Methodology matters: characterization of glioma through advanced MR imaging

Schmitz Abecassis, B.

2 Chemical Exchange Saturation Transfer for Preoperative Glioma Characterization

Bárbara Schmitz-Abecassis
Ece Ercan
Mina Kim
Laura Mancini

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2.1 Overview

CEST imaging enables the acquisition of information from proteins, peptides, and small molecules, which are not detectable with conventional MRI due to their low concentration in tissue. Specifically, CEST selectively saturates the magnetization of solute molecules with exchangeable protons that resonate at a frequency different from water[33]. This saturation results in a decrease in water magnetization, creating a new contrast associated with the solute pool[48]. By exploiting the chemical exchange of exchangeable protons, CEST obtains indirect high-resolution images from the solute pool[49]. In a typical CEST sequence, a saturation period is followed by data acquisition[48], and the whole module is repeated while varying the saturation frequencies. Results are usually shown using a Z-spectrum, which presents the measured normalized water intensity as a function of saturation frequency[48].

Amide Proton Transfer (APT)-CEST imaging is the most studied CEST technique and refers to effects observed around 3.5 ppm downfield from water[33]. APT-CEST is attributed to the slow-exchanging amides in proteins and correlates strongly with pH[33]. The nuclear Overhauser effect (NOE) is another CEST effect that arises from mobile macromolecules, observed at around -3.5ppm[49].

Amine protons at 2 and 3 ppm from water that exchange at intermediate and fast rates, respectively, are found in important molecules, such as creatine, glutamate, and proteins. The detection of these exchanging pools has potential practical applications in the brain (tumors and associated epilepsy), muscle, and heart, motivating the development of appropriate CEST methods[50].

Glucose CEST (glucoCEST) relies on the injection of exogenous D-glucose to study tissue perfusion parameters, such as blood volume, blood–brain barrier (BBB) permeability, as well as tumor malignancy, without the need for a GBCA injection. This method provides more reliable results at 7T than at 3T[51].

Isolating CEST contrast *in vivo* while controlling for multiple confounding effects requires advanced post-processing. A range of techniques is available, resulting in several potential metrics with which to describe the CEST effect. Asymmetry analysis (MTRasym) is an inherently simple approach, and its efficiency and ease of use have made this method popular in patient studies. However, different methods have been developed in response to the challenges encountered with MTRasym. These challenges include a macromolecular contribution due to the asymmetry of magnetization transfer effects and the contribution of NOE effects. Although a detailed description of these methods is beyond the scope of this paper, it is important to mention the most promising ones: water saturation shift referencing (WASSR); the three-offset method (APT*); MTRREX; the apparent relaxation due to exchange (AREX); the apparent APT ratio (APTR*); and Z-spectrum modulation as a combination of direct water saturation and solute pools of interest[49].

2.2 Clinical application

Because of its ability to reflect molecular changes, APT-CEST is used to study tumor microenvironment and metabolism *in vivo*[49], as demonstrated in Figure 1.

Cancer cells often exhibit structural, physiologic, and molecular changes and have an altered metabolic profile compared to healthy cells. Especially in high-grade gliomas, the level of peptides and mobile proteins is substantially increased compared to surrounding tissue[52]. An elevated protein content entails increased chemical exchange between the solute and bulk water. A good correlation has been demonstrated between endogenous protein profiles and APT-weighted signals in gliomas[53]. Studies that have assessed APT-CEST have shown a sensitivity to differentiate tumor grades, with increased contrast in higher grades, and the ability to detect tumor aggressiveness[54]. However, different studies have also shown that suppressing NOE contrast, often decreased in glioma compared to healthy-appearing brain tissue, allowed more reliable characterization of the enhancing lesions of glioblastomas and differentiation between glioma grades, considering the IDH mutations and MGMT methylation status[55]. Investigating CEST contrast in relation to molecular and genetic markers is in line with the most recent 2021 WHO classification[10].

