Clinical routine acquisition protocol for 3D relaxation-compensated APT and rNOE CEST-MRI of the human brain at 3T

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Purpose: The value of relaxation-compensated amide proton transfer (APT) and relayed nuclear Overhauser effect (rNOE) chemical exchange saturation transfer (CEST)-MRI has already been demonstrated in various neuro-oncological clinical applications. Recently, we translated the approach from 7T to a clinically relevant magnetic field strength of 3T. However, the overall acquisition time was still too long for a broad application in the clinical setting. The aim of this study was to establish a shorter acquisition protocol whilst maintaining the contrast behavior and reproducibility.

Methods: Ten patients with glioblastoma were examined using the previous state-of-the-art acquisition protocol at 3T. The acquired spectral data were retrospectively reduced to find the minimal amount of required information that allows obtaining the same contrast behavior. To further reduce the acquisition time, the image readout was accelerated and the pre-saturation parameters were further optimized.

Results: In total, the overall acquisition time could be reduced from 19 min to under 7 min. One key finding was that, when evaluated by the relaxation-compensated inverse metric, a contrast correction for B1-field inhomogeneities at 3T can also be achieved reliably with CEST data at only one B1 value. In contrast, a 1-point B1-correction was not sufficient for the common linear difference evaluation. The reproducibility of the new clinical routine acquisition protocol was similar to the previous state-of-the-art protocol with limits of agreement below 20%.

Conclusions: The substantial reduction in acquisition time by about 64% now allows the application of 3D relaxation-compensated APT and rNOE CEST-MRI for examinations of the human brain at 3T in clinical routine.

KEYWORDS
APT, cancer, CEST, MRI, proteins, rNOE
1 | INTRODUCTION

Chemical exchange saturation transfer (CEST) MRI is an emerging molecular imaging technique enabling the detection of biomolecules in vivo with a resolution comparable to conventional MRI. The CEST experiment consists of a frequency-selective pre-saturation, and a subsequent fast MR image readout of the water proton signal. The acquired signal in living tissue is a complex interplay of various individual contributions from different chemically exchanging protons but also from the water signal itself. The most prominent contributions in vivo are the amide proton transfer (APT) signal and the related nuclear Overhauser effect (rNOE) resonating at a frequency offset relative to the water signal $\Delta \omega$ of $\pm 3.5$ and $-3.5$ ppm, respectively. Both are mainly associated with the content of mobile proteins and peptides within cells. In addition, the APT signal also depends on pH, and the rNOE signal is also related to the average molecular size as well as the protein folding state, making them of particular interest for the diagnosis of a variety of diseases, such as cancer, stroke, or neurodegenerative diseases.

There are several different methods aiming at the extraction of the APT or rNOE signal from the background of concomitant effects. The most widespread is APT-weighted (APTw) imaging based on the asymmetry analysis $\text{MTR}_{\text{asym}}$ at $\pm 3.5$ ppm (ie, the difference between the negative and positive frequency offset) and a presaturation field strength $B_1$ of around $2 \mu T$. Although the value of APTw imaging has been demonstrated in multiple clinical studies, nonetheless, the specificity to the actual APT signal remains to be clarified. Due to the evaluation by the $\text{MTR}_{\text{asym}}$ approach, superimposing contributions from the rNOE as well as from the asymmetric semi-solid magnetization transfer (ssMT) cannot be excluded. Even though the contribution from the rNOE is to some extent suppressed for the typically used high $B_1$ of $2 \mu T$ in APTw imaging, still the influence of a strongly decreased rNOE remains unclear. Alternatively, also a separation of the APT and rNOE signal is possible using a multi-pool Lorentzian-fit analysis and the calculation of the individual contrasts by the Lorentzian difference (LD) metric. For the LD evaluation, complete spectra need to be acquired at various frequency offsets leading to a considerable increase in acquisition time. To allow a spectral separation of the individual signals also a lower $B_1$ of around $0.7 \mu T$ is required impeding a direct comparison to APTw imaging, but still giving insights into the underlying contributions that also will be labeled at higher $B_1$. In addition, CEST signals (ie, the APTw as well as the LD APT and rNOE signal) have been shown to be intrinsically diluted by spill-over effects from the direct water saturation (DS) leading to an interfering dependency on $T_1$ and $T_2$ of water. This intrinsic dependency on the water relaxation properties can be corrected by the approach of relaxation-compensated CEST-MRI. In comparison to the linear LD, the relaxation-compensated contrast $\text{MTR}_{\text{rec}}$ is calculated by the inverse difference of the individual contributions (eg, APT and rNOE). Increasing the specificity of the CEST contrast to one particular contribution is particularly promising with respect to a higher diagnostic accuracy.

