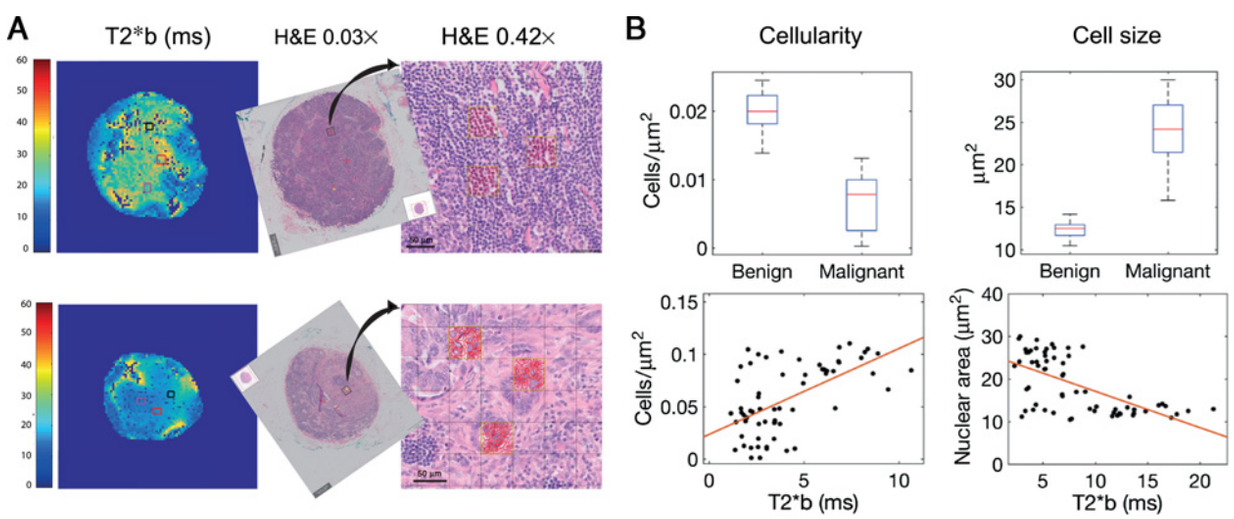

Figure 4.
A, T_2^*b parametric maps and corresponding QuPath cell detection tool applications are exemplified for a benign (top) and a malignant lymph node (bottom). **B,** Cellularity and cell size differences between benign and malignant lymph nodes are depicted in the boxplot above and Spearman correlations between them and T_2^*b are depicted in the scatterplot below ($\rho = 0.52$ for cells/μm^2 and $\rho = -0.56$ for nuclear area, both considered moderate and both with $P < 0.01$).

