Methodology matters: characterization of glioma through advanced MR imaging

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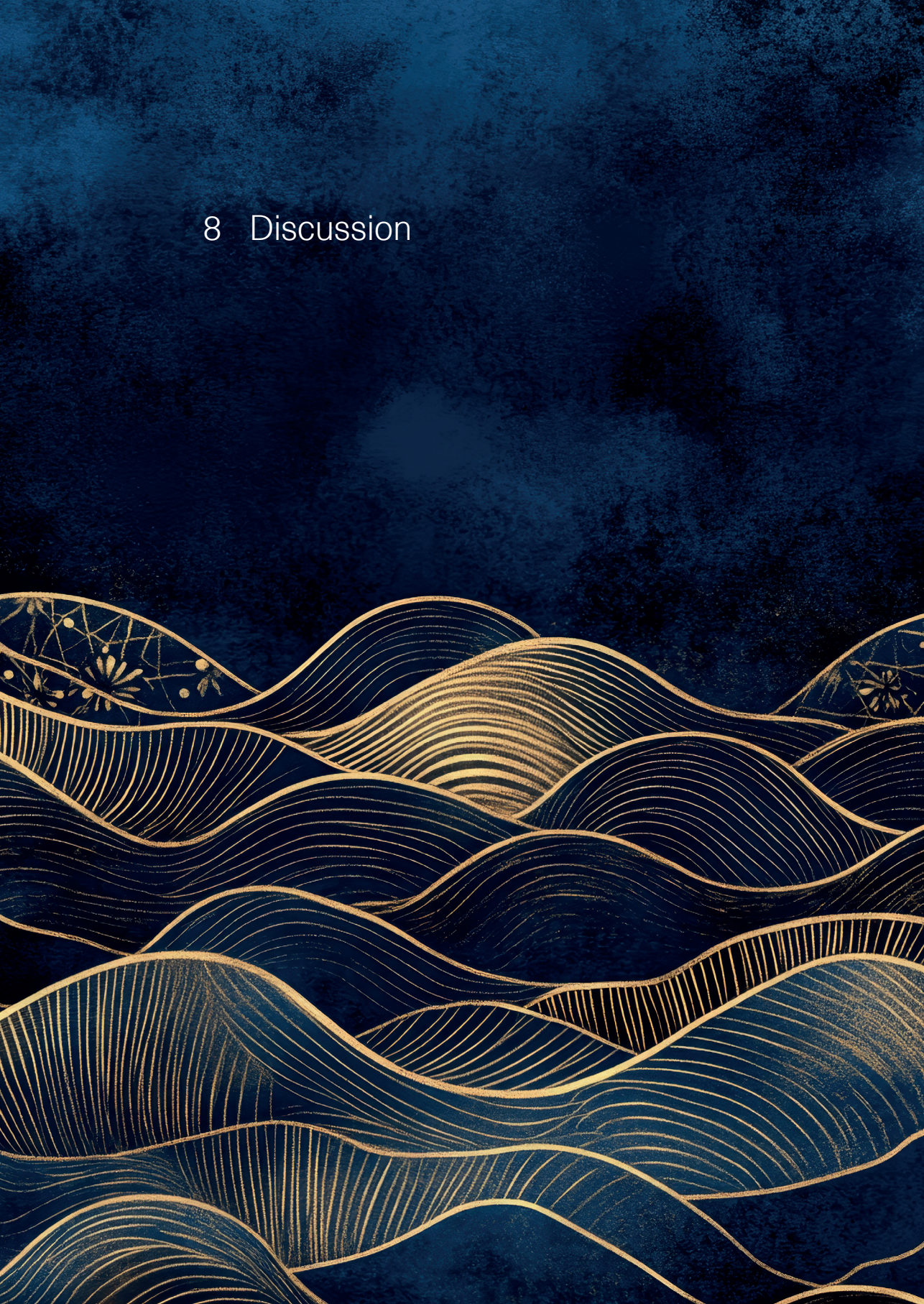
8 Discussion



In this discussion key findings from the individual chapters are addressed and contextualized within the broader body of literature. The first few chapters addressed isolation of CEST pools in the brain, the contribution of different metabolites to the CEST contrast of amines at 2 and 3 ppm in the human brain, and the 2 ppm amine 7T CEST contrast in gliomas. The two latter chapters described the added value of higher-quality ultra-high field T_2 weighted images compared to their current clinical counterpart, and the use of structural and perfusion tumor characteristics to evaluate tumor recurrence and overall survival in patients with glioblastoma.

