Novel imaging markers for neuroinflammation in multiple sclerosis

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Chapter 4

Summary and general discussion

Introduction

In this thesis we studied novel *in vivo* imaging tools for evaluation of neuroinflammation in multiple sclerosis (MS). The first half of the thesis is oriented on the clinical applicability of high field magnetic resonance imaging (MRI). The second half is of a more exploratory nature, studying the possibilities of positron emission tomography (PET). As MRI has already been used as a paraclinical tool for diagnosis and follow-up of MS patients for two decades,[1, 2] research questions are less related to the usefulness of MRI, but more regarding ways to improve the technique. On the contrary, PET imaging in MS is relatively new and many questions still need to be addressed before it can be used in everyday clinical practice. Here we present a summary of our findings and discuss their relevance in relation other recent developments.

Summary: Clinical MRI

In chapter 2 we present the results of a prospective multi-centre study, imaging 66 clinically isolated syndrome (CIS) patients at both standard 1.5 Tesla (T) MRI and high field 3T MRI during the first 2 years after diagnosis. In addition, 26 healthy control subjects (HC) were included in this study. Intuitively, one would expect the number of lesions detected to increase as the field strength increases and this has been confirmed by previous studies.[3-5] However, the clinical relevance of this increased lesion detection has remained uncertain. Moreover, to improve the applicability to everyday clinical practice, all centres used acquisition protocols based on local optimised scanning protocols.

In chapter 2.1 we describe a central reading with eight different raters using a subset of the CIS and HC scans. To determine the inter-rater agreement on lesion detection, all raters independently scored the number of lesions per scan per anatomical region (periventricular, juxtacortical, deep white matter, infratentorial and spinal cord). Overall the agreement on the involvement per anatomical region was moderate to good for both field strengths, with the agreement being lowest for juxtacortical lesions. Secondly, we divided the group of raters into experienced (neuro-radiologists) and less-experienced (radiology residents and PhD students). The inter-rater agreement on lesion detection decreased for 3T for the less-experienced raters compared to the experienced raters. It appears that the effect of experience is more important for correct interpretation of high field MRI, compared to 1.5T. Therefore, this study illustrates that field strength does not impact the agreement of lesion detection, but high field MRI does require additional training.

Subsequent application of the 2010 revisions of the McDonald criteria for diagnosis of MS,[6] also had a moderate to good inter-rater agreement, which was not substantially influenced by field strength. However, these diagnostic criteria presented a new challenge. Even though all eight raters were well familiar with the diagnostic criteria, a good working knowledge of these complex criteria was not without doubt. This supports the simplification of the diagnostic criteria in 2017.[7]

The full cohort is analysed in chapter 2.2 with a consensus score between three different raters from the VUmc. This multi-centre and multi-vendor cohort study confirms the increased lesion detection demonstrated in previous single-centre studies. The improved lesion detection at 3T was seen at the periventricular, (juxta)cortical and deep white matter regions. For the healthy controls no difference in lesion detection between the two field strengths, only tendency towards an increase in deep white matter lesions. In addition to evaluation of the different brain regions, at baseline also spinal cord scans were acquired at both field strengths. This is important as spinal cord lesions are not only relevant for the fulfilment of the criteria for dissemination in space and time, but are also predictive of conversion to clinically definite MS.[8, 9] As spinal cord is prone to various artefacts due to breathing and swallowing of the patient and pulsation of blood vessels and the cerebral spinal fluid,[8] it is important that this did not decrease the lesion detection at high field MRI.
Subsequent to the lesion detection, the diagnostic criteria for MS were applied. There was no difference in fulfilment of the criteria between the two field strengths for either the 2010 or 2017 revisions of the McDonald criteria or the criteria proposed by the MAGNIMS study group in 2016.[6, 7, 10] From that we can conclude that the clinical relevance of the increased lesion detection rate at high field MRI is very limited in the diagnostic workup of patients with CIS. However, it is important to realise the effect of field strength on lesion detection when scanning at different vendors during the follow-up of an individual patient.

In conclusion, there is no real added clinical benefit in opting for either one of the field strength for the diagnostic workup of CIS patients.