Relaxation-compensated CEST-MRI of the APT and rNOE signal has already been demonstrated to provide valuable information for various neuro-oncological clinical questions. Examples are the differentiation of histologic and genetic subtypes of glioma, or the early assessment and prognostication of treatment response. Recently, we translated the approach of relaxation-compensated CEST-MRI from an ultra-high magnetic field $B_0$ of $7T$ to a clinically relevant magnetic field strength of $3T$. However, the acquisition time was still too long preventing a broad application in clinical routine. Therefore, the aim of this study was to shorten the acquisition time whilst maintaining the contrast behavior and reproducibility. The application of relaxation-compensated CEST-MRI in clinical routine would allow examining larger patient cohorts, and thus, an in-depth assessment of its clinical relevance in comparison to common approaches, such as APTw imaging.

To reduce the acquisition time, spectral CEST data of 10 brain tumor patients was retrospectively reduced, iteratively re-evaluated and checked for the minimal amount of required information that allows obtaining the same contrast behavior. In addition, the 3D image readout was shortened under the condition of preserving the field of view (FOV) and temporal signal-to-noise ratio (tSNR). Finally, also the pre-saturation parameters were further optimized. The maintenance of the contrast behavior and the reproducibility was verified in healthy volunteers.

2 | METHODS

2.1 | Subjects

Ten patients with newly diagnosed glioblastoma (World Health Organization grade IV) were examined before therapy. The 3D tumor region in each patient was segmented by selecting the area of abnormal signal intensity on T1 Gadolinium contrast-enhanced (CE) images (Figure 1A,E, magenta lines). In addition, two healthy volunteers were scanned for image readout optimization and reproducibility analysis. Examinations were approved by the local ethics committee of the Medical Faculty of the University of Heidelberg and are in accordance with the relevant guidelines and regulations. Written informed consent was received from the subjects before the examination.
2.2 CEST-MRI

Examinations were performed on a 3T whole-body MR scanner (MAGNETOM Prisma, Siemens Healthineers, Erlangen, Germany) using a 64-channel receive head/neck coil and the integrated transmit body coil. For 3D CEST-MRI, a ‘custom’-built pulse sequence with a snapshot CEST image readout was used.

2.2.1 Clinical routine acquisition protocol

The proposed clinical routine acquisition protocol is based on the recently published state-of-the-art protocol for relaxation-compensated CEST-MRI at 3T. It uses the same pulse sequence as well as the same evaluation workflow, but with refined parameters with respect to a minimal acquisition time. The new protocol comprises one CEST scan and one WASABI (simultaneous mapping of water shift and $B_1$) scan for mapping of $B_0$- and $B_1$-field inhomogeneities. For both scans, the same pulse sequence with an identical image readout but different pre-saturation parameters was used.

Pre-saturation of the CEST scan was realized by 140 Gaussian-shaped radiofrequency (RF) pulses of mean amplitude $B_1 = \text{flip angle}/(\gamma \cdot \text{length}) = 0.7 \mu T$ (ie, maximum $B_1 = 1.44 \mu T$), 20 ms length, 5 ms interpulse delay, resulting in a 80% duty cycle and a total duration of 3.5 s. Z-spectra (the normalized water proton signal as a function of $\Delta \omega$) were sampled at 47 frequency offsets in unequal steps between ±200 ppm (the complete list of frequency offsets can be found in the Supporting Information, which is available online). For normalization, one additional fully relaxed image was acquired at the beginning after a relaxation interval of 12 s.

