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Magnetic resonance spectroscopy in pseudoprogression of brain tumours: A review

ARTIGO DE REVISÃO

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Abstract

Magnetic Resonance Imaging (MRI) has an important role in diagnosis and follow-up of brain tumours. MRI is based on the interaction between an external magnetic field and atoms with odd atomic mass number. In Magnetic Resonance Spectroscopy (MRS), a modality of MRI, spectra of metabolites of tissues are obtained. In brain tumours, the spectra present elevation of choline, low N-acetyl-aspartate and low creatine. When a patient is diagnosed with a brain tumour, the standard treatment is surgical resection, as possible, radiotherapy and chemotherapy. During follow-up, scans can show features of tumour recurrence without true tumour progression. Such changes resembling true tumour recurrence are referred as pseudoprogression. Criteria have been developed to assess treatment response based on MRI scans. In some cases, standard MRI cannot differentiate adequately tumour recurrence from pseudoprogression. Magnetic resonance spectroscopy shows high sensitivity and specificity in the diagnosis of recurrence or pseudoprogression, but imaging protocols are not established, it is technically demanding and there are no established metabolite ratios. Magnetic resonance spectroscopy can be a useful technique in neuro-oncology, especially in association with other advanced MRI techniques once imaging protocols are established.

Key-words: Magnetic Resonance Spectroscopy, MRS, Magnetic Resonance Imaging, Pseudoprogression, Brain Tumours

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Abbreviations

ADC	Apparent diffusion coefficient
Cho	Choline
CNS	Central Nervous system
Cr	Creatine
CT	Computerized Tomography
DSC	Dynamic Susceptibility Contrast
FID	Free Induction Decay
FLAIR	Fluid-Attenuated Inversion Recovery
IDH	Isocitrate Dehydrogenase
MGMT	O (6)-methylguanine-DNA methyltransferase
MRI	Magnetic Resonance Imaging
MRS	Magnetic resonance spectroscopy
NAA	N-acetyl aspartate
NOS	Not Otherwise Specified
PET	Positron Emission Tomography
PRESS	Point Resolved Spectroscopy
PSF	Point Spread Function
R-2-HG	R-2-hydroxyglutarate
RANO	Response Assessment in Neuro-Oncology
RECIST	Response Evaluation Criteria in Solid Tumours
RF	Radiofrequency
SNR	Signal-to-noise Ratio
SPECT	Single Photon Emission Computed Tomography
STEAM	Stimulated Echo Acquisition Mode
SVS	Single Voxel Spectroscopy
TE	Echo Time
TI	Time of Inversion
TMZ	Temozolomide
TR	Repetition Time
WHO	World Health Organization

Introduction

Pseudoprogression is an imaging phenomenon identified in patients with a brain tumour submitted to treatment.

There is not an established definition for pseudoprogression. It is radiologically described as new or enlarged contrast enhancing areas after therapy without true tumour growth (1). It is more challenging to standardize a clinical definition of pseudoprogression since the clinical presentation varies from patient to patient (1). Incidence rates among studies may vary from 9 to 30% (1).

The pathophysiology of this process is not fully understood, although it may arise from increased vascular permeability derived from on-going cytotoxic treatment (2), resulting in radiological alterations.

Identification of pseudoprogression may be difficult, since it may be interpreted as true disease progression and vice-versa. In magnetic resonance imaging (MRI) with gadolinium, both entities may show similar alterations, such as increased contrast enhancement and mass effect (3).

In order to evaluate tumour response to therapy, clinical and imaging criteria were introduced, such as MacDonald criteria (in 1990), for patients diagnosed with gliomas (4). In 2010, Response Assessment in Neuro-Oncology (RANO) criteria were published, seeking an improvement to the previous criteria (4,5).

Imaging in this context is quite helpful in accessing response to treatment. Several modalities are available such as Computerized Tomography (CT), Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) and MRI techniques. The latter may be divided in contrast patterns, perfusion (dynamic susceptibility contrast and dynamic contrast enhanced), diffusion weighted imaging and spectroscopy (3) and it is based on specific atoms' behaviour when submitted to an external magnetic field.

Magnetic resonance spectroscopy is based on the same principle of the magnetic resonance, but it takes advantage of the fact that the signal emitted by a certain atom is influenced by its chemical environment (6). Thus, it is possible to obtain spectra of specific metabolites and its quantification (in an absolute or relative way) and compare them between brain tissue and several pathological entities, namely brain tumours.

This review aims to go through the biophysical basis of the magnetic resonance imaging and spectroscopy and the role of magnetic resonance spectroscopy in the diagnosis of pseudoprogression, and to explore its advantages and disadvantages in the clinical practice.

Methods

The search for this review was conducted on PubMed and on TRIP database, with the searching terms of “MRS” and “Pseudoprogression”, “Spectroscopy” and “Pseudoprogression”, “Magnetic resonance spectroscopy” and “Pseudoprogression”. The search was limited to the last 10 years (2009 to 2019) and it was conducted until 31st of December, 2019.

The search resulted in 113 papers. The 113 papers were analysed and selected by the following criteria: *in vivo* studies, magnetic field strength from 1, 5 to 3 T and adult age.

A total of 24 articles fulfilled the previous criteria, 16 revision papers, 3 meta-analysis papers and 5 research papers.

Nuclear Magnetic Resonance and Magnetic Resonance Imaging

Nuclear magnetic resonance is based on the interaction of certain nuclei and an external magnetic field which results in emission of energy. The energy emitted can be captured by a specific equipment to obtain an image (7).

This phenomenon was first described in 1946 by Bloch, Purcell (8) and colleagues, when it was found that hydrogen nuclei resonate (7,9).

Physics and spin

Nuclei with odd atomic mass number, such as hydrogen-1, carbon-13, sodium-23, and phosphorus-31, have a magnetic momentum. The magnetic resonance imaging systems used in clinical practice establish hydrogen as a source of resonance since it is the most abundant atom in nature, its signal is easy to detect because of its large magnetic momentum and it is part of the water molecule, which is the largest constituent of the human body (9).

MRI systems are configured to hydrogen, and since this atom is made of a proton, the nuclei, and a peripheral electron, the proton will be used as the signal font. Although the hydrogen atom has an electron with a spin, this does not contribute to the magnetic resonance signal (10).

Subatomic particles and molecules have spin, a quantic mechanic property. Nuclei have the property of nuclear spin, since they can spin around of its axis (11).

Angular momentum of nuclear spin is a vectorial property equivalent to linear momentum but with associated rotation. It is described by the equation:

$$\vec{L} = \vec{r} * \vec{p},$$

where $\vec{L}$ is the angular moment of the proton, $\vec{r}$ the vector of the proton and $\vec{p}$ the linear momentum (10). The angular momentum can be altered by an external torque, but in terms of quantity it stays constant for each particle and it is a conservative quantity (9).

There is also a magnetic moment associated to charged particles moving, making the proton, a positive charged particle, behave like a magnetic dipole, creating a magnetic field in its surrounding (7). Without any external magnetic field, protons are distributed randomly, making the net magnetization zero. On the contrary, when subjected to a strong external magnetic field, the protons align with it (10).

The alignment of the protons generates a polarization, i.e. the net magnetization is not zero. Protons rotation can be parallel to the field or anti-parallel, being the first the lower sate of energy and consequently, the preferred state (10). Not all protons align, because

polarization competes with thermal energy of the system. For a magnetic field of 1.5T (Tesla), 10 out of 1 million nuclei are polarized and only the protons that align create the signal able to create an image. With stronger fields polarization is also more significant and better signals are obtained (9).

The external field tries to torque the protons in line, but since they have an angular momentum, they move around the direction of the magnetic field imposed. This movement is called precession and the frequency of the movement can be described by the Larmor equation:

$$\omega_0 = \gamma_0 * \beta_0 ,$$

where ω_0 is the Larmor frequency, γ_0 is the gyromagnetic ratio (or intrinsic magnetic moment, constant for each atomic particle, being 42.58 MHz/T for protons) and β_0 the external magnetic field (7,9,10).

