Quantification and validation of imaging biomarkers in preclinical models of Alzheimer’s disease
(Towards the use of biomarkers for translational medicine and therapy development)

Absolute Beginner’s Guide to Translational Neuroimaging
Friday Sunrise Educational Course – Quantification & Validation of MRI Data

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Objectives: To describe MRI biomarkers used in preclinical evaluation of Alzheimer’s disease. To show how these markers can be validated and quantified. Provide some examples of their use in the evaluation of anti-Alzheimer therapies.

1. Introduction
Alzheimer’s disease (AD) is a severe dementia that leads to the withdraw from social life. Today, it represents 50 to 75% of the 24.3 millions demented persons in the world (Ferri, Prince et al. 2005). This emphasizes the public health issue that it represents.

This disease is characterized by two main microscopic lesions that must be evidenced to provide a full diagnosis: neurofibrillary tangles (NFT) and senile plaques. NFT are intraneuronal accumulation of abnormally phosphorylated tau proteins. Senile plaques are aggregated extracellular deposition of amyloid beta peptides (Aβ). Aβ accumulates also in the perivascular space of the blood vessels leading to cerebral amyloid angiopathy (CAA) in almost 80% of the patients (Vinters, Wang et al. 1996). Recently, several studies also suggested a critical role of intracellular amyloid deposits (Gouras, Tsai et al. 2000) and highly toxic soluble oligomeric forms of amyloid (Lacor, Buniel et al. 2004) in the physiopathology of AD. All these primary lesions lead to neuronal dysfunctions followed-up by dendritic and neuronal losses. One of the most used hypothesis to explain the disease is the amyloid cascade hypothesis. It suggests that amyloid is at the origin of the lesions associated to AD (Hardy and Selkoe 2002).

To date there is no curative treatment against AD. The only treatments that are available modify the symptoms but do not modify the course of the pathology. Many disease modifying treatments such as therapies targeting the amyloid and tau pathologies are in investigation (Margialasche, Solomon et al. 2010). The development of these treatments relies on translational medicine (Fig. 1).

The goal of translational medicine is to evaluate in animal models the effects of therapies on specific targets (for example amyloid or tau pathology) or on functional outcomes and to use results in animals to predict effects in humans. The most widely used models are transgenic mice (Duyckaerts, Potier et al. 2008). Models of amyloidosis are based on the overexpression of mutated forms of amyloid precursor proteins (APP) and possibly of mutated presenilin (PS1 or PS2) genes (Delatour, Le

Cudennec et al. 2006). Transgenic mice overexpressing pathological tau proteins are models of NFT (Oddo, Caccamo et al. 2003). Spontaneous models of AD are also used (Dhenain 2001). In particular, aged primates develop alterations such as cerebral amyloid deposits (Bons, Mestre et al. 1991), tau pathology (Schultz, Hubbard et al. 2000), cerebral atrophy (Dhenain, Chenu et al. 2003), and cognitive impairments (Bartus and Dean 1985; Picq, Aujard et al. In Press).

Invasive markers such as histological or biochemical measures of amyloid or tau loads are widely used in animals. Imaging biomarkers can be much more powerful preclinical measures as they can potentially be followed-up in animals and humans. However, these biomarkers should be pertinent and quantitative. Here, we will present some of the markers used to quantify Alzheimer pathology and evaluate anti-AD therapies at the preclinical level. We will first describe biomarkers of tau and amyloid pathology. Then we will focus on functional markers that might reflect the efficiency of evaluated drugs. Toxicology markers will be presented in the last part of the presentation.

2. Detection and quantification of cerebral atrophy: a marker of tau pathology

At the macroscopic level, brains from AD patients are characterized by a severe atrophy leading to dilation of the ventricular system and a widening of cortical sulci (Valk, Barkhof et al. 2002). In the early stages of the disease, the atrophy process affects mainly medial temporal areas including the hippocampal formation. It is a marker of disease progression in clinical trials (Albert, DeCarli et al. 2005) and is mainly correlated to the progression of the tau pathology (Whitwell, Josephs et al. 2008).

Cerebral atrophy has been largely studied in mouse models of AD. Most of the early studies were based on manual segmentation methods followed by volumetric measurements. These methods require a priori hypotheses about atrophied structures. Alternatively, automatic methods such as voxel based morphometry (VBM) (Sawiak, Wood et al. 2009) or deformation based morphometry (DBM) (Lau, Lerch et al. 2008) can be powerful, automated techniques to detect anatomical differences between populations. For example, the comparison of neuroanatomical differences between APP/PS1 model of amyloidosis and wild-type littermates with these methods was able to detect morphometric differences localized at the level of the hippocampus, cortex, olfactory bulbs, stria terminals, brain stem, cerebellum, and ventricles (Lau, Lerch et al. 2008). Interestingly in this study, a strong correlation between DBM measures and manually segmented volumes was established. This suggests that both methods are reliable. However, the great interest of automatic methods relies on their automatic character which allows exploring cerebral atrophy without a priori knowledge. The methods have also the advantage to be user-independent and automatic. They also detect atrophies in regions that can not be outlined easily such as the entorhinal cortex (Sawiak and Dhenain 2011).