The WASABI spectrum was prepared by one rectangular RF pulse of amplitude $B_1 = 3.7 \mu T$ and 5 ms length. The spectra were sampled at 25 frequency offsets in equal steps between

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**FIGURE 1** 3D image data (ie, 1 out of effective 12 slices) of two representative patients with newly diagnosed glioblastoma examined at 3T. The tumor area is highlighted by the magenta ROIs. A,E, T1 Gadolinium CE images acquired during clinical examination. B,F, Conventional APTw CEST-MRI based on the asymmetry analysis. C,D,G,H, Relaxation-compensated CEST-MRI of the APT and rNOE signal acquired with the state-of-the-art acquisition protocol ($B_1 = 0.7 \mu T$). I-K, ROI averages and SD for the different types of CEST contrasts illustrated above.
constructed B1 = 0.7 µT). For the B1-correction the additional
fundamental information of the original publication25). For normalization, also one
additional fully relaxed image was acquired at the beginning.

Images were acquired using a 3D spiral-centric reordered
gradient-echo acquisition (ie, snapshot CEST30) with 220 × 181 × 56 mm3 FOV, 112 × 92 × 14 matrix, 2 × 2 × 4 mm3
resolution, GRAPPA acceleration of 2, echo time (TE) = 1.99 ms, repetition time (TR) = 3.9 ms, 700 Hz/pixel acquisition bandwidth, 6° flip angle and an elongation factor of 0.5.

In a first step, all images (ie, CEST and WASABI data)
were co-registered using a rigid registration algorithm in the
Medical Imaging Interaction Toolkit (MITK).32 Subsequently,
and the relative B1 map from the WASABI scan (recon-
tinued publication of the state-of-the-art protocol25 (ie, the same
protocol of Zhou J, et al17,35: Four rectangular RF pulses
of amplitude B1 = 2µT, 200 ms length and 95% duty cycle. Z-spectra were sampled at 16 frequency offsets being ±4 (1),
±3.75 (2), ±3.5 (2), ±3.25 (2), and ±3 (1) ppm, where the
number in the parenthesis specifies the repetitions for signal
averaging. Between each offset a relaxation interval of 2 s
was included for signal recovery. For normalization one ad-
ditional fully relaxed image was acquired at the beginning
after a relaxation interval of 12 s.

For correction of B0 inhomogeneities the field map from
the WASABI scan of the state-of-the-art protocol (Section
2.2.2) was used. Likewise, all images were co-registered
using the same registration algorithm in MITK. The cor-
rected Z-spectra were used to calculate the asymmetry mag-
etization transfer ratio MTR asym = Zref − Z of the APTw
signal at +3.5 ppm.

2.2.3 | APT-w acquisition protocol

Conventional APTw imaging was performed with the same
pulse sequence and the identical image readout as for the
state-of-the-art protocol (Section 2.2.2). Pre-saturation pa-
rameters were set according to the established acquisition
protocol of Zhou J, et al17,35. Four rectangular RF pulses
of amplitude B1 = 2µT, 200 ms length and 95% duty cycle.
Z-spectra were sampled at 16 frequency offsets being ±4 (1),
±3.75 (2), ±3.5 (2), ±3.25 (2), and ±3 (1) ppm, where the
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etization transfer ratio MTR asym = Zref − Z of the APTw
signal at +3.5 ppm.

2.3 | Correlation analysis

For quantification of the induced contrast changes by the
shortening of the state-of-the-art acquisition protocol, a
correlation analysis with the local B1 field was performed
(Figures 2 and 3, Supporting Information Figures S1 and S2).
The change in contrast per relative B1 was determined by the
slope of a least squares linear regression. To enable a direct
comparison of the dependence on the local B1 field for dif-
ferent contrasts (ie, MTRrex vs. LD for the APT and rNOE
signal), the slopes were normalized by the according average
state-of-the-art contrast. Healthy gray (GM) and white (WM)
matter, as well as CE lesions were evaluated separately to
exclude systematic biases from the different types of tissue.
Regions of interest (ROIs) for GM and WM were segmented

±1.8 ppm. Between each offset a relaxation interval of 2.5 s
was included for signal recovery. For normalization, also one
additional fully relaxed image was acquired at the beginning.

Images were acquired using a 3D spiral-centric reordered
gradient-echo acquisition (ie, snapshot CEST30) with 220 × 181 × 56 mm3 FOV, 112 × 92 × 14 matrix, 2 × 2 × 4 mm3
resolution, GRAPPA acceleration of 2, echo time (TE) = 1.99 ms, repetition time (TR) = 3.9 ms, 700 Hz/pixel acquisition bandwidth, 6° flip angle and an elongation factor of 0.5.