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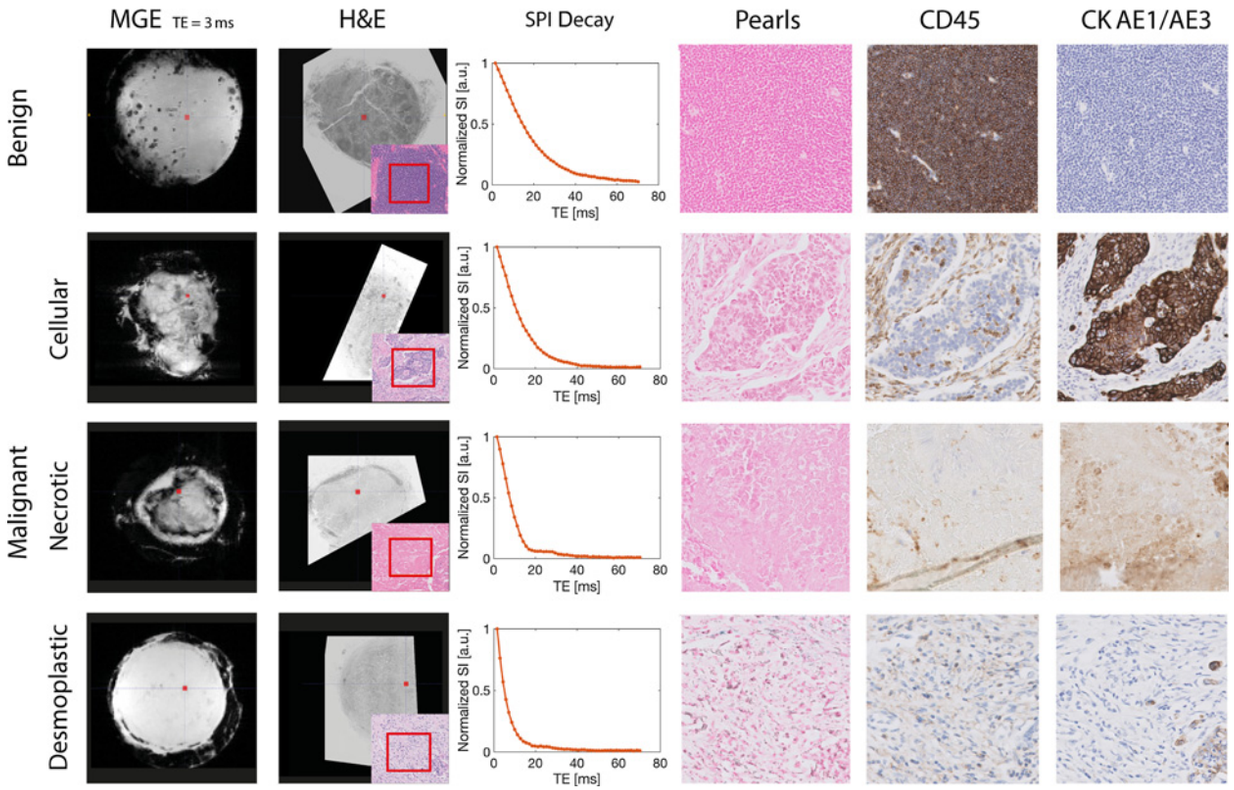

Figure 5.

When selective ROIs were defined, clear variations in SPI decay behavior were present. Highly cellular areas in secondary follicles from benign nodal tissue show a slower initial decline, whereas in tumor tissue, this decline tends to occur much faster and a clear oscillation between 20 and 40 ms becomes apparent. Pearls coloration was negative in all selected fields (iron particles stained in dark blue using Pearls coloration). CD45 is expressed in the cellular membrane of all leucocytes and benign lymph node tissue stains heavily, as expected. CK AE1/AE3 is a cytokeratin combination expressed in epithelial cells, including malignancies of epithelial origin such as colorectal adenocarcinoma. Although all malignant lymph node areas were positive, the area of high cellularity shows more pronounced staining, and the corresponding early decay slope is higher when compared with other malignant patterns.

median = 3.59 ms; iqr = 6.17 ms; $P = 3 \times 10^{-3}$). Both T_2^*a and T_2^*b were shorter in malignant lymph nodes, in accordance with the *ex vivo* data. For a cutoff of 58.5 ms, T_2^*a presented with an AuROC of 0.70 and for a cutoff of 53.2, T_2^*b presented with an AuROC of 0.76. The combination of T_2^*a + T_2^*b resulted in an AuROC of 0.79 (Fig. 7C). The performance of these metrics was superior to both that of T_2^* derived from the monoexponential model (0.64) and that of the T_2-WI–based analysis (0.67 and 0.69 for the two radiologists observing the data, ICC = 0.70, considered good). Adding the T_2^*a + b SPI analysis to the T_2-WI assessment increased the AuROC from 0.65 to 0.80 for Reader 1 and from 0.66 to 0.87 for Reader 2 (Fig. 7C). The observed increase was statistically significant ($P = 8.6 \times 10^{-4}$ and $P = 3 \times 10^{-4}$ for Readers 1 and 2, respectively). However, the performance of the combination was not significantly improved when compared with the plain T_2^*a + b SPI analysis ($P = 0.90$ and $P = 0.26$ for Readers 1 and 2, respectively).