8.1 Prospects of CEST for glioma imaging at 7T

CEST's sensitivity for detecting endogenous components such as metabolites and proteins without the need of external contrast agents, has made CEST to become an attractive non-invasive technique for *in vivo* imaging of glioma. However, the biggest challenge lies upon its relatively poor specificity, given the many competing effects resulting in CEST signal changes in complex biological tissues. A first step to maximize the benefits of CEST can be to acquire the data on an ultra-high field system. This offers significant advantages namely, improved SNR, higher spectral and spatial resolution and an enhanced CEST effect[199]. Specifically for glioma imaging, a few groups have explored APT- and NOE-CEST contrasts and studied how these imaging modalities complement existing techniques[47], as well as how it aids in tumor diagnostics and grading[42–44]. However, until a few years ago only one group had explored amines as an alternative CEST contrast for human *in vivo* examinations, aiming to identify the epileptogenic area of lower grade gliomas[45]. Given the existing pre-clinical studies[63,64,97,200], but the lack of studies in glioma patients at UHF[133], we decided to further explore this issue.

An essential step before moving to patient studies is to select the most optimal acquisition parameters to target the desired CEST pool of interest. Our initial work of Chapter 3 and 4 had therefore the primary focus on exploring different methods to optimally visualize different CEST pools of interest. Chapter 3 was inspired by previous work, where VDMP had been used as a CEST filtering technique to target the desired contrast of interest based on the exchange rate of each pool[69,74,83,201]. Our work extended the previous simulations and the *in vivo* investigations in human subjects. Our findings showed that VDMP is a robust CEST editing technique which allows to filter MTC contrast and, based on the proposed acquisition parameters, the sequence can be sensitized to either slow or fast exchanging CEST pools. It could be interesting to apply this technique in, for instance, glioma patients to understand whether APT, NOE and amine CEST contrasts can be reliably measured without the influence of MTC. On the other hand, our practical experience demonstrated that this sophisticated technique is time consuming and requires adequate post-processing knowledge. Especially because of the time constraints we had for our clinical protocol, it was not possible to include these experiments in the protocol of our patient cohort. It is known that at higher field strengths the T_1 relaxation times are longer, which brings the benefit of enhanced contrast, but unfortunately it comes also at the cost of longer acquisition times.

In Chapter 4 we focused on optimizing the amine CEST contrast in the human brain to achieve the most sensitive possible measurements within a reasonable acquisition time. This came at the compromise of a limited offset frequency range coverage (between -1500Hz and 1500Hz) with larger step sizes of 136.4 Hz. For example, a previous study has evaluated the feasibility of imaging creatine-weighted CEST in the human brain, by acquiring data for 17.5 minutes[89]. In our experience, during a multimodal MR scanning session, acquiring data for this long to yield data to image a single CEST pool, becomes unrealistic. With this in mind we managed to achieve maximum contrast sensitivity to the pool at 2 ppm with B_1rms = 2.14μT & t_{sat} = 1500ms in 03:38 minutes. Acquisition parameters were first optimized through simulations and *in vitro* experiments. By correlating our CEST results from the pools at 2 and 3 ppm with MRS quantified metabolites, we observed that glutamate had a significant contribution in both gray and white matter. Given the broad nature of the 3 ppm pool, vastly present in glutamate, we cannot entirely rule out the possibility of some contamination of the pool at 2 ppm, explaining our observations.

Our findings were viewed as controversial, particularly due to the use of the MTR asymmetry analysis method. Alternative approaches, such as AREX, which rely on Lorentzian fittings, could potentially correct for confounding factors, like T_1 and MTC[106]. However, we observed a broad line shape of the intermediate exchanging amines at 2 ppm, which has also been previously reported[123]. Our multimodal protocol included two CEST protocols. Given the limited total scan time, we chose to include larger step sizes for the sampling points and restrict the frequency offset. In the end a tradeoff between the number of data points (signal intensity as a function of frequency offset relative to water resonance) and acquisition time was made to allow CEST protocols specific to different CEST pools. Acquiring fewer data points can make it more challenging to then fit the acquired *in vivo* human brain data to Lorentzian line shapes. On the other hand, other studies investigating 2 ppm amines and creatine in the human muscle - where creatine concentrations are higher - have successfully used Lorentzian lines shapes to fit their data[118]. This could suggest that with greater metabolite concentrations enough signal becomes available that would superimpose any competing effects. It should be remembered that CEST is an indirect imaging method sensitive to protein and metabolites signal through the signal of water. The solute of interest needs to be present in sufficient concentrations to be sure we are not measuring noise or artefactual signals when applying sophisticated post-processing methods.