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±3.75 (2), ±3.5 (2), ±3.25 (2), and ±3 (1) ppm, where the
number in the parenthesis specifies the repetitions for signal
averaging. Between each offset a relaxation interval of 2 s
was included for signal recovery. For normalization one ad-
ditional fully relaxed image was acquired at the beginning
after a relaxation interval of 12 s.

For correction of B0 inhomogeneities the field map from
the WASABI scan of the state-of-the-art protocol (Section
2.2.2) was used. Likewise, all images were co-registered
using the same registration algorithm in MITK. The cor-
rected Z-spectra were used to calculate the asymmetry mag-
etization transfer ratio MTR asym = Zref − Z of the APTw
signal at +3.5 ppm.

2.2.2 | State-of-the-art acquisition protocol

As a reference, the recently published state-of-the-art protocol
for relaxation-compensated CEST-MRI at 3T was used.25 For
the sake of completeness, all changes with respect to the new
clinical routine acquisition protocol (Section 2.2.1) are speci-
fied in the following; equal sequence parameters or equal eval-
uation steps are not listed again. The state-of-the-art protocol
comprises two CEST scans at different B1 and one WASABI
scan.

Pre-saturation of the two CEST scans was realized by
148 Gaussian-shaped RF pulses of mean amplitude B1 = 0.6
or 0.9 µT (ie, maximum B1 = 1.23 or 1.85µT), resulting in a
total duration of 3.7 s. Z-spectra were sampled at 57 fre-
quency offsets in unequal steps between ±250 ppm (the com-
plete list of frequency offsets can be found in the supporting
information of the original publication25). For normalization,
two additional fully-relaxed images were acquired at the be-
inning and end of each CEST scan. The WASABI spectra
were sampled at 31 frequency offsets in equal steps between
±2.0 ppm. Between each offset a relaxation interval of 3 s
was included for signal recovery.

Images were acquired with a 220 × 179 × 48 mm3 FOV,
128 × 104 × 16 matrix, 1.7 × 1.7 × 3 mm3 resolution, TE = 2.75 ms, TR = 5.5 ms, 340 Hz/pixel acquisition bandwidth
and 7° flip angle. In addition to the MTRRex contrast, also
the linear LD = Zref − Z contrast6,23 of the APT and rNOE
signal was calculated. To correct for B1 inhomogeneities,
the final CEST contrasts from the two scans at different B1
were combined by means of the two-point contrast-correction
method34 (reconstructed B1 = 0.7µT).

2.2.3 | APT-w acquisition protocol

Conventional APTw imaging was performed with the same
pulse sequence and the identical image readout as for the
state-of-the-art protocol (Section 2.2.2). Pre-saturation pa-
rameters were set according to the established acquisition
protocol of Zhou J, et al17,35. Four rectangular RF pulses
of amplitude B1 = 2µT, 200 ms length and 95% duty cycle. Z-spectra were sampled at 16 frequency offsets being ±4 (1),
±3.75 (2), ±3.5 (2), ±3.25 (2), and ±3 (1) ppm, where the
number in the parenthesis specifies the repetitions for signal
averaging. Between each offset a relaxation interval of 2 s
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the WASABI scan of the state-of-the-art protocol (Section
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2.3 | Correlation analysis

For quantification of the induced contrast changes by the
shortening of the state-of-the-art acquisition protocol, a
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(Figures 2 and 3, Supporting Information Figures S1 and S2).
The change in contrast per relative B1 was determined by the
slope of a least squares linear regression. To enable a direct
comparison of the dependence on the local B1 field for dif-
ferent contrasts (ie, MTRrex vs. LD for the APT and rNOE
signal), the slopes were normalized by the according average
state-of-the-art contrast. Healthy gray (GM) and white (WM)
matter, as well as CE lesions were evaluated separately to
exclude systematic biases from the different types of tissue.
Regions of interest (ROIs) for GM and WM were segmented
by an automated thresholding using a quantitative T1 map and the borders [1.4, 2.2] s and [0.8, 1.2] s, respectively. Calculation of the cohort mean and SD yielded the group average percentage contrast change per relative $B_1$ presented in Table 1.

