Chapter 7. Summary

The still limited knowledge of complex neurological disorders permanently stimulates scientific research for which various neuroimaging technologies are employed. Among these technologies, PET and SPECT play an important role. Two examples that clearly show the complexity of neurological disorders are Alzheimer's disease (AD), particularly in the stage preceding dementia also termed as mild cognitive impairment (MCI), and relapsing neuromyelitis Optica (NMO). Among the relevant questions without conclusive answers in these diseases, alterations in brain perfusion occupy a special place.

The aim of this thesis was to demonstrate that brain perfusion SPECT can help to clarify important questions regarding the prodromal MCI stage of AD and relapsing NMO. This is not only pertinent considering our limited understanding of these neurological diseases but also because brain perfusion SPECT is a low cost and worldwide accessible technology.

In Chapter 1, a general introduction of brain perfusion SPECT, including a novel image analysis based on graph theory, is provided. MCI and relapsing NMO are also briefly described in this chapter as well as relevant questions related to brain perfusion in these diseases. Chapters 2 to 4 focus on MCI research questions, while chapters 5 and 6 on those related to relapsing NMO. Finally, chapter 7 presents the concluding remarks and future research directions. In what follows, the main findings presented in this thesis are summarized.

In Chapter 2, the role of brain perfusion SPECT in the prediction of AD dementia in MCI patients as compared to FDG-PET was reviewed. The review showed that current evidence and guidelines support the use of brain perfusion SPECT in both clinical and research settings as a valid biomarker of neuronal injury in MCI due to AD, equivalent to FDG-PET but less expensive and more accessible worldwide. The first part of this chapter also provided a state-
of-the-art review of the role of FDG-PET in the prediction of AD dementia in MCI patients, with a particular focus on the predictive power of FDG-PET as compared to structural MRI.

In Chapter 3, another relevant question was investigated relating to cerebrovascular reactivity (CVR) in patients with MCI. CVR is also known as the cerebrovascular reserve and describes the ability of cerebrovascular structures to increase cerebral blood flow (CBF) above a basal condition in response to a vasodilatory challenge. Although there is increasing evidence that patients with AD dementia have decreased CVR, this is less clear in patients during the prodromal MCI stage. This question is important in AD research because it could have implications for early diagnosis and treatment. Previous unclear findings may partly be explained since current standard analysis may not reflect subtle (early) abnormalities of CVR. Furthermore, considering the complexity of the cerebral microvasculature network the standard analysis of CVR might not reflect subtle network-related alterations since it relies on the analysis of individual regions (or the whole brain) rather than on the interaction between them (i.e. connectivity). Recently, graph theoretical analysis of correlation networks based on FDG-PET and structural MRI data has shown its potential to reveal subtle network-related pathological processes in MCI. The same method can also be applied to brain perfusion SPECT data of MCI patients. Hence, using graph theoretical analysis, this chapter investigated vasodilatory-induced changes in the topology of the CBF correlation (CBF\textsubscript{corr}) network to study possible network-related CVR abnormalities in MCI. For this purpose, four CBF\textsubscript{corr} networks were constructed: two using brain perfusion SPECT data at baseline and under the vasodilatory challenge of acetazolamide (ACZ), obtained from a group of 26 MCI patients; and two equivalent networks from a group of 26 matched cognitively normal controls. The results showed that the control and MCI group networks display different patterns of topological ACZ-induced changes, especially at the global level. In addition, the observed regional differences included the medial prefrontal cortices and inferior parietal
lobe, which represent areas involved in MCI's cognitive dysfunction. In contrast, no substantial differences were detected by standard CVR analysis. These results suggest that graph theoretical analysis of ACZ-induced changes in the topology of the CBF\textsubscript{corr} networks allows the identification of subtle network-related CVR alterations in MCI, which couldn't be detected by the standard approach.

On the other hand, in the context of brain perfusion SPECT, connectivity is a concept grounded in group-based correlation networks (as FDG-PET and structural MRI). Therefore, topological metrics derived from the CBF\textsubscript{corr} network cannot be used to support diagnosis and prognosis individually. However, recently, methods to extract the individual patient contribution to metrics of group-based correlation networks were developed although not yet applied to MCI patients. In order to address this question, Chapter 4 investigated whether the episodic memory of amnestic MCI patients correlates with the individual patient contributions to topological metrics of the CBF\textsubscript{corr} network. To enable this analysis, we first compared topological metrics of the CBF\textsubscript{corr} network corresponding to 24 amnestic MCI patients with those of a network of 26 cognitively healthy controls. Since the methodology applied for extracting the individual patient contribution is based on global network metrics, the analysis was restricted to such metrics. In particular, the global network modularity was examined as it has recently been demonstrated that it is more sensitive to the effects of the AD process as compared to other used metrics. The global and the mean local efficiencies, which are typically used as metrics of network integration and segregation, respectively, were also analyzed. As a secondary aim, changes in the metrics corresponding to the MCI group network after one-year follow-up were also explored, including the association between the individual patient contributions and the global cognitive function as measured by Mini-Mental State Examination (MMSE). The results showed that the global network modularity increased while the global efficiency decreased, both at baseline and at follow-up, in the MCI group.
network as compared to the control group network. Most importantly, it was found that episodic memory inversely correlates with the patient contribution to global network modularity at baseline. Moreover, similar to episodic memory at baseline, the MMSE at follow-up showed a negative correlation with the individual patient contribution to the global network modularity. Thus, these findings highlight the potential of this methodology to develop a CBF connectivity-based biomarker at the individual level since episodic memory decline is the hallmark and major symptom of MCI patients that progress to dementia.