Summary: Microglia- and immuno-PET

The molecular imaging technique PET enables the *in vivo* quantification of pathophysiological processes. This enables the *in vivo* identification of molecular processes involved neuroinflammation (and neurodegeneration), adding to the information conventional MRI provides us on the results from these processes: T2 MS lesions, enhancing lesions and atrophy. Moreover, PET can quantify low-grade neuroinflammation not detected by conventional MRI, as this does not result in blood-brain barrier disruption and therefor gadolinium enhancement. This can provide unique information on disease severity and disease sub-type as early as the diagnosis, or possibly as part of the diagnosis. Furthermore, the effect of treatment could be evaluated quickly, responding to the growing demand for tailor made or personalised medicine. In addition, this could be a highly valuable tool in the development of new drugs. This would require PET tracers and PET analysis that specifically, sensitively and reliably answer clinical questions. Current research is aimed at developing such methods.

To date, the most important PET marker for neuroinflammation is the 18kDa-translocator protein (TSPO), upregulated on the mitochondria of microglia.[11] The dynamic and complex process of microglia activation is a hallmark of neuroinflammation in MS.[12, 13] In chapter 1.2 a list of studied TSPO radiotracers has been provided, but with the rapid expansion of this field of research, this list is certainly not conclusive. In chapter 2.1 we describe a proof of concept study in progressive MS patients using the second generation TSPO tracer [^{18}F]DPA714. Compared to the first generation TSPO tracer [^{11}C]PK11195, [^{18}F]DPA714 has an increased bioavailability to brain tissue and its lower lipophilicity leads to less non-specific binding (to e.g. plasma proteins) and therefore a higher signal-to-noise ratio.[14-16] In this study we demonstrate that the optimal model for quantification of [^{18}F]DPA714 in MS is the reversible two-tissue compartment model with additional blood volume parameter (2T4k_V_B), equal to previous studies in healthy controls and patients with Alzheimer's disease.[17, 18] As TSPO is not selective to microglia only, with additional binding sites in monocytes, astrocytes and the vascular wall, a new model including the binding to the vascular endothelium has been proposed.[19] This adjusted 2T4k_V_B model includes a slow irreversible vascular binding component and is therefore referred to as 2T4k_V_B_1T1k. It is proposed to improve the model fits and the estimates of [^{18}F]DPA714 binding.[20] Although this new model slightly improved the model fits in our dataset, the reliability of the parameter estimates decreased and the vascular component itself could not be reliable estimated. The more standard 2T4k_V_B model did not only reliable estimate the volume of distribution (V_T), but for larger regions also the binding potential ($BP_{ND} = k_3/k_4$). For all patients an increase in BP_{ND} was seen in MS lesions compared with non-lesional white matter. This increased binding could not be demonstrated using V_T, as the inclusion of non-displaceable uptake diluted the small specific signal of [^{18}F]DPA714. More importantly, regional BP_{ND} values were also increased in non-lesional white and grey brain tissue in patients compared with controls, demonstrating a diffuse and glow-grade inflammation behind an intact blood-brain barrier in progressive MS. Unfortunately, this could only be observed in patients genotyped as high affinity binders according to the rs6971 polymorphism, approximately 50% of the general population.[21]
In conclusion, in progressive MS [^{18}F]DPA714 can identify neuroinflammation not only in MS lesions, but also in the non-lesional grey and white matter, provided that plasma input derived 2T4k_V_B BP_{ND} is used and subjects are high affinity binders when genotyped for the rs6971 polymorphism within the TSPO gene.

Even though the second generation TSPO tracers have an increased bioavailability in the brain and an improved signal-to-noise ratio compared to the first generation tracers, there are still several limitations: the rs6971 polymorphism determining genetic binding affinity, binding sites that are not specific to microglia and TSPO does not differentiate between resting state (M0), pro-inflammatory (M1 activated) and neuro-protective (M2 activated) microglia. Therefore, new PET targets for neuroinflammation have been