The Larmor frequency corresponds to the radiofrequency pulse that must be applied after the protons are aligned with the first magnetic field so that the net magnetization of the protons change. This excitation and consequent emission of energy will be captured as a signal, which will be coded in order to obtain an image (12).

Hardware of MRI system

The MRI system is constituted by a superconducting magnet, a series of coils including a radiofrequency, a receiver and a gradient coil and a computer system able to reconstruct an image (10).

The superconducting magnet creates a homogenous and static magnetic field, B_0, usually of 1.0 to 1.5 T (Tesla), but systems with higher magnetic field strengths are also possible. The magnets require a cooling system, such as liquid helium (11).

Besides the coils needed to correct irregularities of the field, other types are required. The radiofrequency (RF) coil is needed to create pulses of a second magnetic field, at the Larmor frequency for hydrogen and for the magnetic field used. The receiver coil, that usually is the same coil that emits the radiofrequency pulses, receives the time varying magnetic field created by the precessing protons, which in turn is translated into an electric current according to Faraday's law. The gradient coils create linear magnetic field which vary spatially, overlapping the main magnetic field making possible the localization of the signal (7).

Finally, the computerized system reconstructs, analyses and quantifies the image obtained.

The acquisition system must be isolated since the signal created by the precessing protons aligned with the magnetic field are approximately 10^{-12} W and there is a fair amount of interfering devices that may alter the signal and subsequently the image (9). Also, magnetic objects must be kept out of such environment, once they will be magnetically pulled into this system, gain acceleration and provoke damage to this system and to the person scanned. These characteristics oblige that magnetic medical devices, such as some pacemakers or metallic implants are contraindications for MRI scans (11).

Acquisition of the MRI scan

When a sample is placed in the system for scanning, it is emerged in a main magnetic field, B_0, generated by the magnet. Once the magnetic field M_0 is generated, the human body becomes magnetized (9). The protons align with the field and the Z axis is defined, along the direction of the longitudinal axis of the human body. In this case, a net magnetization, M_z, is obtained, constituted by the protons that are parallel to the field (10). They precess around B_0, distributed all around the axis, i.e. in a 360º angle (9).

After this, the RF coil creates another magnetic field, B_1, with the frequency given by the Larmor equation, changing the net magnetization. This second field is brief, and it is named a pulse (9). This pulse will make the spins precess away from the Z axis direction to the XY plane, and this is called excitation.

The RF pulse must be applied in a different direction of the Z axis, for example perpendicular, i.e. at a 90º, to create a change in the magnetization (10). Consequently, the angle of the angular momentum will change accordingly to the angle of the RF pulse administration. This is the flip angle, and it depends on the duration and amplitude of the pulse emitted by the RF coil (9). With higher flip angles and the more perpendicular the angle is to the main field, the magnetic influx trough the receiving coil will be higher too.

With this pulse, longitudinal magnetization will reduce, and transverse magnetization, M_{xy}, will occur. The protons will be in the XY plane, and in the outset of this transverse magnetization, they will be coherently aligned parallel to the RF pulse, all in one side of the Z axis, meaning they will not be distributed in a 360º angle like the previous situation. This is named phase coherence. The precession occurring in the transverse direction of the main magnetic field will, after transformation, create a voltage, named MR signal or echo, that can be captured by the receiver coils and measured (10).

When the RF pulse ends, coherence is lost and the protons separate, but remain in the transverse plane. This is called the spin-spin relaxation or T2 relaxation. This is due to the interaction of protons with their respective magnetic field, which interferes with the precession

speed. As a result, protons precess with different velocities. With this dephasing, the MR signal will decrease until it ends. T2 is a time constant that reflects the time needed for the tissue to dissipate 63% of its magnetization or its coherence in the XY plane (13).

T2* is a time constant related to T2. T2 is related with the interaction between the spins of adjacent precessing protons, and T2* is related to that interaction and also the irregularities of the magnetic field that are patient dependent, such as interfaces between air and tissue (7). Dephasing in with T2* will occur faster (10).

After some time without the influence of the RF pulse, transverse magnetization will be lost too, and protons will return to their first position, parallel to B_0. This results in loss of energy which will be read out as a signal (10). This is called the spin-lattice relaxation, or T1 relaxation. T1 is also a time constant, defined as the time necessary for 63% of transverse magnetization to be obtained (13).

T1 and T2 constants differ from different tissues, making these properties a source a contrast. The T1 and T2 relaxations occur at the same time but they are independent and T2 is much shorter than T1 (9).

These two time constants will provide T1-weighted images and T2-weighted images. However, the weighted imagens don't depend only on these two time constants, but also on TR – repetition time, and TE – echo time.

TR is the time between sequential excitations, i.e., the time the scanner waits until it creates another RF pulse and TE is the time the scanner waits after the RF pulse is emitted to detect the signal. For that reason, TR is related to T1 and TE is related to T2 (10). TR and TE are parameters that can be chosen in order to maximize contrast (9).

Image contrast

Intrinsic image contrast can be obtained with T1 and T2 relaxation, previously mentioned, and also with proton density.

Regarding T1-weigted images, tissues with long T1, such as fluids (7), will lose energy to the surroundings at a slower rate, i.e., they take longer to regain longitudinal magnetization, M_z, and this tissue will appear darker in the image. Tissues with short T1, such as fat (7), will regain the longitudinal magnetization faster, having a strong signal and it will appear brighter (10). Protons that are more strongly bound will lose their energy faster, like the hydrogen in adipose tissue (12).

However, TR must be considered, because if a long TR is chosen, almost all tissues will have recovered the longitudinal magnetization and tissues will appear similar in the image. Short TR maximizes the difference of signals between different tissues (10).

Concerning T2-weighted images, tissues with long T2 will, like fluids, stay in phase longer, with a stronger signal, and the image will be brighter. Tissues with a short T2 will dephase faster, generating a weaker signal and a darker image (7,10).

In this case, TE must be taken into consideration too. If a short TE is chosen, all tissues are starting to dephase and if the signal is captured as soon after the RF is applied, the differences in the rate of dephasing of each tissue will not be maximized. Therefore, short TE does not take advantage of T2 contrast and longer TE must be chosen (10).

Proton density weighted images can be achieved when a long TR is chose, reducing the T1 contrast and a short TE is also chosen, reducing the T2 source of contrast. These images are useful in some pathologies, such as edema, infection, inflammation or some tumours (7), and specific anatomical structures, such as tendons, bone or brain (10).

Other parameters that contribute to the image contrast are flow of fluids, diffusion or perfusion (13).

A source of extrinsic image contrast used frequently is gadolinium chelate. This substance will decrease the T1 relaxation time of the near protons (9). It's injection in the blood stream will alter the time constant of the protons of the blood. In the brain, the blood brain barrier will not allow its passage for the cerebral tissue, evaluating the integrity of the membrane (7).

Spatial localization

Localization of the signal is possible if each proton has a different Larmor frequency, which in turn depends on the strength of the magnetic field (10).

By creating magnetic fields (on order of mT) overlapped with the main field (on order of T), that vary from –Z to +Z, the Larmor frequencies at which the protons precess will also vary. This sections the body into slices. The gradient coils mentioned earlier create the overlapped magnetic fields to vary the strength of the main magnetic field.

Within the slice, the RF pulse is the same, and gradients in the other two directions must be applied after the slice selection, so that the location of the precessing protons is obtained.

A frequency encoding gradient is applied during the formation of the signal (7), usually in the X axis , and it is also called read-out gradient (13), where protons are encoded with

different frequencies according to their location. This gradient will make the protons on the side of the stronger magnetic field precess with a higher frequency and protons on the side of the weaker magnetic field precess slower. The higher or lower amplitude of the signal emitted will be related to the localization of the protons (11).

