Neonatal CNS Ischemia: Physics Perspective

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Introduction

Perinatal hypoxic-ischaemic brain injury in the term infant remains a significant problem throughout the world. Neonatal encephalopathy (NE) is the clinical manifestation of the ensuing disordered neonatal brain function and occurs in 1-3 per 1000 live term births in the UK and other developed world countries (1). Serious consequences follow moderate to severe NE; these include death in 10-15%, cerebral palsy in 15% and other significant cognitive, developmental and behavioural problems in 40% of survivors (2). The financial and human costs to the family and society are thus very high; an expert group led by the Chief Medical Officer in 2000 estimated that preventing 10% of birth related adverse events would save £20 million a year in the UK.

Recently, a meta-analysis of three large pragmatic trials has shown that therapeutic hypothermia reduces death or disability at 18 months with a risk ratio of 0.81 (95% CI 0.71-0.93) and a number needed to treat of 9 (5-25) (3). This clearly points to the urgent need to improve neuroprotective interventions, as hypothermia offers just an 11% reduction in risk of death or disability from 58% to 47% (3).

We and several groups around the world are therefore continuously trying to further develop neuroprotection in this patient population, based on animal studies, and to translate it into phase I/II clinical trials (4). One of the most difficult problems to solve in this case is the choice of biomarker to use, both in the animal models as well as in the patients. In addition, any advance of this field will rely on relatively large phase II (typically 100-200 babies) clinical trials. For this reason, advanced MRI/S technologies (MRS, advanced diffusion-based schemes, Arterial Spin Labeling, etc...) are being proposed as potential biomarkers for such studies. Note that these methods will pose additional problems, as they will require dedicated Quality Control (QC) and robust processing pipelines due to the typical issues of this patient population, such as e.g. motion artifacts.

Therefore, in this lecture, I will present some of these advanced methods, from the most established to the most experimental, and its potential to be used as biomarker in clinical trials, including the dedicated QC methods needed to ensure proper implementation across multiple hospitals. In the next section, a summary of each of these methods can be found.

MRI & MRS methods

MRS

One of the oldest biomarkers used in NE has been the ratio of lactic acid (Lac) over N-Acetyl-aspartate (NAA), as measured by ^{1}H magnetic resonance spectroscopy (MRS). These two metabolites are among the four most visible

metabolites measured by MRS: Creatine, Choline, NAA and Lactate. All of these metabolites are typically in the range of 1-10 mM, and provide a robust signal, with long-enough T_2 relaxation times to allow its detection by MRS (5). Generally, Creatine (Cr) measured by MRS represents the sum of both Cr and the phosphorylated Cr (PCr), and remains constant for most cases as Cr and PCr are found in stoichiometric ratios depending on the energy level of the cells (5). Choline (Cho) is a complicated metabolite to understand but is mostly related to the level of myelin and its potential damage in the brain (5). NAA is thought to only be present in functioning neurons, and will therefore decrease with neuronal death (5). Finally, Lac is the product of anaerobic metabolism, by direct transformation of pyruvate into lactate, once normal mitochondrial function is impaired (5).

Recently, thalamic Lac/NAA has been shown to be the most robust MR predictor of neurodevelopmental outcome of all MR measures: when acquired in the first 14 days, the metabolite ratio accurately predicts outcome with a sensitivity and specificity of 82% (95% confidence interval 74-89%) and 95% specificity (95% CI 88-99%) (6). It is noteworthy to state that the same biomarker can be used in preclinical studies on piglets, and has been shown to correlate very well with cell death at 48 h (4,7). As such, it is a very important translational biomarker, allowing for a rapid and well-assessed early translation of potential novel therapies (i.e. Xenon (7) or melatonin (4)) into the clinics.

Diffusion

Interestingly, diffusion weighted imaging (DWI) has not shown in NE the same utility as in adult stroke (6). For this reason, more advanced methods have been used in clinical trials, based on the diffusion patterns in white matter. These methods are based on Diffusion Tensor Imaging (DTI), a method allowing estimating the principal directions of water diffusion through the brain (8).

In particular, Tract Based Spatial Statistics (TBSS) of the posterior limb of the internal capsule has been shown to provide a sensitive quantitative measure which allows group-wise comparisons in babies (9,10). TBSS is an automated observer independent method of aligning fractional anisotropy (FA) images from multiple subjects to make non-biased assessments of localised changes in the major white matter tracts. A recent TBSS study (11) from 43 subjects who underwent hypothermia for NE showed a difference between those with favourable and unfavourable outcome at a median age of 18 (range 12-28) months and a significant linear correlation between FA values and DQ, locomotor, personal-social, hearing and language, eye-hand coordination and performance sub-scale scores throughout the whiter. DTI analysed by TBSS therefore provides a qualified biomarker to assess the efficacy of neuroprotective therapies.

