Chapter 5

General discussion, conclusions, future directions

The general objective of this thesis was to explore possible ways to improve the weak clinical-radiological associations of SVD in order to increase understanding of SVD features. When features of SVD can be determined with more specificity, it will eventually be possible to predict the clinical impact of SVD in an elderly individual. In this chapter, the main findings of the studies presented in this thesis are summarized and discussed. Finally, some recommendations for future research will be presented.

Part 1. Clinical impact of SVD in cross-sectional MRI studies.

As many studies on clinical impact of SVD on MRI have used a cross-sectional design, we first aimed to increase specificity on cross-sectional assessment of SVD. The various used visual rating scales and volumetric methods for WMH may differ in specificity leading to inconsistencies between studies.[1,2] It has been shown that visual rating scales correlate well with WMH volume, but, from a clinical point of view, it is necessary to investigate their validity, i.e. associations with clinical measures. Furthermore, as an independent contribution of lacunes on cognitive consequences has inconsistently been found, it may be possible that the location of strategic lacunes plays a modifying role in the association.[3-5] In the first two studies of this thesis (c*hapter 2*), which were performed on baseline data of the LADIS-study, we investigated the effect of the complexity of WMH assessment and possible strategic locations of lacunes on clinical consequences.

Chapter 2.1

The first study compared a simple visual rating scale (Fazekas scale),[6] a detailed visual rating scale (Scheltens scale)[7] and semi-automated volumetric assessment of WMH[8] in relation to gait and balance disturbances and general cognitive function. We found that associations between the extent of WMH and the clinical variables were significant and comparable across methods of WMH measurement, also after adjustment for possible confounders. In congruence with previous studies, the correlations were only of modest strength.[9,10] This study shows, that simple and complex measures of WMH have equal validity with respect to these clinical parameters and that the weak clinical-radiological associations could not be improved by using more detailed methods of WMH assessment. We would suggest that a simple visual rating scale for WMH is sufficiently informative in clinical practice. For research settings, it is conceivable that volumetric methods remain desirable, as these methods are more precise than visual rating scales.[8,11]

Chapter 2.2

We then investigated whether the location of lacunes in distinct subcortical structures has an independent influence on cognitive performance. Lacunes were counted in the following 5 areas: lobar white matter, putamen / pallidum, thalamus, internal / external capsule, infratentorial areas. General cognitive performance (MMSE and modified Alzheimer Diseases Assessment Scale) and specific

cognitive domains (memory, executive functions, speed and motor control) were assessed. Our results show that the presence of lacunes in the thalamus was independently associated with lower MMSE and worse performance on speed and motor control and executive functions. The presence of lacunes in putamen / pallidum was associated with worse memory and speed / motor control performance. Results were adjusted for several possible confounders including WMH severity. It is concluded that, in non-disabled elderly subjects with WMH, the location of lacunes within subcortical grey matter is a determinant of cognitive impairment, independent of WMH severity. These findings extend on previous studies that have identified the thalamus as a strategic location for memory and executive functions.[5,12]

From these cross-sectional MRI studies, we can conclude that the impact of lacunes can be more specifically predicted, when some strategic locations are taken into consideration. However, more detailed assessment of SVD on cross-sectional T2-weighted MRI did not lead to sufficiently improved specificity, as correlations with the investigated clinical symptoms remained of modest strength.

Part 2: Longitudinal studies of SVD.

From the studies of part 1 can be concluded, that the possibilities to specify SVD in cross-sectional MRI studies are limited, because they do not provide information on temporal relationships and causality. Several longitudinal studies reporting WMH change over time have been published, but longitudinal data on the appearance of new lacunes are scarce and risk factors are unknown.[13-15] In *chapter 3*, we therefore set out to perform longitudinal MRI studies, using baseline and follow-up MRI data from the LADIS-study, in order to evaluate the natural course of SVD over time in initially non-disabled elderly subjects. We further aimed to characterize the risk factors for progression or occurrence of SVD. These temporal relationships may provide more insight in the pathogenesis of SVD.