In the y axis, a phase encoding gradient is used. This is applied after the excitation occurs, but before the signal is received (9). The gradient in the y axis will make the protons dephase along its variation (13). It is chosen a specific strength of the magnetic field at a time and several excitation and signal read-out sequences must be performed (11). This is defined by the TR time, mentioned above.

With these three gradients defined in the three directions, a square is obtained, named a voxel. Even though the z, x, y directions are defined for the application of the gradients, these are arbitrary but all three axes must be orthogonal (9).

After the reception of the signal, the information will be stored in a line of the k-space, a two dimension matrix that contains the information. Several readings must be performed to fill the k-space with information. In the horizontal is the frequency data and in the vertical, the phase data (10). In the centre of the k-space are the signals with more strength and low spatial frequency, responsible for contrast (12) and peripherally are the weaker signals and high spatial frequency (13).

The Fourier transform, a mathematical process, is applied to the signal, which is a complex wave expressed in the time domain (10), to separate the signal in the time domain into the spatial components (7) so that the image can be obtained.

Formation of echoes (signals) – Pulse sequences

Acquisition of images implies excitation, spatial encoding, echo formation and sampling, or signal reception (7). Variations of this acquisition are called pulse sequences. These steps are repeated several times to create an echo.

After the application of the RF pulse of 90º, if other pulses are not applied, the generated signal will be a free induction decay – FID – which is a sum of all the frequencies from the sample analysed (6).

Spin echo sequence is the commonest used pulse sequence. Refocusing of the protons is necessary so that they continue phasing and dephasing and a signal can be emitted – spin echo, depending on TE (10). The refocusing pulse is a 180º pulse given at half of the TE and after the initial 90º pulse. The first will produce a change in the direction at which the

protons spin, in the XY plane and spins will regain coherence and the signal will be stronger. This sequence is repeated according to TR.

Fast spin echo sequence is commonly used in the clinical practice. It uses several 180º refocusing pulses after the first repetition but before the second one, and so several echoes are received, with different TE. Also, each echo is encoded with a different phase gradient. Several lines of the k-space can be filled within one sequence. This will make the image acquisition faster but the contrast can be compromised (9).

Inversion recovery sequence begins with a 180º pulse, to change the magnetization into the -Z direction. With the recovery of the longitudinal magnetization, a 90º pulse is applied, and given the inversion time, the time between the 180º and the 90º pulse, tissues that did not regain the longitudinal magnetization, after the 90º pulse, they will not be in the transverse plane, and so they will not emit a signal. This sequence is used when some tissues need to be suppressed (10).

Fluid-Attenuated Inversion Recovery (FLAIR) **sequence** is a type of inversion recovery used with long inversion time. This is set for when free water is at the transverse plane, moment when the 90º pulse is given. Therefore, water will not generate a signal and it can be supressed in the resulting image. This sequence is useful to supress the signal from the cerebrospinal fluid to detect signals from adjacent structures (10).

Gradient echo is a sequence that spares the application of the 180º refocusing pulse, allowing for some time to be saved. The TR can be shortened. The flip angle is an important source of contrast in this sequence, where low flip angles will give T2* weighted images and higher angles (up to 90º) will give T1 weighted images (9). A frequency gradient is applied in order to create an echo. Firstly, a negative gradient is used to cease phase coherence and secondly, a reverse gradient is applied to recover phase coherence. Because of the short TR values, the longitudinal magnetization may not be complete when the next sequence is started. This will make the signal fainter (10).

Signal-to-noise ratio

Signal-to-noise ratio, SNR, is a ratio between signal intensity and the background noise, i.e., factors that make signal weaker. This is a criteria that translates the quality of the image (11) High values of SNR are preferable. High SNR implies compromising some features such as resolution (10).

Image reconstruction

As mentioned earlier, the Fourier transform will be applied to the signal. The signal is a complex wave (10) that contains the information.

For the reconstruction, direct methods can be used, such as the Fast Fourier Transform or parallel imaging or, for more complex mathematical operations, indirect methods, such as iterative methods (14). The latter is slower than the direct methods because of the iteration itself, however, it is advantageous for more demanding reconstruction.

The iterative method, instead of transforming the obtained signal, tries to theorize the solution of the transformation, then compared to the real data to readjust some eventual inaccuracy (14).

On the other hand, there is the Filtered-back projection, an analytical method, which obtains shadows of the objects after applying a filter to obtain a better image (11).

Artefacts

Artefacts are components that appear on an image created by confounding factors, created the by the MRI system or related to the patient (10).

Among the system artefacts the following can happen: aliasing/wrap-around artefact, truncation or Gibbs artefact, zipper artefact, moire or fringe artefact and partial volume density artefact.

Partial volume density artefact happens when the structures to be scanned are smaller than the voxel size and the spatial resolution. The average of the signals that differ in intensity, from within the voxel, will be the information obtained for that voxel, which means that signal will be lost and so will be the resolution (10).

Among the patient related artefacts, the following can happen: motion artefact, flow artefacts and chemical shift artefact.

Chemical shift artefact takes place at water and fat interfaces, because protons precess at different speeds between those two. Protons within the fat will precess slower comparing with the ones in water. The frequencies will be different and this is interpreted as differences in position, and the fat containing tissues will not be at their correct place (10). This can be abolished with a “fat-suppression” technique.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy, MRS, allows metabolic analysis of the selected tissues through spectra.

An important concept for MRS is chemical shift. Every proton subjected to the main magnetic field will experience it in a different way, because of its surroundings. The electrons that are near the proton will shield it and make the effective magnetic field differ from the B_0 (6), and consequently, the frequency at which the protons precess will differ. The signal generated will be different from molecule to molecule, and the resulting spectra will reflect the different metabolites of the tissue.

The main magnetic field must be as homogenous as possible, so that the signal generated has its origin in the chemical shift and not on the possible irregularities of the main field (7). The correction of the field is obtained with the use of shimming coils.

Frequency spectra are the final result of MRS. The different localized peaks observed, created by different molecules, are referred as ppm (parts per million) of B_0 of a particular substance (6,7). The area under the peak is representative of its concentration, usually in mM.

Concentration of metabolites is about 5000 to 10000 times lower than the concentration of water, and the signal generated by water is greater than the signal from metabolites. Due to this fact, water and fat signals must be supressed and the voxel of interest must have a higher voxel size than the voxel size used in MRI to obtain a signal (9).

MRS acquisition

The MRS data acquisition starts with anatomical images by MRI of the region of interest. The generated images will assist in the choice of the region from which spectra will be obtained (15).

There are two main methods used to obtain a spectrum: single voxel spectroscopy and multivoxel spectroscopy. Multivoxel spectroscopy is also called chemical shift imaging, spectroscopic imaging or magnetic resonance spectroscopy imaging.

Single voxel spectroscopy

Single voxel spectroscopy (SVS) defines a specific voxel to be analysed in the tissue, and its size is chosen by the user (6).Voxels with a smaller size will require more repetitions so that the signal is sufficient to be captured.

The sequences used in SVS to generate signals are Stimulated Echo Acquisition Mode (STEAM) through magnetization with three 90º RF pulses generates an echo or Point

Resolved Spectroscopy (PRESS) with higher signal intensity compared to the first, it measures spin echo, created by a first 90º pulse with two 180º RF pulses following (6). PRESS is used more frequently in clinical practice because of better SNR and less movement artefacts (16).

In STEAM or PRESS, gradients in all directions are applied to select the specific voxel in the same way MRI uses the gradients for localization.

The time between the different pulses will define the TE. For quantification of metabolites, a combined short TE and long TR will minimize the loss of signal from T1 and T2 relaxation (15).

With SVS, the localization of the signal is more precise, there is a better homogeneity, and it is more reproducible (16).

Multivoxel spectroscopy or Chemical shift imaging

Multivoxel spectroscopy uses a grid of voxels, in a chosen region, which are analysed simultaneously. It provides a metabolite image with better spatial resolution than SVS. Single Voxel Spectroscopy is used when a quantification is intended and multivoxel spectroscopy is used when spatial distribution of the metabolites is of interest (15).