The potential usefulness of APT-CEST for presurgical applications relies, in particular, on early detection and, consequently, propagation of more targeted treatment strategies, especially in the group of patients who do not show typical contrast enhancement on conventional T_1-weighted imaging, although they harbor HGGs[56]. Recent work by Warnert et al. aimed to use APT contrast to image non-enhancing gliomas and to more accurately distinguish tumorous from healthy tissue, based on tumor heterogeneity[57]. Heterogeneous APT-CEST contrast was detected within these tumors, with a greater effect size of APT-CEST[57]. Understanding the cause for the intra-tumoral contrast differences could include retrieving biopsies from APT-hyperintense lesions to correlate with histopathological observations and improve overall diagnosis[58].

Given the popularity and large body of work performed around this technique, recently published work has attempted to homogenize the application of APT-CEST in available clinical systems[59].

2.3 GlucoCEST

Since tumor cells utilize a glycolytic metabolic pathway, there will be an increase in glucose consumption. As such, glucoCEST imaging has been suggested to depict the saturation exchange between glucose-hydroxyl protons and water between 1.2 and 3 ppm[51]. Recent studies in glioma patients showed that the glucoCEST signal from dynamic glucose injection may reflect local blood flow, vascular permeability, and volume of the extracellular space, somewhat similar to what DCE T_1-weighted MR does although the correlation between DCE and dynamic glucoCEST cannot be fully understood at the moment[60].

2.4 Amine CEST

Together with the glycolytic metabolism, the hypoxic microenvironment that is considered one of the major driving forces of tumorigenesis leads to intra- and extracellular acidosis in solid tumors, and these intracellular pH changes (pHi) may be evaluated using amine-CEST[61]. In addition, it has also been shown that increased levels of amine protons can be detected in regions of an active tumor where mobile glutamine (Gln) and other neutral amino acids are a major source of fuel for malignant tumors, and transport systems are often amplified to increase Gln consumption[62].

Specifically, the amine CEST contrast at 2 ppm has been shown to correlate with Cr distribution in brain tumors, which is an essential metabolite in the process of converting adenosine diphosphate (ADP) to adenosine triphosphate (ATP)[63]. A decrease in Cr CEST contrast was correlated with increased aggressiveness, and significant differences between the tumor and healthy brain regions have been observed, which most probably reflects the abnormal metabolism of gliomas in different malignancy states[64].

It has also been suggested that the amine and amide concentration-independent detection (AACID) signal from the ratio of the CEST effects generated by amide and amine protons from endogenous tissue proteins may be used to evaluate intracellular pH changes (pHi) in stroke[61].

Moreover, the amines of glutamate (Glu) resonating at around 3 ppm have been shown to also play a role in CEST contrasts of gliomas. Neal et al. have shown that an increase in Glu concentration in the peritumoral area of diffuse gliomas is a result of altered Glu homeostasis[45]. Altered Glu concentrations were associated with higher glioma aggressiveness, described by the enhancement on contrast-enhanced scans[45].

2.5 Validation

CEST, including APT, has not yet been widely implemented in clinical settings for glioma imaging. However, in a recent consensus publication, updated implementation guidelines have been defined. There has also been an effort from the industry to develop a clinical sequence, which has resulted in a commercially available APT-CEST product for clinical use. Yet, cross-vendor reproducibility has not been widely investigated. Most studies have, so far, focused on technical validation and, to some extent, have included clinical validation; however, a sizable multi-site comparison is still missing. Another challenge includes the lack of standardized diagnostic cut-off criteria, which would be essential for wide clinical use. Last, implementation, including data analysis and post-processing, would require special training and expertise. Once these translational challenges are tackled, CEST could be an interesting technique to adopt in glioma imaging.

2.6 Summary

In conclusion, CEST has shown potential as a novel technique that can provide unique endogenous contrast. APT-CEST yields the most promising results, evidenced by its popularity and high research output. Other CEST-based contrasts that derive from amine and glucose still need to demonstrate their value in larger cohorts. Overall, CEST is still in need of multi-site, multi-vendor clinical validation before it can be adopted for widespread glioma imaging in clinical practice.