Chapter 3.1

We first determined the most reliable and sensitive method for measuring progression of WMH over time. WMH change was scored by four experienced raters using three conventional visual rating scales (Fazekas scale,[6] Age-Related-White-Matter-Changes scale,[16] Scheltens scale;[7] scored on baseline and follow-up scan) and two dedicated visual progression scales (Rotterdam progression scale[17] and Schmidt progression scale[18]). The visual rating scales were compared with volumetric assessment (baseline and follow-up measurement). Change scores for the cross-sectional visual rating scales and volumetry were calculated by subtracting follow-up scores from baseline scores. We found that all visual scales showed significant change of WMH over time, but the sensitivity was highest for both of the progression scales. Volumetric change of WMH correlated best with the Rotterdam Progression scale. Although all scales were reliable for assessment of WMH cross-sectionally, WMH progression

assessment using visual scales was less reliable, except for the Rotterdam Progression scale which had moderate to good reliability. In conclusion, to determine change in WMH, dedicated progression scales, especially the Rotterdam Progression scale, are more sensitive and have higher correlations with WMH volume change than conventional cross-sectional scales.

Chapter 3.2

We then evaluated SVD progression over three years follow-up in the LADIS-study using the Rotterdam Progression scale for WMH progression and counts for new lacunes. We described their natural course, regional distribution at baseline and follow-up and risk factors for progression over time. Results showed that both WMH and lacunes progress over time, mostly in subjects who have WMH or lacunes at baseline. Our findings extend on previous studies, because we found that not only WMH but also lacunes progress over time, especially in subjects with SVD at baseline.[14, 19] Previous cross-sectional studies suggested that mainly the group of subjects with severe WMH experience clinical effects.[20] We found that SVD also progressed in subjects with moderate WMH. This finding may have clinical consequences, as the moderate WMH group could eventually progress to severe WMH and consequently develop clinical symptoms in the following years. Possible intervention or prevention of risk factors should therefore be directed at both patients with moderate and severe WMH. We further found that previous stroke, diabetes and blood glucose independently predicted WMH progression. Risk factors for the occurrence of new lacunes were male gender, hypertension, systolic blood pressure, previous stroke, body mass index, HDL and triglyceride levels. Previous studies, however, described various other risk factors for WMH progression and showed that relationships between baseline predictors and WMH change are complex.[18, 21, 22] The found vascular risk factors as predictors for the progression of SVD support previous studies that arteriolosclerosis and ischemia play a role in their pathogenesis.[23] In this study, we further described that WMH progressed mostly in the subcortical white matter (WM), where WMH were also most prevalent at baseline. The comparable regional distribution at baseline and follow-up suggests that the change was mainly due to increase of existing WMH. The majority of new lacunes appeared in the subcortical WM, whereas baseline lacunes were equally prevalent in the basal ganglia. The regional distribution of lacunes seemed to shift over time, leading to the hypothesis that basal ganglia lacunes have a different pathogenesis than subcortical WM lacunes.

Chapter 3.3

We therefore investigated regional differences in MRI characteristics and risk factor profiles of incident lacunes to provide more insight in the pathogenesis of lacunes. Incident lacunes were characterized with respect to: 1) brain region (subcortical WM, basal ganglia or infratentorial region), 2) the emergence within pre-existing WMH at baseline, suggesting the development of a cavity within the WMH (yes/no), 3) the (change in) WMH size surrounding the incident lacune and 4) specific risk

factors. Results show that incident lacunes in the subcortical WM occurred more often within pre-existing WMH and were mostly accompanied by new and expanded WMH, compared to incident basal ganglia and infratentorial lacunes. Risk factors for incident subcortical WM lacunes were history of hypertension and stroke, whereas atrial fibrillation predicted incident basal ganglia/ infratentorial lacunes. This study shows that there are differences in risk factor profiles and relationship with WMH between subcortical and basal ganglia/ infratentorial lacunes. We postulate that subcortical WM lacunes develop slowly in an area with already compromised perfusion, i.e. pre-existent WMH, due to increasing hypoxia/ischemia, leading to frank infarction. In contrast, the pathogenesis of lacunes in the basal ganglia and infratentorial region may involve a more acute occlusion of a single arteriole.