The sequences used in multivoxel spectroscopy to obtain a signal are similar to the ones used in MRI. First, a slice is selected with a gradient and the RF pulse is applied to that slice. Second, a phase encoding gradient is applied, and with each repetition, a different amplitude of the phase encoding gradient is chosen. However, no frequency encoding gradient is used (17).

In MRI, the frequency encoding gradient is applied for spatial localization. However, if this gradient is applied in multivoxel spectroscopy, the signal will have a frequency that is influenced not only by the chemical shit, but also the frequency encoding gradient. The frequency gradient can't be applied in MRS, and the phase-encoding gradient is used repeatedly for localization (17).

According to the type of multivoxel spectroscopy – 1D, 2D or 3D, phase encoding in one, two or three directions can be applied for localization of the voxel. The 2D multivoxel is used more frequently, because time of acquisition is not as long as the 3D multivoxel.

STEAM or PRESS sequences can also be applied in multivoxel spectroscopy to preselect the area of interest. After this selection, the grid and its voxels are chosen and only then the multivoxel pulse sequences are applied.

An important concept to be considered in multivoxel spectroscopy is Point Spread Function (PSF), which is a function that reflects the influence of the surrounding voxels on the

voxel analysed. The signal localization is obtained with a limited number of phase encoding gradients, and after the Fourier transform, the signal is affected by the adjacent voxels' signals. This is called "voxel bleeding" (17). This may be avoided with a higher number of phase encoding steps, which will also increase acquisition time. An equilibrium must be obtain in order to minimize "voxel bleeding" and to have a reasonable scanning time. This phenomenon does not occur in SVE.

Point Spread Function (PSF) is also affected by the lipid and water signal, which is higher than metabolites' signals. The suppression of undesired signals can be achieved with volume pre-selection, outer volume suppression, which saturates the surroundings of the voxels of interest (17) and water suppression techniques. Spectra without water suppression are also obtained for posterior comparison and corrections (15).

Multivoxel spectroscopy is time consuming and several modalities have been explored to minimize acquisition time, such as k-sampling reduction, Turbo multivoxel (with multiple echo formation) and multi-slice multivoxel (analysis of several slices at the same time), in a similar manner as some MRI pulse sequences elucidated before.

K-sampling reduction works by turning the k-space into a circle, instead of a square, and the outer circle is filled with zeros after acquisition so that the Fourier transform can be applied. This also reduces the PSF, by suppressing data from surrounding voxels, but the spatial resolution worsens. (17)

Multivoxel spectroscopy results can be presented in a form of a spectra or a metabolic map, in which the various concentrations of metabolites are coded with a grey-scale and overlapped with the anatomic imagen of the region at study (17).

Post processing

The data acquired from both techniques of MRS is also stored in k-spaces and some manipulation of the data can be performed to maximize the final result.

In SVS post processing, the following strategies are used: time domain multiplications, zero-filling, Fourier transform, and phasing and baseline corrections. Regarding multivoxel spectroscopy, data is first multiplied with a filter and then the Fourier transform is applied. After this step, the procedure is equal to SVS post processing (15).

In order to quantify the metabolites, the area under the curve of the peaks in the spectrum must be calculated. For this, there are software programmes with complex algorithms which preform this quantification (15).

Quantification of metabolites can be performed in an absolute or relative manner. The first is more demanding in terms of technique and the latter uses ratios. Absolute quantification uses water as a reference, and water concentration must me assumed, which might induce errors between diseased and normal tissues, and even between different tissues, because of its differences in water content. This quantification increases scanning time since T1 and T2 must be minimized (6). Another reference can be used, which is called a phantom that is an external solution with a known concentration. The problem with this type of reference is that the field applied will be different from the one the patient in scan experiences and the quantification will be affected (6).

In the relative quantification, metabolite ratios are used, having creatine as a reference. Once again, creatine levels are regarded as constant between diseased and normal tissues (6).

With all the variables above, it is possible to choose different settings and these variations between different investigations will result in different metabolite concentrations, which will difficult the comparison. There are no standardized methods for multivoxel spectroscopy yet (18).

Spectra and cerebral metabolites

The number of metabolites estimated to exist in the human brain go from 2000 to 20000 (15) and not all of them can be identified through MRS, since the identification depends on a concentration threshold. Metabolites vary with age and there are some substantial differences when analysing the spectra form a new-born, a child, an adult or an elder (18).

The most protruding peaks in a spectrum from brain tissue of an adult at a magnetic field strength of 1.5T and a TE of 30 ms (6):

N-acetyl aspartate (NAA), with a peak at 2.0 ppm, encompasses not only NAA but also N-acetylaspartyl glutamate. The signal is generated from methyl groups of these compounds (15). NAA is associated with neuronal integrity (9,19). In a brain tumour, NAA will be reduced or absent, since it’s a compound present in neurons and the tumours are constituted in its majority of glial cells (18).The NAA peak can be smaller or absent in other pathologies, such as dementias, infections and temporal lobe epilepsy (7).

Creatine (Cr) peaks, derived from creatine and phosphocreatine, are present at 3.0 ppm - from methyl groups, and 3.91 – from methylene groups (15). Cr is related to energy metabolism. In a tumour, Cr is reduced due to necrosis and rapid consumption of energy because of higher cell multiplication (18).

Choline (Cho) peak is at 3.2 ppm and the signal is derived from free choline and phosphocholine (15). Cho is a marker of membrane turnover (9) and cell proliferation (19). Absolute quantification of Cho may be inaccurate and ratios are preferred – Cho/NAA or Cho/Cr (18).In brain tumours, infarction and inflammation, Cho levels are higher (15).

As mentioned before, TE influences the peaks on the spectrum. With a short TE (40 ms), myoinositol, glycine and lipids are better observed (15). With an intermedium TE (135-144 ms), the lactate peak is better observed and with a long TE (270-288 ms), NAA, Cho and Cr are evidenced. TR influences the signal too. Long TR (2500 ms) with a combination with a short TE deliver adequate data for spectrum formation. (16)

Myoinositol, at 3.6 ppm, is a simple sugar that glial cells create. An increase in myoinositol is associated with a higher number of glial cells (15).

Lipids, at 1.3 ppm, usually don't appear in normal tissue. They can be detected if the external tissues to the selected voxel are not supressed adequately or they can have a pathologic meaning – the presence of lipid is associated with radiation necrosis, brain tumours or metastasis (15,20).

Lactate doublet at 1.3 ppm is not usually seen in normal brain tissue and is related to the change in metabolism, to anaerobic and to high degree of glycolysis. It can appear on behalf of hypoxia, in brain tumours, in necrotic tissues, and in brain cysts (16).

Other metabolites are possibly identified, such as glutamate, glutamine or GABA. The signals of these metabolites are smaller than the metabolites referred above (15).

In brain tumours, an elevation of choline is present, due to an increase in membrane synthesis. A reduction of N-acetyl-aspartate is also present, since the majority of brain tumours derives from non-neuronal cells as well as a reduction of creatine due to alterations of energy metabolism (15). Myoinositol has a high peak in low-grade gliomas, and it decreases as the grade gets higher. Lactate peak can be present in brain tumour spectrum but it is not related with the grade of tumour. Lipids appear in necrotic regions of malignant tumours (15).

Brain tumours and pseudoprogression

Epidemiology

According to the Central Brain Tumour Registry of the United States' (CBTRUS) report of Central Nervous System (CNS) primary tumours (2012-2016), the annual age-adjusted incidence rate of brain tumours was 23.41 per 100 000 population, being glioblastoma the most common malignant tumour (14% of all tumours) and meningioma the most common non-malignant tumour (37.6% of all tumours) (21). The reported five-year survival rate of malignant brain tumours was 35.8% and non-malignant was 91.5% (21).

In a systematic analysis for the study of the burden of diseases, global burden of brain tumours was assessed between 1990 and 2016, which evidenced an increase in the incidence rate of CNS tumours of 17.3% between those years, pointing out east Asia, western Europe and south Asia with the highest incidence rates, by this order (22).