2.4 | tSNR analysis

The tSNR of the image readout was calculated voxel-wise by the ratio of the mean and the SD of 10 consecutively acquired images. For signal recovery a relaxation interval of 12 s was included before each measurement. Average tSNR values in Figure 4D were determined using all voxels of the 3D images within the brain.

2.5 | Reproducibility analysis

Reproducibility of the final CEST contrasts was evaluated by a Bland-Altman analysis of two directly succeeding measurements without repositioning of the volunteer in between. All images from the two measurements were co-registered using the same registration algorithm in MITK as for the routine evaluation workflow described above (Section 2.2.1). For each Bland-Altman plot, the bias was calculated as the mean of the relative differences between the two measurements, and the limits of agreement (LOA, i.e., 95% confidence intervals) as 1.96 times the SD. For normalization, the average of the two measurements was used. In the ideal case of perfect reproducibility and no noise, the bias and LOA would be zero. The analysis was performed on a voxel-by-voxel basis.

3 | RESULTS

To demonstrate the functionality of the recently proposed state-of-the-art acquisition protocol, patients with newly diagnosed glioblastoma were examined. The relaxation-compensated $MTR_{\text{Re}}$ contrast showed the expected contrast behavior with a partially hyper-intense APT and a uniformly
A hypo-intense rNOE signal in T1-CE lesions (Figure 1, magenta ROIs) compared to normal appearing WM (NAWM). In addition, also conventional MTR asym-based CEST-MRI was performed, likewise exhibiting a hyper-intense APTw signal in tumors. Although, also substantial local differences between the MTR\textsubscript{Rex} APT and the MTR\textsubscript{sym} APTw signal were apparent (cf. Figure 1B,C and F,G). Most striking was the difference between the two signals in the edema area of...
patient 1 on the right side of the CE lesion (Figure 1A-C). In addition, an opposing contrast behavior of the \( MTR_{\text{asy}} \) APTw and the \( MTR_{\text{Re}} \) rNOE signal was found. A detailed statistical analysis will be presented in a forthcoming study, as the focus here is on the technical further development of the acquisition protocol. The overall acquisition time for the state-of-the-art protocol was \( t_{\text{acq}} = 18:49 \) min comprising two CEST scans at different \( B_1 \) (0.6 and 0.9 µT) with an acquisition time of \( t_{\text{CEST}} = 7:34 \) min each and one WASABI scan of \( t_{\text{WASABI}} = 3:41 \) min duration.

In order to check for the minimal amount of required information to obtain the aforementioned CEST contrasts, the acquired spectral CEST data were retrospectively reduced and re-evaluated. In addition to a visual comparison of the contrast differences to the state-of-the-art protocol also a quantitative analysis of the correlation with the local \( B_1 \) field was performed. First, CEST data at only one \( B_1 \) value (ie, 0.6 µT) was used for the correction of \( B_1 \)-field inhomogeneities, shortening \( t_{\text{acq}} \) by already 40% to 11:15 min (Figures 2C and 3C). Please note that, in order to allow for a direct comparison to the case of no \( B_1 \)-correction (Figures 2B and 3B), all CEST contrasts in this retrospective analysis were reconstructed at \( B_1 = 0.6 \) µT instead of the previously used \( B_1 \) of 0.7 µT (compare Section 2.2.1 and 2.2.2). In comparison to the state-of-the-art protocol, almost no differences were apparent for the \( MTR_{\text{Re}} \) contrast a correction for \( B_1 \)-inhomogeneities at 3T could also be achieved reliably with CEST data at only one \( B_1 \) value.