The main results from studies of cerebral atrophy suggest that mouse models of amyloidosis display an atrophy of various brain regions. However, the atrophy process can involve amyloid-free regions (midbrain, internal capsule, white matter fiber tracts such as the corpus callosum and fornix (Dodart, Mathis et al. 2000; Gonzalez-Lima, Berndt et al. 2001; Weiss, Venkatasubramanian et al. 2002; Redwine, Kosofsky et al. 2003; Delatour, Guegan et al. 2006) while cortical brain areas with high amyloid burden are not atrophied (Delatour, Guegan et al. 2006). These alterations are often observed in young animals and show no further deterioration in older mice. Because of their early occurrence, these lesions might thus be viewed as a neurodevelopmental deficit rather than an age-related brain shrinkage induced by progressive deposits of Aβ. The lack of clear relationship between amyloid load and cerebral atrophy can justify why cerebral atrophy has not been used as a biomarker of amyloid load in preclinical therapeutic trials. On the contrary, studies on mouse models of tau pathology have shown a severe age-associated cortical and hippocampal atrophy pattern (Yang, Xie et al. 2011), thus suggesting that cerebral atrophy is a marker of the tau pathology. Cerebral atrophy might thus be used as a marker of tau pathology in preclinical therapeutic trials.

3. Detection and quantification of amyloid load

Amyloid deposition is a very early event in the course of AD as it occurs up to 20 years before the clinical occurrence of the disease (Sperling, Aisen et al. 2011). It is thus the target of most of the current therapeutic trials against AD. Being able to follow-up amyloid plaques by imaging methods is thus of major interest. In humans, currently, amyloid plaque imaging relies mainly on positron emission tomography (PET) and on the use of specific radioligands (PIB (Klunk, Engler et al. 2004) or AV45 (Choi, Golding et al. 2009)).

In animals, these radioligands are less able to detect amyloid plaques than in humans (Kluck, Lopresti et al. 2005). Also, the resolution that can be reached by PET does not make it easy to detect amyloid plaques. On the contrary, MRI is able to detect individual amyloid plaques. Approaches based on the natural contrast of the plaques or on dedicated targeted or non targeted contrast agents have been explored. In the absence of contrast agents, the plaques appear as dark spots in T2, T2*-
Because of the wide interest for amyloid plaque imaging by MRI, one can expect that many innovative methods will be developed in the future. Several steps can be used to validate a new method of amyloid plaque detection. First one needs to register MR images with histological sections. Most of the studies are based on manual registration of 3D MRI images on 2D histological images and on the recognition of specific landmarks such as blood vessels or amyloid plaques on both images (Dhenain, Privat et al. 2002). Another option is to register 3D MR images with 3D histological images (Lebenberg, Herard et al. 2010). The validation of the ability of MRI to detect amyloid plaques can also be based on correlative studies between the amyloid load detected on histological sections and the amyloid load detected on MR images (Jack, Wengenack et al. 2005; Petiet, Santin et al. In press (doi:10.1016/j.neurobiolaging.2011.03.009)).

Once amyloid plaques have been detected, it is critical to quantify their load in the brain. As for cerebral atrophy, several methods have been proposed. The easiest one is based on the counting of the plaques within regions of interest inside the brain (Petiet, Santin et al. In press (doi:10.1016/j.neurobiolaging.2011.03.009)). This method does not need strong image processing skills but is time-consuming. That is why it can not be applied in a routine environment to test new drugs. More sophisticated methods have also been developed. For example Chamberlain developed a method based on the subtraction of filtered images (2D median filter) and original images. This leaves mainly small size structures (amyloid plaques) that can be selected by using a thresholding method (Chamberlain, Wengenack et al. 2010). Some authors have also experimented more sophisticated protocols based on segmentation parameters using support vector machines (SVM) in an unsupervised way (Vapnik 1998). This approach uses simulated flooding (watersheds) to define and count low intensity regions surrounded by higher intensity neighbors (Iordanescu, Venkatasubramanian et al. 2009). Some other methods are based on the evaluation of relaxation parameters that are modulated by amyloid plaques (Scholtzova, Wadghiri et al. 2008). Finally, voxel-based analysis (VBA) allows to compare cohorts of animals in a statistical un-biased quantitative way (Sigurdsson, Wadghiri et al. 2008).

Interestingly, the quantification of amyloid plaques by MRI is now used to evaluate drug efficacy (Scholtzova, Wadghiri et al. 2008; Yang, Dai et al. 2011).

4. Brain function: Examples of quantitative measures of perfusion and axonal transportation

In humans, impaired cerebral perfusion is reported in AD patients (Alsop, Detre et al. 2000; Johnson, Jahng et al. 2005). Such alteration, if detected in animals might be a non-invasive marker of...
disease progression and more importantly, a marker of functional improvement associated to an efficient treatment.