In summary, Chapters 3 and 4 show that brain perfusion SPECT combined with graph theoretical analysis is feasible and useful for investigating problems of complex neurological diseases, such as the CVR alterations in MCI, as well as for clinical use from the perspective of the brain connectivity.

Regarding the other main topic of this thesis, previous neuroimaging studies have shown that structural brain abnormalities in NMO are more frequent than earlier described. Still, more research considering multiple aspects of NMO is necessary to better understand these abnormalities. A clinical feature of relapsing NMO (which is the most common form of the disease) is that the incremental disability is attack-related. Therefore, an association between the attack-related process and neuroimaging might be expected. In addition, the immunopathological analysis of NMO lesions has suggested that CNS microvasculature could be an early disease target, which could alter brain perfusion. Brain tissue volume changes accompanying perfusion alteration could also be expected throughout the attack-related process. Hence, the aim of Chapter 5 was to investigate in relapsing NMO patients, the assumed associations between regional brain white (WMV) and grey matter volumes (GMV) and/or perfusion on one side, and the number of optic neuritis (ON) attacks, myelitis attacks and/or total attacks on the other side. Because disease duration and NMO-IgG serological status could also be related to brain structural and functional changes, possible associations of...
these two clinical variables with regional brain tissue volumes and perfusion were evaluated as well. For this purpose, high-resolution structural MRI (T1-weighted) and brain perfusion SPECT images were obtained in 15 relapsing NMO patients that were in the stable phase of the disease. The data were then analyzed using voxel-based correlation analysis as implemented in the statistical parametric mapping (SPM8) toolbox. The results showed that among analyzed covariates of interest, only the number of ON attacks showed significant correlation with regional brain tissue volumes (WMV and GMV) and perfusion. On the one hand, negative regional correlations of WMV, GMV, and perfusion with the number of ON attacks, involving important components of the visual system, were found, which could be relevant for the comprehension of incremental visual disability in relapsing NMO; and on the other hand, a positive regional correlation of perfusion with the number of ON attacks, starting from lower than normal values, and mostly overlapping the brain area where the WMV showed a negative correlation was also found. This provides evidence that brain microvasculature is an early disease target and suggests that perfusion alteration could be important in the development of brain structural abnormalities in relapsing NMO.

As established in Chapter 5, the number of ON attacks negatively correlates with regional WMV and GMV in patients with relapsing NMO; while negatively correlating with brain perfusion in some regions and positively in other regions. This suggested that the number of ON attacks per patient may be a confounding factor when comparing patients with controls and should thus be taken into account during voxel-based analysis, especially if the patient sample includes patients with both smaller and larger numbers of ON attacks. Therefore, in Chapter 6 a voxel-based analysis was performed, which included the potential confounding effect of the number of ON attacks suffered by the patients. For this purpose, a group-wise comparison of regional WMV/GMV and perfusion differences between patients and controls was performed, by assessing these differences in two subgroups of patients grouped according
to the number of ON attacks and for the whole patient group. The subgroup I was composed of nine patients with one or two ON attacks; and the subgroup II consisting of six patients with three or four ON attacks. A non-parametric voxel-based analysis was used considering the size of sample subgroups. The results showed that subgroup I presented no volume reductions, contrary to subgroup II that showed an unequivocal reduction, especially extensive in WMV. We also found hypoperfusion in different brain regions in different subgroups. The results were quite different for the whole patient group. Thus, this study highlights that the number of ON attacks per patient in each group significantly influences the results, which indicates that this clinical variable is a potential confounder when comparing releasing NMO patients with controls, for both tissue volumes and perfusion by voxel-based statistical analysis, and calls for attention in future studies.

In summary, chapters 5 and 6, using brain perfusion SPECT combined with voxel-based analysis, provide for the first time evidence that brain microvasculature is an early disease target in relapsing NMO and suggest that perfusion alterations could be important in the development of brain structural abnormalities in this disease. Moreover, Chapter 6 provides a new methodological insight that could be useful for future clinical neuroimaging studies, including brain perfusion SPECT, in relapsing NMO.

In conclusion, the findings presented in this thesis show that brain perfusion SPECT can help to clarify important questions regarding the prodromal MCI stage of AD and relapsing NMO, which could have significant clinical implications and, therefore, the potential for practical applicability.