In a second step, the sampling of the Z-spectra was selectively reduced by about 19% from 59 to 48 frequency offsets (ie, numbers include also the reference scans for normalization), shortening \( t_{\text{CEST}} \) to 6:04 min and \( t_{\text{acq}} \) to 9:45 min (Figures 2D and 3D). The frequency offsets were successively reduced and also several different combinations of the omitted frequency offsets were tested before we committed ourselves to the presented reduced sampling. In total, four and six data points were excluded in the spectral range of selective CEST signals (ie, between \( \pm 15 \) ppm) and the ssMT, respectively. The remaining sampling points were required to achieve a reliable separation of the different contributions to the Z-spectrum by the applied four-pool Lorentzian-fit model. Nonetheless, in comparison to the state-of-the-art protocol, the reduced sampling partially led to significant differences for very low contrast values (Figures 2H and 3H), which however mainly correspond to the fluid parts of the brain (ie, cerebral spinal fluid [CSF], vessels, necrotic tissue). In contrast, in the solid parts of the brain, which are of main interest in radiology, the contrast differences were negligible. In GM, WM, and CE lesions the average contrast change per relative \( B_1 \) was very similar to the case with the original sampling and again approximately one order of magnitude lower in comparison to the case of no \( B_1 \)-correction (Table 1, light-gray rows). Likewise as for the original sampling, a \( B_1 \)-correction could not be achieved reliably for the LD metric (Supporting Information Figures S1 and S2) showing a dependence on the local \( B_1 \) field in the same order of magnitude as in the case of no \( B_1 \)-correction (Table 1, dark-gray rows).
To further shorten $t_{\text{acq}}$, the 3D image readout was optimized to minimize the readout time ($t_{\text{readout}}$) while preserving the FOV and $t_{\text{SNR}}$. An increase of the acquisition bandwidth by a factor of two allowed a significant reduction of $t_{\text{readout}}$ of approximately 27%, but at the same time led to a decrease of the $t_{\text{SNR}}$ (Figure 4A,B). This decrease was compensated by slightly increasing the voxel size and simultaneously decreasing the matrix size (Figure 4C), which shortened $t_{\text{readout}}$ by additional 19% (Figure 4D). The number of slices was decreased by two (ie, from 16 to 14), reducing the aliasing artifacts of the snapshot CEST approach in slice direction. As a result the effective number of 12 slices that could be evaluated meaningfully was preserved while saving the acquisition of two unnecessary slices. The final effective coverage in slice direction was 48 mm. The image readout optimization led to an overall further shortening of $t_{\text{acq}}$ by around 20% to 7:43 min, comprising $t_{\text{CEST}} = 4:52 \text{ min}$ and $t_{\text{WASABI}} = 2:51 \text{ min}$.

In a final step, also the signal preparation of the CEST and WASABI scan was shortened. For the CEST scan, the pre-saturation duration was slightly reduced by about 5% from 3.7 to 3.5 s, leading to a final $t_{\text{CEST}}$ of 4:38 min. The preservation of the state-state condition for application of the relaxation-compensated inverse MTR$_{\text{Rex}}$ metric, was investigated by simulations (Supporting Information Figure S3). In GM, WM, and CE lesions still more than 95% of the steady state was reached for the reduced pre-saturation duration. Only in CSF the steady-state condition was not completely fulfilled, which however, again is typically not of main focus in radiological applications. For the WASABI scan, first the sampling of the Z-spectra was reduced from 32 to 26 frequency offsets (ie, numbers include also the reference scan for normalization), and second the relaxation interval between each offset was shortened from 3 to 2.5 s. This allowed an approximately 25% faster WASABI scan with duration $t_{\text{WASABI}} = 2:08 \text{ min}$. Consequently, the final $t_{\text{acq}}$ of the fully optimized clinical routine acquisition protocol for relaxation-compensated CEST-MRI of the APT and rNOE signal was 6:46 min. Maintenance of the contrast behavior in comparison to the state-of-the-art acquisition protocol was verified in a healthy volunteer (Figures 5 and 6 upper vs bottom row) demonstrating a very similar gray-white-matter contrast. The reproducibility of both acquisition protocols was good with LOA below 20% for the rNOE (Figure 5C,F) and also APT contrast (Figure 6C,F).