Studies by MRI in transgenic models of amyloidosis have outlined perfusion alterations (Weidensteiner, Metzger et al. 2009; Faure, Verret et al. 2011; Poisnel, Herard et al. In Press (DOI information: 10.1016/j.neurobiolaging.2011.09.026)) and changes of hemodynamic response (Mueggler, Sturchler-Pierrat et al. 2002). To date, in most studies, the quantification of the perfusion is based on measurements within regions of interests. Although these functional markers are of great interest, they are not routinely used during therapeutic evaluations.

Functional alterations can also be evaluated at the level of the neurons by using manganese enhanced MRI (MEMRI). MEMRI relies on manganese administration either directly into the brain or in a systemic way (Smith, Kalthoff et al. 2007). Manganese is an analog of calcium that has paramagnetic properties. During neuronal physiological activation, extracellular manganese enters neurons through voltage-gated Ca\(^{2+}\) channels (Koretsky and Silva 2004). In addition, intracellular manganese is transported along axons and across synapses thus allowing for tract tracing as shown in the olfactory (Pautler, Silva et al. 1998) or optic (Watanabe, Michaelis et al. 2001) systems. The transport of manganese occurs along microtubules via fast axonal transport. Thus MEMRI has been proposed as a way to dynamically measure the rates of manganese transportation (probably within mitochondria), which is reflective of fast axonal transport rates. In a first study, Smith et al. injected manganese in the olfactory system of mouse models of amyloidosis. They showed a significant decrease in axonal transport rates when the Aβ levels increase even before plaque formation. After plaque formation, the decline in the transport rate becomes even more pronounced (Smith, Kalthoff et al. 2007). The neuronal transportation alterations can be related to intraneuronal Aβ accumulation (Wirths, Muthuap et al. 2001), to a direct effect of Aβ on actin or axonal swelling (Wirths, Weis et al. 2006) or to an effect of oligomeric Aβ (Pigino, Morfini et al. 2009). More recently, the same authors have shown that treatments that reduce the amyloid load lead to a recovery of the axonal transportation. Thus MEMRI is able to detect recovery of function in reaction to a therapy against amyloidosis (Massaad, Amin et al. 2010; Smith, Paylor et al. 2011). MEMRI has also been used to evaluate modulation of axonal transportation induced by tau pathology (Bertrand, Hoang et al. 2011). It can also be used to evaluate functional interactions between genes such as presenilin and tau (Peethumngongsin, Yang et al. 2010).

MEMRI is thus a unique marker of neuronal transportation. However, methods of quantifications of axonal transportation by MEMRI are still an ongoing field of research. First methods were based on single-scan protocols (Smith, Paylor et al. 2011). More recently methods based on the follow-up of several eight MR sessions (from one hour to 7 days after MnCl\(_2\) administration) have been proposed (Bertrand, Hoang et al. 2011). The signal on each scan can then be fitted to models to determine the “peak value”, “time-to-peak” and “maximal slope” parameters (Bertrand, Hoang et al. 2011). The physiological values of these parameters must still be fully understood (calcium uptake, transport of mitochondria and vesicles, synaptic transmission, neuronal density…?) and compared to a gold standard method such as videomicroscopy that is mainly used on cell cultures or living brain slices.

5. Toxicologic biomarkers

Toxicology is another point of great interest in the evaluation of therapies against AD. Indeed, several treatments against AD failed in clinical trials because of side effects such as microhaemorrhages (Boche, Zotova et al. 2008) or encephalomyelitis (Orgogozo, Gilman et al. 2003).

Microhaemorrhages can be detected on T2*W images by looking at hypointense spots either without using any contrast agent (Luo, Rustay et al. 2011)(Fig. 3) or by looking at peripheral blood-borne macrophages after intravenous administration of iron oxide nanoparticles (Beckmann, Gerard et al. 2011). The detection of microhaemorrhages by MRI is currently used to evaluate the effects of therapies such as passive amyloid immunotherapy (Beckmann, Gerard et al. 2011; Luo, Rustay et al. 2011). As for amyloid plaques, the validation of the methods is based mainly on manual registrations of 3D MR images and histological sections. The quantification of the microhaemorrhages is based on manual counting of hypointense regions on MR slices.
Vasogenic edema can also be detected by MRI in animals (Penet, Viola et al. 2005; Serduc, van de Looij et al. 2008). However, so far, vasogenic edemas induced by anti-alzheimer therapies have not been detected by MR evaluations.

6. Conclusion
In this course, we will illustrate that MRI can evaluate the modification of targets of anti-alzheimer therapies (amyloid, tau), that it can detect potential functional improvements following the modification of these targets and that it can reveal side effects of these treatments. Currently the quantification of MR parameters is largely based the evaluation of regions of interest and manual counting of information from these regions of interest. This method is time consuming and can not be applied during routine evaluation of drugs at a large scale. High throughput, user-independent, non-a-priori, and automatic methods are thus developed to speed-up the evaluation of parameters from MR images.

References


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