Discussion

Lymph node staging is crucial for clinical decision-making in the great majority of malignancies. In the particular case of rectal cancer, lymph node involvement before surgery may be considered an indication for neoadjuvant therapy (18) but lymph node classification is limited on the basis of standard imaging, namely high-resolution T_2-weighted MRI (4, 5). Lymph node characterization can be improved using nontargeted imaging agent methods such as MR lymphography with USPIO nanoparticles, which have been applied with very high accuracy (19, 20, 21) and AuROC above 0.90 (22, 23). Very good results have also been reported using Gadofosveset, a gadolinium-based intravascular contrast agent (AuROC curve of 0.96; ref. 24). The drawback of these techniques is that they involve the administration of exogenous contrast agents with their implicit adverse reactions. Moreover, these contrast agents are not available for clinical use, making these techniques impractical.

In this study, we hypothesized that the microstructural changes associated with tumor infiltration—namely, the presence of malignant epithelial cells, much larger and more densely packed than native leucocytes—would cause susceptibility-induced magnetic field perturbations that could be picked up using SPI—an MGE sequence acquired up to very long TEs. Our histology-inspired numerical simulations clearly revealed that the hypothesis is viable and that MGE signal decays nonexponentially with

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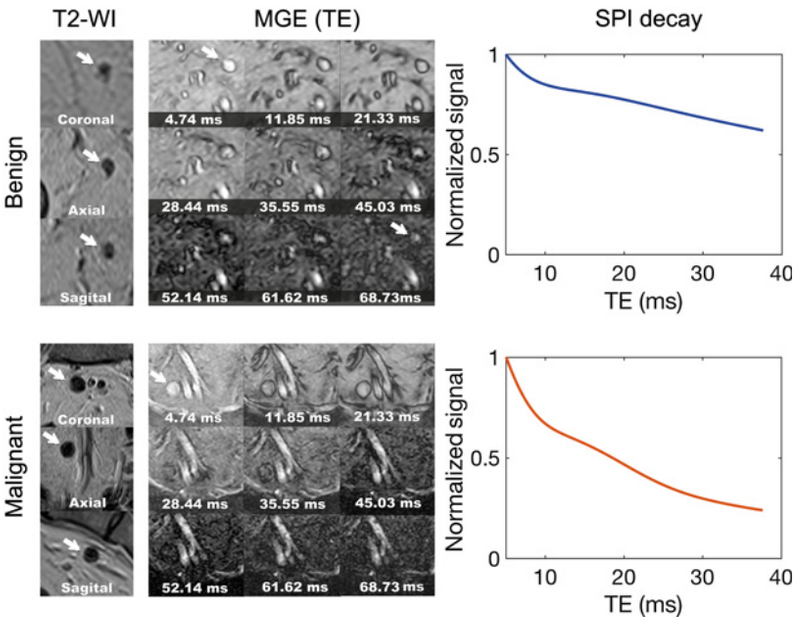

Figure 6.
Examples of a benign and malignant lymph node as depicted in multiplanar high-resolution T_2-WI and MGE clinical images with increasing TE (only 9 TEs were depicted for simplicity, but the full range of TEs is presented in Supplementary Fig. S1). A clearly faster loss of signal intensity with increasing TE is observed in the malignant lymph node. The corresponding single-slice whole-node ROI-based decay curves again show nonmonoexponential behavior.

potentially oscillating features. We then applied SPI at ultrahigh field 16.4 T MRI to perform a "virtual histology" with sufficient resolution to actually resolve infiltrated regions versus normal node tissue. Our experimental findings further corroborated our hypothesis: the SPI decays in the 64 lymph nodes dissected from the total mesorectal excision specimens of patients with rectal cancer were dramatically different in benign and malignant tissue, both with respect to the phenomenological signal decay, which was slower for normal nodes, but also quantitatively, from the ensuing extracted parameters. Interestingly, we found three different types of malignant infiltration into the lymph nodes. Patterns included clumps of adenocarcinoma cells tightly packed together, areas of desmoplastic reaction, and areas of necrosis/cystic degeneration. These patterns tend to coexist in varying proportions within and across lymph nodes but we were able to show, albeit qualitatively only, that the three different kinds of infiltration may be characterized by different SPI curves. While the prognostic impact of these histologic divergences is unknown, it may be worth exploring in the future to further assist in the decision-making framework.