Types of tumours – WHO 2016 classification

The World Health Organization (WHO) introduced in 2016 a review of its classification of brain tumours, having in mind the molecular and genetic features of brain tumours, besides the histopathological patterns considered in previous editions. This new classification aims to categorize diagnosis with more accuracy with the aid of molecular and genetic patterns in specific tumour subtypes (23).

The major groups of CNS tumours of the WHO 2016 classification are: Diffuse astrocytic and oligodendroglial tumours, Other astrocytic tumours, Other gliomas, Choroid plexus tumours, Neuronal and mixed neuronal-glial tumours, Tumours of the pineal region, Embryonal tumours, Tumours of the cranial and paraspinal nerves, Meningiomas, Mesenchymal, non-meningothelial tumours, Melanocytic tumours, Lymphomas, Histiocytic tumours, Germ cell tumours, Tumours of the sellar region, Metastatic tumours (23) (see table in appendix).

As mentioned above, the most common primary malignant tumour of the CNS is glioblastoma and the primary non-malignant is meningioma. Metastasis, however, are the most common tumour of the CNS (24). For this review, only the most common primary tumours will be addressed, with a focus on glioblastoma.

Diffuse astrocytic and oligodendroglial tumours categories encompass neoplasms with similar histological features but also Isocitrate Dehydrogenase (IDH) mutations.

Within this division are: diffuse astrocytoma (IDH-mutant, IDH-wildtype, NOS), anaplastic astrocytoma (IDH-mutant, IDH-wildtype, NOS), glioblastoma (IDH-mutant, IDH-

wildtype, NOS), diffuse midline glioma (H3 K27M-mutant), oligodendroglioma (IDH-mutant and NOS), anaplastic oligodendroglioma (IDH-mutant and NOS), oligoastrocytoma (NOS) and anaplastic oligoastrocytoma (NOS) (23,24) (see table in appendix).

Glioblastoma is the most common astrocytoma (24) and was previously referred as glioblastoma multiforme. These tumours can be IDH-mutant, IDH-wildtype (when there is no mutation) or Not Otherwise Specified (NOS) (when it is not possible to attain conclusion whether the mutation is present or not) (23).

Isocitrate Dehydrogenase is an enzyme in Krebs' cycle. The gene of this enzyme can be mutated in some tumours (24). There are IDH1 and IDH2 mutations, being the first the most common and both being related with a better prognosis (24).

IDH-wildtype tumours, the subpopulation with no detectable mutation, is more frequent (about 90% of glioblastoma cases), related to *de novo* glioblastoma, normally in patients over 55 years (23), being associated with a worse prognosis.

IDH-mutant tumours (10% of glioblastoma cases) are more common in younger patients and are usually a progression of a former low-grade glioma (23,25), which is why they are referred as secondary glioblastoma (24). These two entities are classified as being grade IV WHO.

As mentioned before, the most common non-malignant CNS tumour is meningioma. Meningiomas have their origin in the meninges, derived from arachnoid cells (25) and the majority grows insidiously with well-defined margins (24). Their grades vary from I to III, being grade II described as atypical meningioma and grade III as anaplastic meningioma - a rapidly proliferating entity, with a malignant behaviour.

Symptoms

The symptoms that accompany brain tumours depend on its location and rate of growth (25). Progressive neurological deficit, headaches and seizures are among common symptoms and signs (24).

According to the International Classification of Headache disorders (3rd edition), headaches may appear in 32% to 71% of the patients with brain tumours (26) and can be caused by increased intracranial pressure, compression of structures sensitive to pain, alterations of vision (due to compression or invasion of the structures related to sight) or psychogenic (24). It is more frequently associated with rapidly proliferating tumours (25) and localization of the headache is frequently linked with the location of the tumour - supratentorial or infratentorial tumours.

Seizures are more frequent in supratentorial, cortical tumours and in the temporal lobe (25) and can be the first symptom. In adults with no previous seizure episodes, tumour diagnosis must be excluded (24).

MRI scans of brain tumours

Magnetic resonance imaging scans of brain tumours vary with type of tumour. Low-grade tumours usually present with no or mild peritumoral vasogenic edema and high-grade tumours can have surrounding edema and/or mass effect (9).

Astrocytomas scans show hypointense mass compared with normal brain tissue on T1-weighted images and hyperintensity on T2-weighted and FLAIR images (9). This hyperintensity is due to perilesional edema (27).

Low-grade gliomas have defined margins. Glioblastomas have less defined margins, peritumoral edema (9), necrosis, edema, increased permeability with various patterns of enhancement and hypervascularity (28). The infiltration that accompanies these tumours is best seen in FLAIR imaging (28).

High-grade gliomas, in T1-weighted images with gadolinium, are more contrast-enhanced, compared with low-grade, where only less than 10% demonstrate contrast-enhancement (9,28).

Treatment

Grade III and IV brain tumours' treatment encompasses total surgical resection if possible, radiotherapy and chemotherapy (29,30). Current treatment of glioblastoma consists of surgical gross total resection, if possible, followed by Stupp protocol, comprising radiotherapy and temozolomide (TMZ).

Surgery aims at total resection of the tumour, although this is not always possible, mainly because eloquent areas may be affected. With surgical resection, histology can be obtained, mass effect and symptoms can be minimized and, ultimately, reduce the size of the tumour so that radiotherapy and chemotherapy can have maximum effect (25).

Radiotherapy volume is selected base on T2 weighted or FLAIR images, with a 2 to 3 centimetres margin (24,25). A total dose of 60 grays (Gy) is administered, divided in fractions of 2 Gy daily for 5 days per week, over 6 weeks (31).

Temozolomide is administrated concomitantly with radiotherapy, 75 mg per square meter of body surface area per day, for 7 days a week from the first day of radiotherapy until the last day. This is followed by adjuvant temozolomide, 150-200 mg per square meter of body surface area, for 5 days, in a 28 days cycle, up to 6 cycles (31).

In the CNS, chemotherapy drugs have to cross the blood-brain barrier, which is possible due to their lipid-soluble nature. Several factors can impair their diffusion, such as edema surrounding the tumour, and for that, corticosteroid use can diminish the edema (25).

Alkylating agents, such as temozolomide, cause arrest of the cell cycle and DNA fragmentation (32). Temozolomide is a prodrug that is stable in acid environments, reason why it can be administered orally. It is quickly absorbed and when the pH is higher than 7, it goes through a series of chemical reactions, giving rise to monomethyl triazenoimidazole carboxamide (MTIC). which will methylate the DNA (33).

Recently, antiangiogenic agents, such as Bevacizumab, an anti-VEGF (anti-Vascular Endothelial Growth Factor) monoclonal anti-body, have been added to treatment options as a second line in chemotherapy treatment.

Treatment-related changes and Pseudoprogression

During follow-up, the images obtained may present features related to tumour progression. However, other alterations may appear as well, without being related to true progression, but rather related to treatment. Consequently, they are sometimes referred to as treatment- related changes (34).

Radiation necrosis is a local reaction of the tissue that has been subjected to radiotherapy. It can either be acute (during the time of radiotherapy), subacute (after radiotherapy in a window of 3 to 9 months) or chronic (35). The first two are related to vascular alterations, such as vasodilation, endothelial damage and blood brain barrier impairment. Oligodendrocytes may also be affected by radiation, with demyelination as a possible treatment side effect (29). Chronic radiation necrosis is related to necrosis, fibrosis, reactive gliosis and hyalinization of the vessels (34). Radiation necrosis has a reported frequency of 5-25% (1), and is related to a poorer prognosis compared with pseudoprogression (34).

Pseudoresponse may occur when antiangiogenic agents are used, and it is identified as a reduction in the enhancement of the tumour in the scans almost immediately after its administration. The edema that delimits the tumour may be diminished when FLAIR is used (29). This is due to reduction in the vascular permeability (36) and it may be misinterpreted as an improvement of the disease (37)

Pseudoprogression

Pseudoprogression is usually identified in the first 3 months after the treatment (29) and it is commonly described as new enhancing areas after therapy in acquired images, without true tumour growth (1).