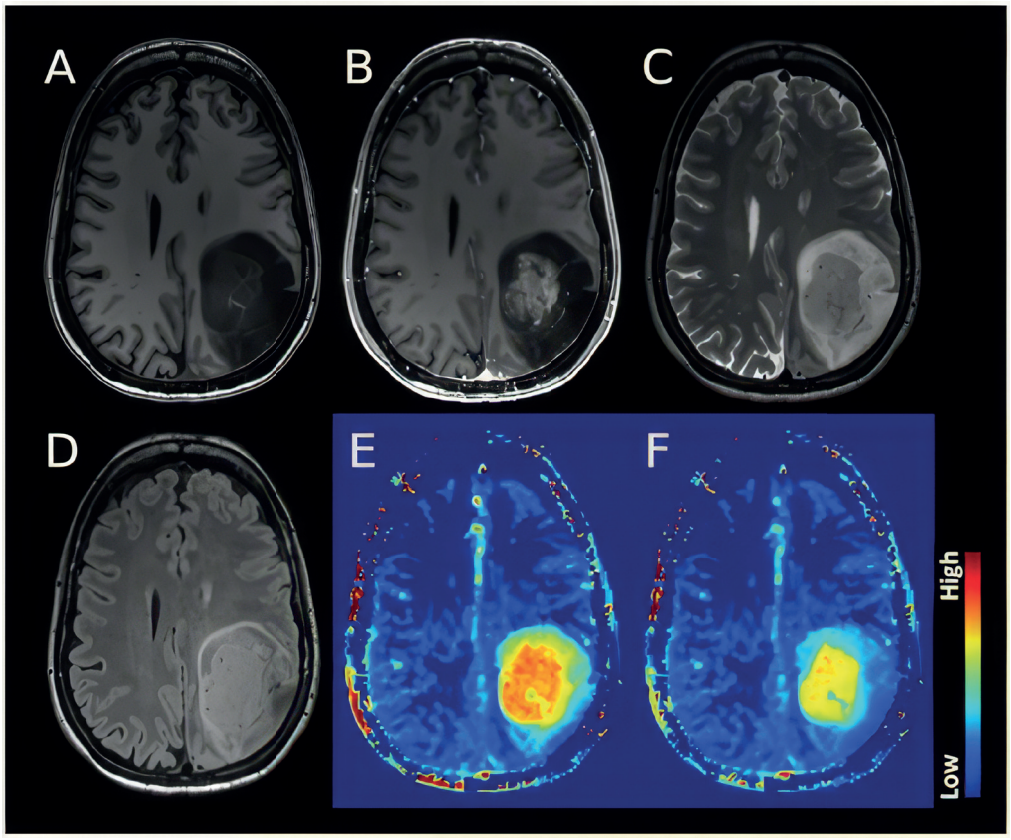

Figure 1. Example of an astrocytoma, IDH-mutant, 1p/19q retained, CNS WHO grade 4. The structural images (A: T_1w, B: T_1w post-Gd, C: T_2w, D: FLAIR) demonstrate a heterogeneous lesion with a rather solid central and well-enhancing part and a peripheral compartment demonstrating some T_2/FLAIR mismatch without overt enhancement. The APT-weighted maps (E: standard APT CEST, F: fluid-suppressed APT CEST (Casagranda S et al. ISMRM 29th An Meet 2021)) show significantly elevated signal in the enhancing tumor, suggesting clearly high-grade features. Notably, the rim zone of the lesion shows variable degrees of APTw signal elevation in the fluid-suppressed images, thus suggesting that this compartment features mixed solid and cystic parts. Interestingly, the anterior rim zone, along with a halo surrounding the enhancing area, demonstrate a mildly elevated APTw signal that indicates likely high-grade metabolic tumor characteristics. The data were acquired on a Siemens 3T Prisma scanner. APTw protocol included DC=91%, B_1rms=2uT, Tsat=2s, and WASAB1 for B_0 correction. WASAB1 and APTw data were processed in Olea Sphere 3.0 software (Olea Medical, La Ciotat, France).