developed. In chapter 2.2 we studied the purinergic receptor $P2X_7$ upregulated M1 activated microglia,[22-24] with the novel tracer [^{11}C]SMW139[25, 26] in five active relapsing remitting MS patients (RRMS) and five healthy controls. [^{11}C]SMW139 has a fast influx into the brain tissue in both patients and controls, but also a fast efflux, resulting in only a small specific compartment. The tracer kinetics can best be described using the model 2T4k_V_B_k_4, with the k_4 is fixed to the whole brain grey or white matter value for the same subject. This adjustment to the more standard model increases the reliability of the parameter estimates, especially for the small regions in which the small specific compartment is difficult to estimate. Using this model, both regional V_T and BP_{ND} values are increased in the patient group compared to the healthy controls. Three of the RRMS patients had gadolinium enhancing lesions large enough for kinetic analysis. In these enhancing lesions V_T is increased compared to the non-lesional white matter. In contrast, BP_{ND} is decreased in these T1 enhancing lesions. This difference results from an increase in the K_1/k_2 ratio in the enhancing lesions, due to disruption of the blood-brain barrier, compensating the decrease in BP_{ND}. This underlines the importance of accurate pharmacokinetic modelling and careful interpretation of the different parameters obtained.[27, 28] The study as presented in this thesis is limited by its small sample size, but the promising results warrant the recruitment of additional subjects to validate these results.

In chapter 2.3 a different application of PET is studied: immuno-PET. In itself this is not a new technique, as it has been used in oncology over the past 20 years,[29, 30] but to our knowledge this is the first application in MS. In this pilot study the monoclonal antibody rituximab was labelled to the long-lived positron emitter zirconium-89 (half-life 78.4 hours). Rituximab effectively reduces the number of relapses and new MS lesions in RRMS through its effect on CD20 expressing B-cells and possibly also T-cells.[31-33] This effect on peripheral immune cells cannot fully explain the early therapeutic effect of rituximab that can be seen as early as 4 weeks after the first dose.[31] A possible explanation would be that rituximab can cross the blood-brain barrier and have a direct effect in the MS lesions. In this study two active RRMS patients underwent a PET scan following injection of [^{89}Zr]rituximab and after 3 and 6 days. During follow-up no cerebral uptake could be demonstrated and therefore the hypothesised mechanism of action of rituximab could not be confirmed.
When interpreting the results it should be noted that the labelled rituximab was administered after the therapeutic dose of unlabelled rituximab. As such we aimed to block the peripheral binding sites with the unlabelled rituximab, in order to allow for the labelled rituximab to enter the brain. In theory, the unlabelled rituximab could already have saturated the cerebral binding site and therefore have prevented the labelled rituximab from specific binding in the brain. Further studies are necessary to confirm the results from this pilot study. Moreover, as this study demonstrates the possibilities and

also the feasibility of immune-PET in MS, this imaging technique could provide us with useful insights into the mechanisms of action of other monoclonal antibodies currently in use or in development for the treatment of MS. In particular, the recently registered anti-CD20 monoclonal antibody ocrelizumab could benefit from this PET technique.[34, 35]

Future perspectives: Advanced MRI

Clinical MRI plays a pivotal role in the diagnosis and follow-up of MS, but it lacks the specificity and sensitivity to identify pathological mechanisms underlying the disease. Advanced MRI techniques have the potential to give us insight into the complex processes involved in the onset and progression of (the different subtypes of) MS. As described in chapter 1.2, many new MRI techniques are being developed to image neuroinflammation (and neurodegeneration) in MS.