The results based on our acquisition parameters and scan duration, suggest that for the purpose of human brain CEST imaging, a fit free approach seems to be the most reliable. Interestingly, for the purposes of clinical application, a recent study that looked at how different quantitative metrics can be utilized for grading gliomas using the 3 ppm CEST pool, has reached similar conclusions[202]. Fitting approaches can be beneficial when imaging data produce a distinct CEST signal with sufficient quality to accurately represent the pool of interest. Otherwise, fitting becomes impractical when dealing with broad signals that do not conform to a Lorentzian shape. While more sophisticated quantification methods are being developed, their application

is currently limited to research settings. To bring CEST closer to clinical use, future research should focus not only on developing robust processing methods but also on ensuring these methods remain scientifically sound, while also practical feasible for clinical application.

In Chapter 5 we explored the clinical application of measuring the intermediate exchanging 2 ppm CEST pool in different regions in glioma patients. Our research question was whether we could measure differences in the CEST contrast of the 2 ppm CEST pool in the different tumor regions of glioma patients. Our aim was not to determine which method is most advisable but to explore their outcomes given the current lack of consensus in the field. Choosing an appropriate quantitative metric was found to be challenging, as each method has inherent limitations. We quantified the CEST effect using both Lorentzian fittings and a fit free approach, i.e. the MTR asymmetry approach. Overall our results showed significantly different 2 ppm CEST pool contrast between tumor regions, also compared to normal appearing white matter. This evidence builds upon previous findings[89,133] suggesting that this proton pool could serve as a marker for distinguishing different tumor components.

One of the prevailing hypotheses is that the CEST pool at 2 ppm contrast originates from creatine[117]. From a biological standpoint, creatine plays a critical role in cellular metabolism by providing phosphate to synthesize adenosine triphosphate (ATP) within cells during aerobic respiration[113]. Elevated creatine levels have been observed in grade 2-3 astrocytomas that later progressed, compared to cases that remained stable[143]. In fact, glioblastomas have been shown to have lower levels of creatine compared to astrocytomas[144]. Another study evaluated the different tumor lesions in more detail, and found reduced creatine levels in poorly perfused areas with high lactate in grade 4 gliomas[140]. In grade 3 gliomas areas with increased CBV were seen to also have higher creatine levels. Another study in grade 3 gliomas showed that overall creatine levels decrease compared to normal appearing white matter, except in areas of elevated metabolism, where creatine levels were higher[203]. Increased creatine concentrations could represent the initial rise in metabolic demand, such as for new blood vessel formation, which later shifts to anaerobic respiration due to extreme energy requirements, resulting in lactate accumulation[143]. This hypothesis aligns well with the physiological understanding of tumor metabolic demands. However, for CEST imaging to non-invasively reflect such processes, it is essential that the contrast correlates directly with creatine concentration.

One of the major challenges in CEST imaging is to verify the specificity of the contrast. One possible strategy is to fine-tune the acquisition parameters to maximize sensitivity to the targeted pool. For metabolite and protein protons to be specifically targeted, it is essential not only to apply the correct pulse frequency but also to use the appropriate B_1 power and saturation time. In Chapter 3, we aimed to address this challenge by implementing a CEST editing technique designed to enhance sensitivity to specific pools of interest while minimizing the competing effects of magnetization transfer. Subsequently, in Chapter 4, we attempted to correlate our CEST imaging findings with MRS metabolite measurements. This allowed us to define optimal acquisition parameters; however, we did not observe a consistent correlation

with creatine concentration in the brains of healthy subjects. It would be valuable to replicate this experiment in patients or perhaps in muscle tissue, where creatine concentrations are known to vary more widely.