4 | DISCUSSION

In this study, the state-of-the-art acquisition protocol for 3D relaxation-compensated APT and rNOE CEST-MRI of the human brain at 3T was shortened to enable its application in clinical routine. In total, the $t_{\text{acq}}$ could be reduced by around 64% from approximately 19 to under 7 min. Therefore, $t_{\text{acq}}$ is roughly in the order of the 5 min which is unofficially the measure of things to be clinically applicable. In comparison to the conventional, most popular CEST application, asymmetry-based APTw imaging at 2µT, with an average duration of around 3-5 min, the presented protocol is less than two times longer. The applicability of relaxation-compensated CEST-MRI in clinical routine now allows also the examination of large cohorts of patients, and thus, an in-depth assessment of its clinical relevance in comparison to common approaches. In this study, already substantial local differences were found between the relaxation-compensated MTR$_{\text{Rex}}$ APT contrast and conventional APTw imaging, as well as an anti-correlation of the MTR$_{\text{Rex}}$ rNOE and the APTw contrast (Figure 1). Although the APT and rNOE signal can both be assigned to originate mainly from mobile proteins and peptides, the observed opposing contrast behavior is not in contradiction to that. As they rely on different magnetization transfer pathways (ie, chemical exchange and intra-molecular spin-diffusion for the APT and rNOE, respectively), their dependency on physiological alterations is not the same. The smaller the proteins are, and thus, the less structure they have, the more amide groups are exposed to the surrounding water leading to a larger APT signal. On the other side, the larger the proteins are, the more efficient are intramolecular spin-diffusion processes, leading to a larger rNOE signal. Thus, a simultaneous increase of the APT and decrease of the rNOE signal can, for example, be explained by the presence of more small mobile proteins and peptides within cells. In addition, the much stronger pH-dependency of the APT signal, as well as the contribution of lipids to the rNOE signal further support our observation of a different APT and rNOE contrast in vivo. Insights into these differences might allow a more precise interpretation of the observed contrast changes on the molecular level and eventually a higher diagnostic accuracy of the underlying physiological cause.

The largest saving in acquisition time was gained using CEST data at only one B1-field value for the correction of B1-field inhomogeneities. In comparison to the previously used two B1-values, this enabled a shortening of $t_{\text{acq}}$ by around 40% without any serious negative impact on the contrast behavior. Interestingly, the one-point B1-correction only achieved reliable results for the relaxation-compensated inverse MTR$_{\text{Rex}}$ metric, but not for the linear LD metric. This is due to the likewise strong dependence of the DS on the local B1-field, which is already intrinsically compensated by the MTR$_{\text{Rex}}$ evaluation. For the LD metric, the counteracting B1-dependency of the DS leads to a complex behavior of the CEST contrast on B125 preventing a simple linear approximation. Across all evaluated types of tissue (ie, GM, WM, and CE lesions) an approximately five times worse residual dependency on B1-field inhomogeneities was found for the LD
in comparison to the MTRRex evaluation (ie, 49.9 ± 13.8% and 9.1 ± 7.9% average percentage change in contrast per relative B1). For common B1-field inhomogeneities across the human brain at 3T of around ±30% this leads to an average dispersion of the CEST contrasts of 30% and 6% for the LD and MTRRex, respectively. In addition to the higher specificity, the better robustness against B1-field inhomogeneities is a further compelling reason for the application of relaxation-compensation in CEST-MRI.

The contrast behavior of the new clinical routine acquisition protocol could be maintained for the APT and rNOE signal in the radiologically interesting parts of the brain (eg, brain matter and CE lesions). Significant differences in comparison to the state-of-the-art protocol occurred only in the fluid parts of the brain (ie, CSF, vessels, necrotic tissue). Here the APT and rNOE signal is particularly low leading to more instabilities in the four-pool Lorentzian-fit. Therefore, the contrast changes can be traced back to a different interpretation of the comparatively weak peaks by the fit depending on the respective sampling of the Z-spectra. The maintenance of the contrast behavior was not only checked for the retrospectively adjusted and re-evaluated data, but also for the final clinical routine protocol with all adjustments being implemented (ie, with the optimized image readout and signal preparation). Also here the APT and rNOE signal exhibited a very similar contrast behavior and equal image quality (ie, differentiability of brain structures and tSNR). The optimized image readout now resembles in several parameters the protocol suggested by Deshmene et al38 and Herz et al.39

In this study, only the relaxation-compensated MTRRex contrast was evaluated, which intrinsically compensates for spillover effects (ie, the dilution of CEST signals depending on other superimposing signals in the Z-spectrum like the DS or the ssMT).4,22,25 The apparent exchange-dependent relaxation (AREX = MTRRex/T1)22 metric, which additionally compensates for the scaling of all CEST signals by the T1 relaxation of water was not investigated. At 3T, the AREX approach seems to overcompensate the T1 relaxation40-42 leading to a similar contrast behavior of the APT and rNOE signal.25,43