Perfusion

Interestingly, perfusion imaging has not been widely used so far in clinical trials, and only a few recent publications have shown the potential for this method to be used in NE (12-14). This is due in parts to the difficulties and potential ethical issues in using Gd-based contrast agents in this patient population. In addition, the non-invasive alternative, Arterial Spin Labeling (ASL), is particularly difficult to use in this patient population. Indeed, ASL is based on the consecutive (interleaved) acquisition of two MRI experiments (15). In the first experiment

arterial water spins are labeled upstream from the tissue of interest by the combined application of field gradients and RF pulses. This labeling usually consists of an inversion of the arterial water magnetization. An image is then acquired after a suitable delay time, during which the RF-labeled arterial water spins have the time to travel to the tissue of interest, and to exchange there with the stationary spins located in the extravascular compartment. In the second experiment, a sham labeling is performed, to set the system in the same conditions as the first experiment, and a second image of the tissue of interest is acquired. Ideally, the only difference between the labeled acquisition and this "control" acquisition is that the latter has no manipulation of the arterial spins. Both labeling and control acquisitions are acquired after a time TI ("inversion time"). The difference between both images will provide a signal proportional to the exchanged water magnetization and therefore water delivered to the tissue at the time TI.

As such, ASL is difficult to use in babies, mainly due to the presence of a very low SNR (babies have a very low cerebral blood flow (CBF)), but also to the fact that motion artefacts are particularly problematic for this type of methods and dedicated pipelines need to be put in place to allow reproducible measures of CBF in babies with NE. Such measures include a very detailed and optimised set of sequence parameters, relatively long acquisition times (±10 min), and advanced motion correction procedures, coupled with automatic rejection of too badly motion-corrupted sets of control and label acquisitions.

pH imaging (MRS & CEST)

Since the 1980s there have been only few studies using ^{31}P MRS as outcome biomarkers in babies following asphyxia, as this is not routinely available on clinical scanners, but the decline in the brain energy measured in this manner in the days and hours after birth strongly correlates with neurodevelopmental outcome and head growth (16).

^{31}P MRS, however, can provide unique information such as brain intracellular pH (pHi) that may be vital to our understanding of the response to hypoxia-ischaemia and lead to possible new avenues of neuroprotection; there is no other non-invasive way to measure absolute pHi. Brain pHi can be measured using the chemical shift difference of inorganic phosphate (Pi) (17), PCr and ATP (18). The pHi value calculated from Pi is thought to reflect the pHi in dead or injured cells whereas that derived from other metabolites may reflect different cell populations. A very important study showed, however, that a brain pHi of 7.15 and above measured in the first 2 weeks after birth had a sensitivity of 71% and specificity of 92% for predicting adverse outcome (19). As such, development of 1H-based estimation of pH would be of extreme interest for this field.

Since this early study, there have been significant advances in MR techniques that enable us to determine brain pHi at higher resolution.

In particular, Amide Proton Transfer (APT), is a technique that has recently been demonstrated to be sensitive to pH changes. APT-based imaging is a variant of magnetization transfer (MT) imaging (20), in which the selective saturation of the magnetization of amide protons is detected indirectly through chemical exchange with bulk water protons. Selective irradiation is possible because there is a composite amide proton resonance at approximately 3.5 ppm downfield from the water resonance, and in the brain, amide protons exchange with water

protons at a rate of about 30 times per second, providing conditions of slow exchange on the NMR time scale (20). The amide pool is small (mM concentration range), but continuous saturation leads to a measurable decrease by a few percent of the large water signal due to a sensitivity enhancement mechanism, analogous to chemical exchange saturation transfer (CEST) (20).

The transfer rate between amide protons and water is pH dependent and follows the base-catalyzed amide proton relationship (20): $pH=6.4 + log_{10} k_{ws}/5.57$ where k_{ws} is the exchange rate of amide protons. This new technique is safe, being similar to conventional magnetization transfer MRI, and has been used to define pHi changes after stroke in adults (21). As such, it shows a great potential for being used as a biomarker in NE as well.

Conclusion

In conclusion, the future for NE is bright, and the use of advanced MRI and MRS techniques will allow further development of neuroprotective interventions in the clinic, especially when used in multi-centre clinical trials. The caveat in this case is of course that the most advanced techniques presented here will first need to be established before being optimised for its use in such trials. However, the oldest techniques listed here (MRS thalamus Lac/NAA and TBSS) are already currently being used in phase II clinical trials.

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