Adding to the research mentioned in the introduction, recent studies have confirmed the importance of the central vein sign detected by susceptibility-weighted imaging (SWI). This thin hypo-intense line or dot in the centre of a white matter lesion, is present in a higher proportion of MS lesions compared to cerebral lesion resulting from small-vessel disease.[36] Therefore, the central vein sign can help differentiate between these two types of white matter lesions. In addition, it can help distinguish MS from mimics such as neuromyelitis optica spectrum disorder (NMOSD), as even at 3T MRI the central vein sign is significantly less frequent in NMOSD.[37]
In addition, SWI can detect a paramagnetic rim at the edge of T2 lesions in a subset of lesions, which colocalizes with ring-like contrast enhancement.[38] Longitudinal analysis at 7 Tesla shows that lesions with a persistent phase rim after the contrast enhancement has resolved, are more likely to expand during follow-up and are more likely to become T1 hypo-intense black holes.[39, 40] Histopathological analyses demonstrates the presence of iron-rich macrophages and activated microglia (after the destruction of iron-rich oligodendrocytes) at the edge of these lesions, causing the hypo-intense appearance on SWI.[39, 40] This identifies those lesions as chronic active lesions, which results in the irreversible tissue damage and the tissue loss. A s such, a paramagnetic rim can be considered a marker of chronic inflammation. Moreover, as lesions with a phase rim are rarely found in MS mimics such as NMOSD, this could potentially be an imaging marker for MS in addition to the central vein sign.[41]
Thirdly, quantitative susceptibility mapping has been proposed as a method to analyse the heterogeneous patterns of cortical lesions, difficult to image with conventional MRI.[42] Future studies could improve our understanding of the cortical demyelination.

Over the past years, perfusion-weighted imaging studies have led to the consensus that cerebral blood flow and volume are increased in active MS lesions and decreased in

normal appearing white matter and (cortical and deep) grey matter, which is associated with disease severity.[43, 44] As such, perfusion MRI appears to be a sensitive tool in the detection of cerebral inflammatory changes in MS and could have a future role in monitoring disease activity in clinical setting. This would require standardization of acquisition protocols, regarding which progress has been made by suggestion technical guidelines for arterial spin labelling.[45] Moreover, the pathophysiology of changes in brain perfusion in MS still needs clarification. An earlier longitudinal study linked the increased perfusion in MS lesion to vasodilation resulting from inflammation.[46] However, the pathogenesis of decreased perfusion in normal appearing brain tissue is uncertain.[43, 44] Additional studies are warranted to understand the roles of different hypothesised mechanisms, such as reduced energy demand due to degeneration and primary or secondary cerebral vascular insufficiency.

Leptomeningeal enhancement (LE) has been of ongoing interest in understanding the pathophysiology of MS. An increasing number of studies have described a causal relation between leptomeningeal inflammation and subpial demyelination and cortical atrophy.[47] LE on post-contrast T2-FLAIR could be an indirect marker for this leptomeningeal inflammation and is frequently seen in MS. In recent 3T studies, LE is observed in 40-50% of MS patients and is associated with cortical atrophy.[48 49] This is more frequent compared to previous studies, which could be related to technical difference in MRI acquisition. Moreover, one 7 Tesla study proposes LE to be even more frequent: in almost all of the 29 MS patients and two of the three healthy controls LE was observed.[50] However, in HC only focal or “nodular” enhancement was seen, whereas three quarters of MS patients had more diffuse or “spread” leptomeningeal enhancement, which is associated with cortical atrophy. This suggest that such nodular LE is physiological not related to MS pathology.
In addition to MS, LE appears to be common in other inflammatory neurologic conditions, such as HTLV (45%) and HIV (21%).[51] However, in these patients LE was not associated with cortical atrophy as seen in MS, probably due to different immunological reactions leading to the leptomeningeal inflammation.
This increasing body of work on LE underlines its relevance in the pathophysiology of MS. Nevertheless, determining the actual prevalence of LE and understanding the connection with cortical atrophy, still need further investigation.

Lastly, only few studies using ultrasmall superparamagnetic particles of iron oxide (USPIO) as a contrast agent have been published over the last few years, largely due to limited availability of the agent. Recent work confirm the relationship between USPIO enhancement and disease severity described in previous work. One study in 25 CIS patients demonstrated an increase in T1 hypo-intense lesions and a higher T2 lesion load after a 2 year follow-up, in patients with both USPIO and gadolinium enhancing lesions at baseline compared to patients with only gadolinium enhancing lesions.[52]

A second longitudinal study in 15 CIS patients reported greater tissue damage after a 3 year follow-up for lesions with initial USPIO enhancement compared to lesions with only gadolinium enhancement.[53] This suggest a different immunological mechanism in USPIO and gadolinium enhancing lesions, which is associated with the severity of tissue damage. Although the results are promising, the body of work studying USPIO in MS still remains limited.