Due to the substantial broadening of CEST signals at 3T in comparison to applications at ultra-high B0 ≥ 7T, there are only two broad clear dips at +3.5 and −3.5 ppm detectable in the Z-spectra.25 As they are approximately of the same spectral width, we sampled them symmetrically with the same amount of data points for each resonance. Due to the broad

FIGURE 5 Reproducibility analysis of the MTRRex rNOE contrast (B1 = 0.7 µT) acquired by the state-of-the-art protocol (A-C) and the new clinical routine acquisition protocol (D-F) in a healthy volunteer. The two scans were acquired directly after one another without a repositioning of the volunteer in between. C,F, Voxel-by-voxel Bland-Altman plot demonstrating the agreement of the two scans. In the ideal case of perfect reproducibility and no noise, all data points (blue dots) would be on a horizontal line around zero.
appearance of CEST signals at 3T, also contributions from other chemically exchanging protons (i.e., amine, guanidinium, or hydroxyl) to the APT signal cannot be excluded. Nonetheless, due to the used low $B_1$ (i.e., 0.7 $\mu$T) and the fixed evaluation at $+3.5$ ppm, it can primarily be associated with the APT. Following previous findings, the ssMT was set to be asymmetric around the DS and to resonate at $-2.5$ ppm. Although in some tissues, as for example, in tumor lesions, a symmetric ssMT was reported, the effect on the APT and rNOE signal in our multi-pool Lorentzian-fit analysis is negligible due to the comparatively broad appearance of the ssMT at 3T (i.e., full-width at half maximum of around 60 ppm). For the fitting of the ssMT also a Lorentzian line shape was assumed, which has been demonstrated to fit best to low-$B_1$ in vivo Z-spectra at 3T, in contrast to a super-Lorentzian line shape which better suits at high $B_1$.

The reproducibility of the new clinical routine protocol was good with LOA below 20%. In comparison to the previous state-of-the-art acquisition protocol the reproducibility remained of the same quality. The LOA values can be understood as a threshold to identify genuine contrast changes from the background of random fluctuations. Please note that, in this study, the LOA was determined on a voxel-wise basis taking no spatial information of the images into account. Consequently, the reproducibility for a complete ROI (e.g., tumor lesion or a specific brain area) can be assumed to be considerably better. The image quality was good enough to reliably differentiate T1-CE lesions and NAWM in brain tumor patients using the $\text{MTR}_{\text{Rex}}$ APT and rNOE contrast (Figure 1). To the best of our knowledge to date, reproducibility in CEST-MRI has only been evaluated in a few studies. In addition, these studies are using widely ranging evaluation methods and also investigating different entities, which is why a direct comparison has to be taken with caution. Only in the male prostate, relative LOA values evaluated in a complete ROI were reported ranging between 17 and 38% for the APT and rNOE signal. In comparison to these values, the reproducibility of our protocol is equal or even better.

In the future, the $t_{\text{acq}}$ could even further be reduced by replacing the WASABI scan for the quantification of field inhomogeneities by a faster technique. Instead of acquiring a $B_0$ map, the spectral position of the DS form the Lorentzian-fit could be used to compensate for $B_0$-field inhomogeneities. Furthermore, a $B_1$ map could also be acquired in less than 30 s using the DREAM (dual refocusing echo acquisition mode) approach. This would enable a $t_{\text{acq}}$ even below 5 min, even further facilitating the application of relaxation-compensated CEST-MRI in clinical
routine. In addition to neuro-oncological clinical questions, the presented acquisition protocol could also be used, for example, for examination of stroke patients or for investigation of neurodegenerative diseases, such as Alzheimer disease.

5 CONCLUSIONS

The presented clinical routine acquisition protocol allows 3D relaxation-compensated APT and rNOE CEST-MRI of the human brain at 3T within a clinically applicable acquisition time of under 7 min. The overall shortening of the protocol by about 64% was achieved by a selected reduction of the acquired spectral data, in combination with an acceleration of the 3D image readout and a further optimization of the pre-saturation parameters. In addition, we demonstrated the advantage of relaxation-compensated CEST-MRI over common approaches with respect to the correction of $B_1$-field inhomogeneities. The new clinical routine acquisition protocol allowed maintaining a very similar contrast behavior as the state-of-the-art and showed a good reproducibility. All in all, this opens the door for relaxation-compensated CEST-MRI in clinical routine.

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