The pathophysiology of pseudoprogression is not entirely clear and it is possible that it results from an inflammatory response and greater vascular permeability (38) as a consequence of cytotoxic treatment (2). It is believed that the combination of the radiotherapy and chemotherapy with temozolomide, and the consequent inflammatory response, can cause the referred enhancement (39).

Incidence rates among studies may vary from 9 to 30% (1) and it is more frequent in patients that have temozolomide schemes in their treatment. A correlation has been confirmed between the use of temozolomide and pseudoprogression incidence, considering low incident rates (about 1%) before the advent of this alkylating agent (37,40).

Incidence of pseudoprogression is also thought to be higher in tumours that have the O (6)-methylguanine-DNA methyltransferase (MGMT) promoter gene hypermethylated (41). The methylation of the gene will inhibit its function, that is to repair the damage imposed by alkylating agents (41). For this reason, hypermethylation of MGMT is related to a better prognosis (27,34). Pseudoprogression may be more frequent in patients subjected to higher doses of radiotherapy (34,39).

Imaging changes when it comes to pseudoprogression are transitory and there is no further action to consider for its resolution (32,36).

Response assessment criteria

In order to evaluate response to treatment, criteria were created throughout time. The first response criteria used was Response Evaluation Criteria in Solid Tumours (RECIST), adapted from tumours outside the central nervous system (4).

MacDonald criteria were introduced in 1990, for patients diagnosed with gliomas, and were based on CT scans initially (5). It comprised maximal perpendicular diameter, duration of the response, corticosteroid use and clinical presentation (4) and classified the response in four categories: complete or partial response, progressive or stable disease (29). These criteria were limited in some aspects, like not considering pseudoprogression as a possible response to treatment.

In 2010, Response Assessment in Neuro-Oncology (RANO) criteria were published, seeking an improvement to previous criteria. In addition to what MacDonald criteria comprised, RANO criteria include consideration of pseudoprogression, MRI - T1 with gadolinium and T2-weighted and FLAIR (fluid-attenuated inversion recovery) (4,5).

Above mentioned response criteria are summarized in Table I.

Table I: Comparison of response criteria

	RECIST	MacDonald	RANO
Measurement	1D contrast enhancement	2D contrast enhancement	2D contrast enhancement
Progression	≥ 20% increase in sum of lesions	≥ 25% increase in product of perpendicular diameter	≥ 25% increase in product of perpendicular diameter
Response	≥ 30% decrease in sum of lesions	≥ 50% decrease in product of perpendicular diameter	≥ 50% decrease in product of perpendicular diameter
Durability of response	Optional	Yes (at least 4 weeks)	Yes (at least 4 weeks)
Definition of measurability	Yes	No	Yes
Number of target lesions	Up to 5	Not specified	Up to 5
T2/FLAIR	Not evaluated	Not evaluated	Evaluated
Corticosteroids considered	No	Yes	Yes
Clinical status considered	No	Yes	Yes
Pseudoprogression considered	No	No	Yes

Table I: Abbreviations: RECIST, Response Evaluation Criteria in Solid Tumours; RANO, Response Assessment in Neuro-Oncology. Adapted from Chukwueke UN, Wen PY. Use of the Response Assessment in Neuro-Oncology (RANO) criteria in clinical trials and clinical practice. CNS Oncol. 2019 Mar 1;8(1):CNS28.

Similarly to MacDonald criteria, RANO classifies the response of the tumour to therapy as: complete response, partial response, stable disease or progressive disease. It is advisable that a MRI scan is obtained within 24 to 48 hours after surgery to establish the baseline image, given the fact that post-operatory contrast-enhancement of resected tumour margin is a common feature after this time period (2). For each category, several items must be confirmed, which are summarized in Table II.

Table II: RANO criteria

Response	Criteria
Complete response	Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks; No new lesions; Stable or improved non-enhancing lesions (T2/FLAIR); No current use of corticosteroids ; Stable or improved clinically;
Partial response	≥ 50% decrease in product of perpendicular diameter of all enhancing lesions, sustained for 4 weeks; No progression of non-measurable disease; No new lesions; Stable or improved non-enhancing lesions (T2/FLAIR) on same or lower dose of corticosteroids; Corticosteroid dose at the time of the scan must not surpass the initial dose at which the baseline scan is obtained; Sable or improved clinically;
Progression	≥ 25% increase in product of perpendicular diameter of enhancing lesions compared with the smallest tumour measurement obtained at baseline (if no response), or obtained at best response, on stable or increasing doses of corticosteroids; Significant increase in T2/FLAIR non-enhancing lesions on stable or increasing dose of corticosteroids; Any new lesions; Clear clinical deterioration not attributed to other causes apart from the tumour; Failure to return for evaluation as a result of death or deteriorating condition; Clear progression of non-measurable disease;
Stable disease	Does not fit the previous categories; Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids that the dose at which the baseline scan is obtained;

Table II: Abbreviations: FLAIR, fluid-attenuated inversion recovery. Adapted from Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated Response Assessment Criteria for High-Grade Gliomas: Response Assessment in Neuro-Oncology Working Group. J Clin Oncol. 2010 Apr 10;28(11):1963–72.

The imaging modalities used for RANO criteria are limited to MRI T1-weigted with gadolinium contrast images, T2-wheignted and FLAIR. Although an effort has been made in order to incorporate pseudoprogression into the criteria, just a few imaging modalities display robust results for this criteria.

Other modalities are currently being studied to differentiate pseudoprogression from tumour progression. Magnetic resonance spectroscopy is one of those modalities.

Magnetic resonance spectroscopy in pseudoprogression

Brain MRI scans have become standard tools for evaluation of response to treatment (32) but T2 and T1 with gadolinium MRI scans cannot always differentiate true tumour progression from treatment related changes (41).

Usually, there is no histopathological confirmation of tumour recurrence because of some limiting factors, such as localization of the tumour or patient status (29,42). Less invasive methods are desirable, with high sensitivity and specificity to diagnose tumour recurrence. Magnetic Resonance Spectroscopy is able to provide specific spatial distribution and metabolite concentration in normal and pathologic tissue (41).

The correct diagnosis of recurrence is also important for the criteria of admission for clinical trials (41) because, with the lack of an efficient diagnostic tool, patients after therapy must wait several months until they can be admitted to clinical trials of glioblastomas (29).

Magnetic Resonance Spectroscopy (MRS), Diffusion-Weighted Image (DWI) or Perfusion-Weighted Image are being currently accessed for their clinical utility as diagnostic tools. These techniques are not taken into consideration by RANO since there are no impactful studies regarding their utility on the diagnosis or even a standardization of protocols for each technique (36,41).

A meta-analysis from 2014 (42) of 18 studies with 455 patients showed a moderate diagnostic performance to differentiate true progression from radiation necrosis using Cho/NAA and Cho/Cr ratios, with a sensitivity of 88% and specificity of 86%.

A recent meta-analysis on the diagnosis accuracy of treatment response (43) concluded that spectroscopy was the technique with higher diagnostic accuracy in the treatment response assessment, showing that MRI had a sensitivity of 68% and specificity of 77% (5 studies, 166 patients); Apparent diffusion coefficients (ADC) showed a sensitivity of 71% and a specificity of 87% (7 studies, 204 patients); Dynamic Susceptibility Contrast, DSC-perfusion showed a sensitivity of 87% and specificity of 86% (18 studies, 708 patients); Dynamic Contrast Enhanced, DCE-perfusion showed a sensitivity of 92% and specificity of 85% (5 studies, 207 patients); Spectroscopy showed a sensitivity of 91% and specificity of 95% (9 studies, 203 patients).

