Imaging patterns of inflammation in Multiple Sclerosis
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Summary

Building on the potential of imaging to further specify the pluriformity of inflammation in MS, this thesis aimed to explore novel MRI patterns of inflammation, using different imaging approaches such as statistical mapping, macrophage tracking, and quantitative imaging.

To establish this, inflammation in MS was studied and discussed on different levels: from the most fundamental level of genetic background (**chapter 5**), through the cellular level covering focal and diffuse cellular activation (**chapters 2 and 3**), the focus moved to macroscopic lesions (**chapter 1**) and the more global approach of their spatial distribution related to measurements of disability (**chapter 4**). This composition of approaches encompasses the borders of the research fields of clinical neurology, radiology, cell biology and immunology, and imaging physics and informatics, introducing hypotheses for further research within these research fields.

In **chapter 1**, newly developed T2 lesions within a time interval were used to determine disease activity. This resulted in the finding that rebound disease activity may occur in Natalizumab-treated MS patients once treatment is suspended, although this may be driven by the group of subjects with short treatment duration. Although an explanation for this rebound phenomenon is currently lacking, the finding of partial immunosuppression giving rise to extra disease activity was previously observed in rats with experimental allergic encephalomyelitis being treated with low-dose cyclosporine A (1). After treatment continuation in the study cohort of chapter 1, disease activity was again halted successfully. This study being performed in a small patient sample, the results need confirmation in a larger cohort before firm conclusions can be drawn. Importantly, the two short reports in **chapter 1** illustrate how T2 lesion development is currently used as a surrogate marker for disease activity in MS.

An MRI marker more specific for acute inflammation is Gd-DTPA (2), which has its limitations as described in the introduction of this thesis (3-6). Gd-DTPA enhanced imaging does not disclose any information on the cellular component of the inflammatory response. Therefore, in **chapter 2**, the use of the USPIO nanoparticle SHU555C was explored as a novel MRI marker for inflammation in focal MS lesions. SHU555C-enhanced imaging showed more enhancing lesions than Gd-DTPA. Spatial and temporal discrepancies between BBB-leakage as demonstrated by Gd-DTPA-enhancement, and cellular infiltration as shown by USPIO-enhancement were demonstrated in our study and have also been reported previously using ferumoxtran-10 (7,8), another USPIO particle differing from SHU555C in size, plasma half-time and ionic charge. Although the exact mechanisms underlying USPIO-enhancement still need further clarification, especially the mechanism of transport over the BBB, and practical limitations hamper current clinical applications, **chapter 2** forms an important step in our understanding of focal inflammation.

In **chapter 3**, SHU555C was used to explore the diffuse inflammatory component of NAWM damage. NAWM inflammation, invisible on conventional MR images, has been reported previously in histopathology (9), introducing diffuse microglial activation as an important factor associated with progressive axonal injury in the NAWM and also with more extensive cortical demyelination. A previous study aiming to explore diffuse inflammation in vivo using Gd-DTPA failed to demonstrate differences between patients and healthy controls (10). **Chapter 3** of this thesis therefore represents the first report to demonstrate diffuse inflammation in vivo.

Returning to focal lesions, in **chapter 4**, the hypothesis was raised that the location of focal lesions may be partly responsible for the moderate correlations between imaging results and

clinical disability. Therefore, these correlations may be improved once lesion location would be taken into account (11,12). Using a GLM approach combined with non-parametric statistical mapping, disability was related to lesion distribution in **chapter 4**. In this study, periventricular lesion location showed to be most important in determining disability, confirming results of a previous report not taking total lesion load (LL) into account (13). Our study, that did include LL in the model, demonstrated that the interplay between lesion location and total lesion burden should not be underestimated. The effect of LL overshadowing relations between lesion location and symptoms has been reported previously (14,15), and fits with our data.

The same method of non-parametric statistical mapping was used in **chapter 5**: Hypothesizing that genetic background may influence immunological properties (16) expressed through spatial distribution of focal abnormalities (17), genotypes in 69 candidate genes were related to spatial lesion distribution in 208 MS patients. Our results, interpreted in the light of previous reports on heterozygosity in the MHC-region (18-21) suggested that heterozygous disadvantages in the MHC-region may play a role in determining lesion expression, but firm conclusions should only be made in more focussed studies.