To simplify the SPI analysis, we assumed that a small number of components could be fit to the data. T_2^*b (the component with longer relaxation time) evidenced the most significantly different values and was much shorter in tumor tissue, though

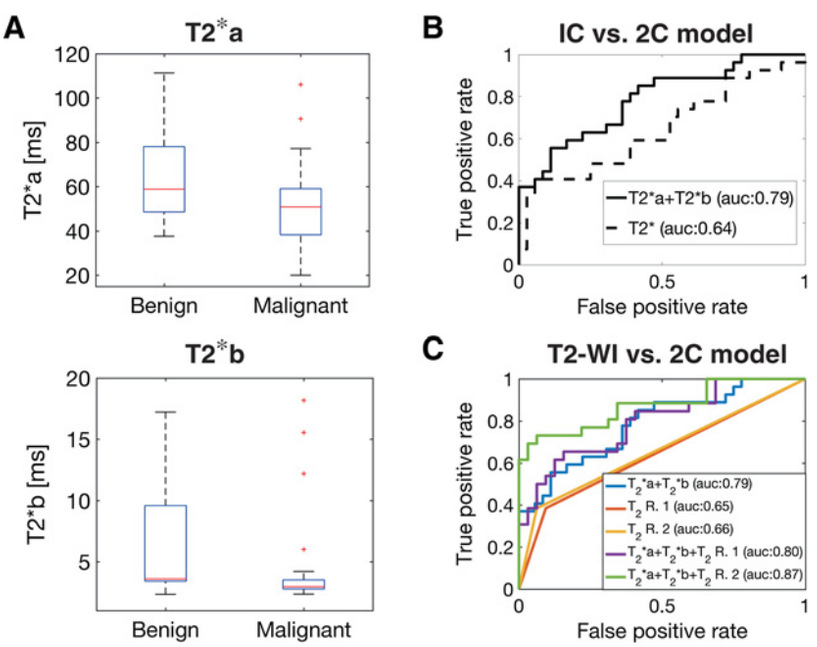

Figure 7.
A, Boxplots depict the significant differences in 2-compartment model-derived parameter values from the clinical dataset. **B,** ROC curves for monoexponential T_2^* and biexponential $T_2^*a + b$ are also depicted, in which a clearly higher AuROC is present for the bi-compartmental model assessment. **C,** ROC curves for T_2-WI and $T_2^*a + b$ SPI analysis are shown, as well as their combination. The combination of the T_2-WI assessment with the SPI analysis performs best, showing the highest AuROC.

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T_2^*a was also significantly shorter. In absolute value, the frequency shift ($\Delta\Omega$) in tumor was higher, which is in line with the shorter relaxation rates observed.

The final step in the *ex vivo* part of the study was corroborating the working hypothesis using histology. Our histology quantification data indicated that microstructural features, namely cellularity and cell size, influenced the extracted SPI parameters. Judging by Pearl coloration, which was negative in both benign and malignant nodes studied, iron particle accumulation does not appear to be the source for the observed differences in the signal decays. Importantly, the shortening of both T_2^* components observed in tumor *ex vivo* were also evidenced in malignant nodes *in vivo*. This suggests that oxygenation state, flow effects, or tissue preparation are unlikely explanations for our results. All these lend further credence to our hypothesis: the microstructure itself induces the capability of differentiating between infiltrated and benign node tissue.