Concerning true tumour progression, classical features of metabolites spectra are an elevation of choline (Cho) and lower levels of N-acetyl-aspartate (NAA) compared with pseudoprogression (1). Radiation necrosis spectra show decreased N-acetyl-aspartate (NAA), no choline elevation and the presence lipid lactate peak (1).

Metabolite ratios are preferred over absolute concentrations (41). The first studies performed using MRS in treated tumours exhibited satisfactory results by using Cho/NAA and Cho/Cr ratios to differentiate true tumour progression from radiation necrosis(1). These ratios are the most extensively studied and validated, so far (36).The meta-analysis mentioned before concluded that Cho/Cr was the best predictor to distinguish tumour progression and treatment related changes (43)

A study with 24 patients showed that, in all relapsed glioblastomas, lipid and lactate peak was higher than 4.8 mM, although this same feature was also present on 33% of pseudoprogression cases (sensitivity of 100%, specificity of 66.7%) (41). All of the patients with pseudoprogression had NAA concentration values higher than 1.5 mM (sensitivity of 75%, specificity of 100%). Relapse showed lower NAA/Cr ratio (cut-off of 0.7 mM, sensitivity 94.4%, specificity 91.7%), and higher Cho/NAA (cut off of 1.4 mM, sensitivity of 100%, specificity of 91.7%) (41).

A recent study (38) by using 3D echo planar chemical shift imaging (CSI, or multivoxel spectroscopy) showed higher Cho/NAA and Cho/Cr in true tumour progression with better space resolution. Higher Cho/NAA values in the peritumoral area of recurrence is associated with a higher degree of infiltration and mapping of peritumoral regions is suggested for the differentiation of true progression from pseudoprogression with 3D echo planar spectroscopic imaging. This study showed a sensitivity of 94% and a specificity of 87%. The results should be interpreted carefully due to small number of patients (24 patients) (38).

Regarding SVS and CSI (or multivoxel spectroscopy), CSI accounts for heterogeneity of the tumour and has better accuracy for detecting tumour (44).

Limitations of MRS

MRS adds significant extra time to standard MRI (42) because concentrations of metabolites are low and the signal generated by these molecules are fainter when compared with higher concentration molecules (34,43,45). Magnetic resonance spectroscopy is technically demanding and parameters like magnetic field strength, MRS technique used (SVS or CSI), TE, TR, time of inversion (TI) for suppression of lipids and water signals in order to get the signal from metabolites (27,43), voxel size, Field-of-View (FOV), slice thickness, matrix size and data analysis programs are chosen by the operand. Personal experience and manual input influence the quality of the spectra obtained (44,46) and MRS requires additional time for post-processing too (45).

The chosen TE influences observed peaks seen in spectra (1). Lipids are better seen in short TE and the lipid lactate peak is seen with longer TE.

Voxel size is larger than MRI and partial volume effects between tumour progression and treatment related changes can happen. Smaller lesions are challenging to be detected by MRS (34,43). When using MRS, the chosen voxel can contain tumour tissue, necrotic tissue and normal brain tissue, which can influence data (38). Single voxel spectroscopy (SVS) is not good for spatial tissue heterogeneity (36)

Ratios and suggested thresholds vary from distinct studies (43). Proposed thresholds of total metabolite concentration vary as much as 50% between different studies and may also differ in technique and type of tumour (1). Institutional definition and validation of the thresholds of MRS are needed (46).

Associating MRS with other techniques, such as ADC, provides better sensitivity and specificity (41) and is therefore recommended (42).

Future perspectives

Combining MRS with other multimodal imaging methods (42,46) may increase its sensitivity and specificity for the diagnosis of pseudoprogression or tumour recurrence.

When it comes to new possible markers, R-2-hydroxyglutarate (R-2-HG), an oncometabolite that accumulates in IDH mutation tumours can be measured for post treatment changes in patients with tumours that are IDH-mutant, and its spectra may present follow-up value (34,45).

In order to make MRS a more widely accepted technique, image acquisition protocols must be standardized and algorithms with minimal user input are needed (45).

Conclusion

Magnetic Resonance Imaging is a well-established field with a significant contribution to the field of medicine and with ever-evolving advanced techniques, such as MRS. It is a versatile field since parameters can be chosen to best suit the final result, the image.

Magnetic Resonance Imaging has a crucial role in modern CNS imaging. Constant updates on standardized and validated procedures us fundamental for a desirable multidisciplinary approach, namely in the field of neuro-oncology.

Pseudoprogression had a higher incidence with the emergence of temozolomide, a standard component of glioblastoma's treatment. Pseudoprogression doesn't require treatment whereas tumour recurrence demands further therapeutic procedures. It is, then, important to distinguish pseudoprogression and true tumour recurrence, ideally with non-invasive methods.

Magnetic Resonance Spectroscopy can be a useful tool in Pseudoprogression, as it adds diagnostic value to MRI scans and MRI advanced techniques, such as diffusion weighted images and perfusion weighed image.

Magnetic Resonance Spectroscopy is time consuming and technically demanding, depending on user and parameters chosen. It is not a standardized technique and metabolite absolute values and ratio cut-offs are not established, giving rise to different results, sensitivity and specificity when comparing different studies.

Protocols need to be established so that MRS can be performed in the context of pseudoprogression and results can be compared between institutions.

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Appendix I – WHO classification of tumours of the central nervous system, 2016,

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	9400/3
Gemistocytic astrocytoma, IDH-mutant	9411/3
Diffuse astrocytoma, IDH-wildtype	*9400/3*
Diffuse astrocytoma, NOS	9400/3
Anaplastic astrocytoma, IDH-mutant	9401/3
Anaplastic astrocytoma, IDH-wildtype	*9401/3*
Anaplastic astrocytoma, NOS	9401/3
Glioblastoma, IDH-wildtype	9440/3
Giant cell glioblastoma	9441/3
Gliosarcoma	9442/3
Epithelioid glioblastoma	*9440/3*
Glioblastoma, IDH-mutant	9445/3*
Glioblastoma, NOS	9440/3
Diffuse midline glioma, H3 K27M-mutant	9385/3*
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9450/3
Oligodendroglioma, NOS	9450/3
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9451/3
Anaplastic oligodendroglioma, NOS	*9451/3*
Oligoastrocytoma, NOS	*9382/3*
Anaplastic oligoastrocytoma, NOS	*9382/3*
Other astrocytic tumours	
Pilocytic astrocytoma	9421/1
Pilomyxoid astrocytoma	9425/3
Subependymal giant cell astrocytoma	9384/1
Pleomorphic xanthoastrocytoma	9424/3
Anaplastic pleomorphic xanthoastrocytoma	9424/3
Ependymal tumours	
Subependymoma	9383/1
Myxopapillary ependymoma	9394/1
Ependymoma	9391/3
Papillary ependymoma	9393/3
Clear cell ependymoma	9391/3
Tanycytic ependymoma	9391/3
Ependymoma, *RELA* fusion-positive	9396/3*
Anaplastic ependymoma	9392/3
Other gliomas	
Chordoid glioma of the third ventricle	9444/1
Angiocentric glioma	9431/1
Astroblastoma	9430/3
Choroid plexus tumours	
Choroid plexus papilloma	9390/0
Atypical choroid plexus papilloma	9390/1
Choroid plexus carcinoma	9390/3
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	9413/0
Gangliocytoma	9492/0
Ganglioglioma	9505/1
Anaplastic ganglioglioma	9505/3
Dysplastic cerebellar gangliocytoma (Lhermitte–Duclos disease)	9493/0
Desmoplastic infantile astrocytoma and ganglioglioma	9412/1
Papillary glioneuronal tumour	9509/1
Rosette-forming glioneuronal tumour	9509/1
Diffuse leptomeningeal glioneuronal tumour	
Central neurocytoma	9506/1
Extraventricular neurocytoma	9506/1
Cerebellar liponeurocytoma	9506/1
Paraganglioma	8693/1
Tumours of the pineal region	
Pineocytoma	9361/1
Pineal parenchymal tumour of intermediate differentiation	9362/3
Pineoblastoma	9362/3
Papillary tumour of the pineal region	9395/3
Embryonal tumours	
Medulloblastomas, genetically defined	
Medulloblastoma, WNT-activated	9475/3*
Medulloblastoma, SHH-activated and *TP53*-mutant	9476/3*
Medulloblastoma, SHH-activated and *TP53*-wildtype	9471/3
Medulloblastoma, non-WNT/non-SHH	9477/3*
Medulloblastoma, group 3	
Medulloblastoma, group 4	
Medulloblastomas, histologically defined	
Medulloblastoma, classic	9470/3
Medulloblastoma, desmoplastic/nodular	9471/3
Medulloblastoma with extensive nodularity	9471/3
Medulloblastoma, large cell / anaplastic	9474/3
Medulloblastoma, NOS	9470/3
Embryonal tumour with multilayered rosettes, C19MC-altered	9478/3*
Embryonal tumour with multilayered rosettes, NOS	*9478/3*
Medulloepithelioma	9501/3
CNS neuroblastoma	9500/3
CNS ganglioneuroblastoma	9490/3
CNS embryonal tumour, NOS	9473/3
Atypical teratoid/rhabdoid tumour	9508/3
CNS embryonal tumour with rhabdoid features	*9508/3*
Tumours of the cranial and paraspinal nerves	
Schwannoma	9560/0
Cellular schwannoma	9560/0
Plexiform schwannoma	9560/0