Another challenge, only partially addressed in this thesis, is achieving B_1 homogeneity. Ideally, the saturation power would remain consistent throughout the field of view. To mitigate inhomogeneities, we used dielectric pads when acquiring the data for Chapters 4 and 5. Despite this, we were unable to fully counteract the observed inhomogeneities. A potential reason for this could be that the calcium-titanate in the pads may have partially dried out, and slight mechanical adjustments were necessary to fit the pads around the subject's head inside the head coil[104]. Parallel transmit or meta-materials might be better solutions, but these were not available for our studies of this thesis.

In terms of acquisition parameters, intermediate and fast-exchanging CEST pools require high total B_1 saturation power. The B_1 should ideally be between 2 and 3 μT, with a total saturation time around 1000 to 1500 ms. However, due to SAR constraints, reaching this level of total saturation power can be challenging at 7T, if not currently unfeasible, resulting in an imperfect saturation profile and increased susceptibility to contamination from competing effects.

These technical considerations highlight the complexities of CEST imaging, despite its great potential. Outlining these challenges creates opportunities for further refinement before scaling up for broader clinical applications.

8.2 Advanced and multimodal MRI for non-invasive glioma characterization

Current clinical challenges that could be addressed by advancing imaging techniques include enhanced imaging to improve delineation of tumor borders and determine the full extent of tumor spread with improved accuracy. Additionally, the timely and accurate identification of true tumor progression could improve prognostic assessments and consequently allow timely treatment adjustments.

In Chapter 6, we addressed the first challenge by aiming to more accurately identify the volume and shape of non-enhancing tumors using high-resolution 7T imaging. Our results showed that high-quality 7T T_2-weighted images significantly improved visualization of T_2 hyperintense lesions extent and shape compared to current clinical T_2-weighted images acquired at 1.5 or 3T. Tissue boundaries were more clearly defined, and involvement of key brain structures, such as the corpus callosum, was more discernible. A particularly striking example was the visualization of optical tract involvement of the tumor, which was unclear in clinical images but clearly visible on the high-quality 7T scans.

The advantages we identified align with previous studies, which, for example, demonstrated improved identification of potential organs at risk using 7T T_2-FLAIR images compared to their clinical counterpart. This could make the delineation of target volume for radiotherapy planning more precise potentially sparing healthy tissue from radiation[37]. Also for the purpose of neurosurgery the superior tissue contrast and enhanced vessel visualization has shown to be appealing[204]. These results, and the ones from our work illustrate the potential that ultra-high field imaging has in the realm of structural imaging. Given its increase in signal to noise ratio (SNR) and improved contrast, due to a stronger net magnetic moment and longer T_1 and T_2 relaxation times of tissues, an improvement in tissue characterization is possible[157].

On the other hand, susceptibility to distortions and motion artifacts are typically more pronounced for ultra-high field imaging than at lower magnetic field strength MRI. In our study, we observed signal loss near the skull base and the center of the brain, which can make evaluating tumors in these regions more challenging. Another challenge of ultra-high field 7T imaging has been observed during image guided neurosurgery. In structural imaging, like magnetization-prepared T_1-weighted images, extracranial shifts (i.e. irregularities appearing outside the skull, deriving from motion, implants, field inhomogeneities, susceptibility artefacts) have been found to introduce a certain degree of unreliability in localization of fiducial markers. The proposed solution involved fusing the 7T images with the clinical ones in the surgical image guidance system[205]. This approach is suggested as a temporary measure until the issue of extracranial shifts is fully resolved. This can serve as an interesting example of how clinical images can complement the high-quality images of 7T, while ultra-high field systems still having certain technical challenges. Translating this cooperative method to our work, the use of high-quality 7T images for tumor assessment could prove to be valuable in ambiguous cases where the extent of the tumor is unclear on clinical MR images. Conversely, in areas affected by distortion or signal loss in 7T imaging, clinical images should be leading in radiological assessment. This

interplay between clinical and ultra-high field images highlights their complementary roles in overcoming current limitations and perhaps making it more feasible to be introduced in clinical practice.