It could be argued that these results, which were obtained at such high magnetic fields, would not necessarily be translatable to the lower magnetic fields existing in clinical settings. However, although it could be expected that the field distribution strongly depends on the strength of the magnetic field, it is worth noting that the MGE phase, which governs the nonmonoexponential decay observed above, goes as $\varphi_{MGE} = \gamma \Delta B \cdot TE$, which suggests that the lower ΔB can be compensated for with higher TE, and still obtain the same phase. We therefore translated the experiment to the clinical setting and scanned 63 mesorectal lymph nodes in patients with rectal cancer both with SPI and the contemporary state-of-the-art high resolution T_2-WI evaluated by two expert radiologists according to current guidelines (17). We found that, in accordance with the *ex vivo* experiment, the MGE signal is nonmonoexponential and, in some cases, also nonmonotonic, and it was best characterized by a biexponential model. We further found that in most instances, T_2^*a and T_2^*b values were significantly lower in malignant lymph nodes. The differences were somewhat less pronounced than in the *ex vivo* experiment, which may be justified by the smaller field strength and by the use of whole-node ROIs, required due to the constraints in clinical image resolution. Still, SPI parameters performed better than visual assessment by expert radiologists, with a higher sensitivity and no loss in specificity. We were therefore able to show that the simple SPI experiment provides indeed information on the microstructure also *in vivo* and adds value to current standard evaluation based on T2-WI. Even though results were slightly inferior to those reported for USPIO and Gadofosveset-enhanced MR imaging, unlike the latter, SPI is a simple, readily available, and adverse-effect–free method that can easily complement T_2-WI during staging pelvic MRI of patients. It should be noted that SPI is expected to have a better performance at 3T or even higher fields that may become more clinically relevant in the future. The separability of the curves is expected to be enhanced with increasing field, as the T_2^*s shorten and the phase shifts increase due to stronger susceptibility perturbation (25–27). Moreover, an increase in signal to noise ratio could facilitate higher resolution for characterizing smaller nodes.

This study of course also had several limitations. First, no lymph node tissue could be retrieved from very small lymph nodes (<3 mm), which biased our sample for larger nodes in both *in vivo* and *ex vivo* experiments. Second, a large heterogeneity in the signal intensity decay patterns of malignant datasets was found. However, these reflected to some extent an inherent variability in tumor-spreading patterns, which as previously stated is hypothesized to carry relevant prognostic information. Third, the numerical simulations were somewhat simplified: the two tissue types assumed ideally spherical cells and that susceptibility does not vary between substrates. In addition, when computing the MRI signal, diffusion effects were ignored, that is, the static dephasing regime was assumed (12, 13). Nevertheless, the simulated signal in this toy model showed similar trends to measured data, suggesting that differences in cellular size and packing density play an important role in the observed SPI contrast.

Finally, it is worth reflecting on the translation of these findings to other lymph nodes not necessarily originating from the mesorectum of patients with rectal cancer. They might be particularly useful for urologic cancers such as prostate and bladder, for which high-mordibity–associated lateral pelvic lymph node dissection is still performed for staging purposes or based on indirect nomogram predictive model scoring rather than strictly to patients with lymph node involvement documented noninvasively (28, 29).

Conclusion

SPI accurately characterizes lymph node tissue and improves specificity toward microstructural characterization. At least in part, the quantitative differences in the extracted parameters were explained by differences in cellularity and cell size between the tissues, as opposed to iron content or oxygenation state. Using the same methodology and without the need for exogenous contrast administration, we found concordant results *in vivo* at 1.5T. When compared with expert visual assessment based on T_2-WI, SPI increased the accuracy of lymph node characterization upon clinical staging, with a significant improvement in sensitivity and no loss in specificity. It may therefore be of added value for patient selection for neoadjuvant therapy. The results given here are a first step that bodes well for future studies aiming to generalize SPI as a lymph-node–specific biomarker.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I. Santiago, R. Theias, N. Loução, A. Beltran, N. Shemesh
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I. Santiago, J. Santinha, A. Ianus, M. J. Barata,
Writing, review, and/or revision of the manuscript: I. Santiago, J. Santinha, A. Ianus, A. Galzerano, M. J. Barata, A. Beltran, C. Matos, N. Shemesh
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Study supervision: N. Shemesh
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Others (pathology assessment): A. Galzerano
Others (macroscopy search for lymph nodes in surgical specimen and optical microscopy analysis of lymph nodes): R. Theias

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Susceptibility Perturbation MRI Maps Tumor Infiltration into Mesorectal Lymph Nodes

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