Appendix I, Table I – From: Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016 Jun 9;131(6):803–20.

Appendix I – Continued - WHO classification of tumours of the central nervous system, 2016,

Melanotic schwannoma	9560/1
Neurofibroma	9540/0
Atypical neurofibroma	9540/0
Plexiform neurofibroma	9550/0
Perineurioma	9571/0
Hybrid nerve sheath tumours	
Malignant peripheral nerve sheath tumour	9540/3
Epithelioid MPNST	9540/3
MPNST with perineurial differentiation	9540/3
Meningiomas	
Meningioma	9530/0
Meningothelial meningioma	9531/0
Fibrous meningioma	9532/0
Transitional meningioma	9537/0
Psammomatous meningioma	9533/0
Angiomatous meningioma	9534/0
Microcystic meningioma	9530/0
Secretory meningioma	9530/0
Lymphoplasmacyte-rich meningioma	9530/0
Metaplastic meningioma	9530/0
Chordoid meningioma	9538/1
Clear cell meningioma	9538/1
Atypical meningioma	9539/1
Papillary meningioma	9538/3
Rhabdoid meningioma	9538/3
Anaplastic (malignant) meningioma	9530/3
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma**	
Grade 1	8815/0
Grade 2	8815/1
Grade 3	8815/3
Haemangioblastoma	9161/1
Haemangioma	9120/0
Epithelioid haemangioendothelioma	9133/3
Angiosarcoma	9120/3
Kaposi sarcoma	9140/3
Ewing sarcoma / PNET	9364/3
Lipoma	8850/0
Angiolipoma	8861/0
Hibernoma	8880/0
Liposarcoma	8850/3
Desmoid-type fibromatosis	8821/1
Myofibroblastoma	8825/0
Inflammatory myofibroblastic tumour	8825/1
Benign fibrous histiocytoma	8830/0
Fibrosarcoma	8810/3
Undifferentiated pleomorphic sarcoma / malignant fibrous histiocytoma	8802/3
Leiomyoma	8890/0
Leiomyosarcoma	8890/3
Rhabdomyoma	8900/0
Rhabdomyosarcoma	8900/3
Chondroma	9220/0
Chondrosarcoma	9220/3
Osteoma	9180/0
Osteochondroma	9210/0
Osteosarcoma	9180/3
Melanocytic tumours	
Meningeal melanocytosis	8728/0
Meningeal melanocytoma	8728/1
Meningeal melanoma	8720/3
Meningeal melanomatosis	8728/3
Lymphomas	
Diffuse large B-cell lymphoma of the CNS	9680/3
Immunodeficiency-associated CNS lymphomas	
AIDS-related diffuse large B-cell lymphoma	
EBV-positive diffuse large B-cell lymphoma, NOS	
Lymphomatoid granulomatosis	9766/1
Intravascular large B-cell lymphoma	9712/3
Low-grade B-cell lymphomas of the CNS	
T-cell and NK/T-cell lymphomas of the CNS	
Anaplastic large cell lymphoma, ALK-positive	9714/3
Anaplastic large cell lymphoma, ALK-negative	9702/3
MALT lymphoma of the dura	9699/3
Histiocytic tumours	
Langerhans cell histiocytosis	9751/3
Erdheim–Chester disease	9750/1
Rosai–Dorfman disease	
Juvenile xanthogranuloma	
Histiocytic sarcoma	9755/3
Germ cell tumours	
Germinoma	9064/3
Embryonal carcinoma	9070/3
Yolk sac tumour	9071/3
Choriocarcinoma	9100/3
Teratoma	9080/1
Mature teratoma	9080/0
Immature teratoma	9080/3
Teratoma with malignant transformation	9084/3
Mixed germ cell tumour	9085/3
Tumours of the sellar region	
Craniopharyngioma	9350/1
Adamantinomatous craniopharyngioma	9351/1
Papillary craniopharyngioma	9352/1
Granular cell tumour of the sellar region	9582/0
Pituicytoma	9432/1
Spindle cell oncocytoma	8290/0
Metastatic tumours	

The morphology codes are from the International Classification of Diseases for Oncology (ICD-O) (142A). Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumours. The classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions.
*These new codes were approved by the IARC/WHO Committee for ICD-O. *Italics*: Provisional tumour entities. **Grading according to the 2013 *WHO Classification of Tumours of Soft Tissue and Bone*.

Appendix I, Table I continued – From: Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016 Jun 9;131(6):803–20.

Appendix II - Grading system, WHO classification of tumours of the central nervous system, 2016,

WHO grades of select CNS tumours

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	II
Anaplastic astrocytoma, IDH-mutant	III
Glioblastoma, IDH-wildtype	IV
Glioblastoma, IDH-mutant	IV
Diffuse midline glioma, H3 K27M–mutant	IV
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	II
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	III
Other astrocytic tumours	
Pilocytic astrocytoma	I
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumours	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
Ependymoma, *RELA* fusion-positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Angiocentric glioma	I
Chordoid glioma of third ventricle	II
Choroid plexus tumours	
Choroid plexus papilloma	I
Atypical choroid plexus papilloma	II
Choroid plexus carcinoma	III
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	I
Gangliocytoma	I
Ganglioglioma	I
Anaplastic ganglioglioma	III
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	I
Desmoplastic infantile astrocytoma and ganglioglioma	I
Papillary glioneuronal tumour	I
Rosette-forming glioneuronal tumour	I
Central neurocytoma	II
Extraventricular neurocytoma	II
Cerebellar liponeurocytoma	II
Tumours of the pineal region	
Pineocytoma	I
Pineal parenchymal tumour of intermediate differentiation	II or III
Pineoblastoma	IV
Papillary tumour of the pineal region	II or III
Embryonal tumours	
Medulloblastoma (all subtypes)	IV
Embryonal tumour with multilayered rosettes, C19MC-altered	IV
Medulloepithelioma	IV
CNS embryonal tumour, NOS	IV
Atypical teratoid/rhabdoid tumour	IV
CNS embryonal tumour with rhabdoid features	IV
Tumours of the cranial and paraspinal nerves	
Schwannoma	I
Neurofibroma	I
Perineurioma	I
Malignant peripheral nerve sheath tumour (MPNST)	II, III or IV
Meningiomas	
Meningioma	I
Atypical meningioma	II
Anaplastic (malignant) meningioma	III
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma	I, II or III
Haemangioblastoma	I
Tumours of the sellar region	
Craniopharyngioma	I
Granular cell tumour	I
Pituicytoma	I
Spindle cell oncocytoma	I

Appendix II, Table I – From: Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016 Jun 9;131(6):803–20.