One of the biggest challenges in glioma imaging is the differentiation between true tumor progression and pseudo-progression. Unfortunately, total resection of glioblastomas is practically impossible due to their infiltrative growth, and despite adjuvant therapies, glioblastomas often quickly recur. Ideally clinicians would be able to visualize true progression in a timely manner, but often times this is not clear from conventional MR imaging, or even when more advanced modalities like perfusion imaging are added to the assessment. Gliomas are biologically heterogeneous, having different physiological processes occurring simultaneously (eg. neoangiogenesis, altered lipid metabolism, etc.)[206]. Combining different imaging data can make it interesting to understand if certain tumor characteristics are present (or absent) in tumors with similar (or different) outcomes. In Chapter 7 we explored a clustering approach to understand if perfusion imaging characteristics together with structural and enhancing pattern features, could group patients with glioblastoma and whether these groups would be predictive of progression and overall survival. Our results showed that this grouping did not differentiate patients with true from those with pseudo-progression, but the groups did exhibit different overall survival times. Our data set was limited to the images retrospectively acquired in the clinical setting and did not include other imaging modalities, such as diffusion weighted images and magnetic resonance spectroscopy. Since glioblastomas are biologically heterogeneous, it could have been interesting to have added radiological characteristics from such other MR images to the model for an even broader representation of tumor characteristics. Also, other imaging modalities such as positron emission tomography (PET) could have been interesting to add to the model as they can account for metabolic activity, which could help to differentiate between active tissue, necrosis and non-tumorous inflammation[207]. Moreover, the method we used included visual scoring which is time consuming and labor intensive. The idea behind visually scoring these images was based on including the expert opinions of radiologists when assessing these images in the clinic. Translating such expert knowledge into automatic, quantifiable features is not easily done. Currently, many studies have focused on machine learning alternatives as less labor intensive methods, where oftentimes tumor genetics' profile and high-order imaging characteristics (eg. gray-level texture) are combined. However, the downside of this approach is that the way in which the algorithms combine this information is not always clear and, importantly, do not represent how radiologists currently assess MR images. The use of machine learning, and in the broader sense, artificial intelligence, for medical purposes poses a very interesting possibility given the rise in medical data and labor time and costs. Perhaps the use of such technology is more suited for applications that are performed more frequently than glioma imaging and have very well-defined specific imaging characteristics, in this way benefiting from more data available. For example, using machine learning to detect very well defined lung cancer nodules on CT is probably more likely to benefit from machine learning applications than the reporting on glioblastoma. However, implementing these tools for clinical decision-making or even prediction of disease outcome

seems, at this day and time, still far away.

8.3 Concluding remarks

The overarching aims of this thesis were to investigate the potential of advanced methods for imaging gliomas, with a particular focus on UHF imaging and CEST.

CEST is a promising non-invasive technique for glioma imaging, benefiting from higher spectral resolution provided by UHF. However, despite the advantage of UHF providing higher spectral resolution, magnetic field inhomogeneities currently hinder CEST's sensitivity and imaging reliability. Another challenge lies in mitigating competing effects, particularly the MTC. The VDMP results demonstrated how this technique can be used to resolve this challenge, specifically by isolating specific CEST effects. Nevertheless, the major limitation of VDMP remains its long acquisition times.

Regarding CEST quantification techniques, there is currently a lack of consensus on the most optimal method. Depending on the data acquired, its acquisition parameters, and the targeted CEST pool of interest, using different quantification techniques will be most optimal.

When investigating and optimizing the acquisition protocol for amines CEST we found that glutamate seems to also contribute to the pool at 2 ppm. Moreover, we showed how the 2 ppm CEST pool contrast significantly differs between tumor lesions.

This thesis has also shown how UHF could be of value for treatment planning including delineating glioma's boundaries and evaluating the presence of infiltration of critical brain structures, given its improved spatial resolution and contrast.

Lastly, this work investigated a clustering analysis model that utilized anatomical and perfusion radiological characteristics to identify MRI phenotypes in glioblastoma patients. The MRI phenotypes were shown to have different overall survival outcomes. However, the underlying technique of manually annotating images to perform clustering analysis can be very time consuming, even though it still only included limited radiological features. These efforts could be expanded and maybe automized to cover the complex nature of glioblastoma more thoroughly.

In sum, this thesis explored several advanced techniques to improve the characterization of gliomas, which might help local treatment planning, differentiate glioma components, and identify sub-groups of patients with glioblastoma with a distinct overall survival. The clinical use of these new techniques not only needs much greater validation which goes beyond the pilot studies conducted during this thesis, but also a careful consideration of practical aspects such as standardization, accessibility and costs before they should be introduced in clinical practice.