Future perspectives: Novel radiotracers

TSPO tracers have formed the cornerstone of neuroinflammation PET studies in MS. However, as mentioned above the TSPO receptor and the TSPO tracer have their limitations. Therefore, novel imaging targets for neuroinflammation in MS are warranted. In regard to the discussed $P2X_7$ receptor, several additional tracers have been developed besides [^{11}C]SMW139. So far, [^{11}C]JNJ-54173717 and [^{11}C]GSK1482160 have shown promising results in non-human primates and clinical evaluation is to be expected.[26, 54]
The other PET target under development mention in the introduction is the adenosine 2A receptor (A_{2A}), which has been of great interest over the past years. Following the somewhat unsuccessful A_{2A} tracer [^{11}C]TMSX, several novel tracers have been evaluated in healthy controls *in vivo* but not in neuroinflammatory or neurodegenerative disease.[55]

Following the purinergic receptor $P2X_7$, recent studies have identified the $P2Y_{12}$ receptor as an additional target for imaging neuroinflammation in MS.[22] In contrast to $P2X_7$, associated with the pro-inflammatory microglial phenotype, the $P2Y_{12}$ receptor is expressed mainly in normal appearing brain tissue in post-mortem tissue and its expression is increased in the remission phase in animal models of MS.[22] Therefore, $P2Y_{12}$ is associated with the M2 or neuro-protective phenotype of microglia. This could provide the possibility of *in vivo* imaging of the dynamic process of microglia polarisation. Currently $P2Y_{12}$ tracers are under development.

An second novel candidate for PET imaging of neuroinflammation is the cannabinoid receptor type 2 (CB2). In contrast to the cannabinoid receptor type 1, which is abundantly expressed in the central nervous system, CB2 is primarily expressed in peripheral organs, with very limited expression on neurons, glial cells and microglia under physiological condition.[56] In neuroinflammatory conditions, CB2 is upregulated on microglia and as such it is proposed as a marker for neuroinflammation. Post-mortem studies have demonstrated an increase in CB2 in MS lesions, but its exact role in the pathophysiology in MS and treatment of MS symptoms with cannabinoids remains uncertain.[57, 58] Various CB2 tracer have recently been developed and studied in animal models.[59] To our knowledge, no *in vivo* MS studies have been performed yet.

A third novel target is the macrophage colony-stimulating factor 1 receptor (CSF1), a tyrosine kinase involved in the regulation of the activity, differentiation and survival of macrophages lineage cells.[60] An increased CSF1R response has been observed in many autoimmune disorders and as such CSF1 receptor inhibitors could offer therapeutic options by regulating the immune response by reducing macrophage and microglial function.[61] Various radiotracers binding to the CSF1 receptor are under development. Results from rodent studies using [^{11}C]JHU11744, [^{11}C]AZ683 and [^{11}C]CPPC have generally been promising,[60, 62, 63] and clinical evaluation is to be expected.

Lastly, radiotracers are being developed for cyclooxygenases-2 (COX-2), rapidly upregulated in neuroinflammation. COX enzyme activation is essential for the formation of cytokines, chemokines and prostaglandins, therefore playing a significant role in inflammation.[64]

To conclude, this results in this thesis and the recent publications mentioned above illustrate the possibilities of PET in imaging the pathophysiological processes of neuroinflammation in MS. The large amount of novel PET targets under development demonstrate the ongoing research regarding tracer specific challenges, such as low signal-to-noise ratio, sensitivity and specificity, genetic polymorphisms and microglia polarizations. Moreover, our results demonstrate the importance of careful methodology of PET studies and accurate modelling of novel PET tracers to obtain reliable results. This is especially true in diseases like MS in which the disruption of the blood-brain barrier and the diffuse inflammation violate the assumptions underlying reference tissue models. The use of different radiotracers, varying patient populations, local scanning protocols and various different methods for data analyses, lead to the heterogeneity of PET research at present. This hinders comparison of different studies and reproducibility. Addressing these problems will help progress PET imaging from the field of research to a clinical relevant biomarker of neuroinflammation. In addition, this would enable a multi-centre approach, which will be beneficial in defining the clinical applicability of these novel imaging markers for neuroinflammation in multiple sclerosis.

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