In conclusion, these studies show that WMH and lacunes progress over time, especially in subjects with SVD and vascular risk factors at baseline. The pathogenesis of SVD may involve multiple processes and may differ between features of SVD and between brain regions.

Part 3: Heterogeneity in underlying pathology of SVD.

Several attempts to increase specificity on cross-sectional T2-weighted imaging, such as the use of more precise assessments, have not been sufficiently successful. Longitudinal MRI studies are informative for investigating the course of SVD over time. They suggest that vascular risk factors play a role and that the pathogenesis of SVD is heterogeneous and complex.[24,25] Still, MRI remains an indirect reflection of the true underlying pathological changes and is not able to show the types and severity of tissue changes involved. As illustrated in the figure, the only way to unravel the underlying pathological changes of SVD on MRI, is by direct comparison of MRI findings with histopathology in the postmortem setting.[26,27] Postmortem MRI and histopathology correlations bridge the discrepancies between MRI and clinical measures, thereby potentially improving the understanding of the impact of SVD on MRI.

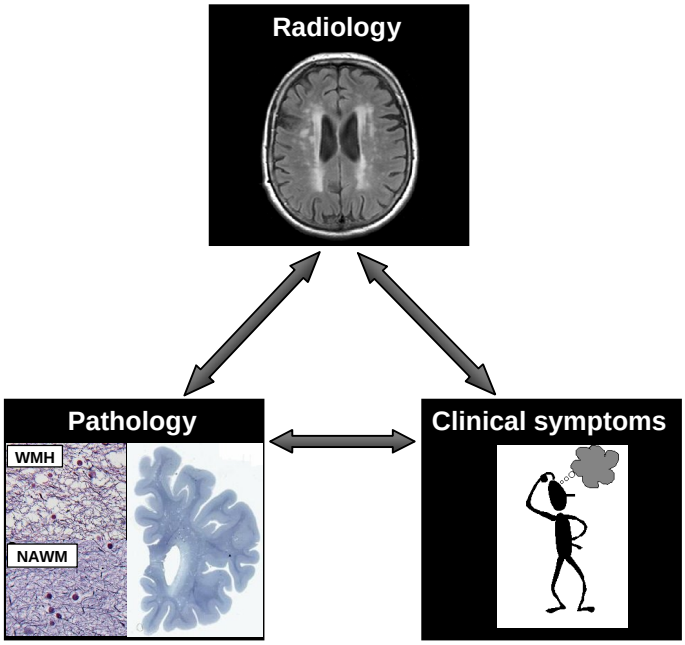

Figure. Postmortem MRI – pathology correlations have an important function of bridging the discrepancies between MRI and clinical symptoms.

Recently, attempts are being made to improve specificity of in vivo MRI scanning by novel quantitative MRI (QMRI) techniques.[28,29] The pathological substrates of QMRI in SVD, however, are still unknown. When the pathological correlates are unraveled, tissue changes in SVD may be translated into in vivo MRI scanning. In this thesis, we evaluated pathological correlates of two QMRI techniques: diffusion tensor imaging (DTI) and T1-relaxation time mapping. DTI quantifies the diffusivity of water molecules with respect to extent and spatial directionality (tissue anisotropy).[30] In normal WM, that contains many fibre bundles, water molecules diffuse more readily along the long axis of the fibres than perpendicular to them. Tissue-specific T1 relaxation times may reflect pathological processes related to intraparenchymal changes in water content.

Chapter 4.1

We first reviewed studies that correlated postmortem MRI and histopathology, in order to elucidate the pathological substrates of the whole range of SVD features. Most of the studies that were reviewed, were only small and descriptive. Recently, a few larger population based studies attempted to unravel the pathogenesis of WMH.[23,31,32] These studies provide valuable data and improve understanding of SVD. Relatively many postmortem studies have shown that heterogeneity exist in pathological substrates underlying WMH: 1) damage to the fibre network range from slight loosening to varying degrees of myelin and axonal loss; 2) glial cell responses include astrogliosis, clasmatodendrosis, loss of oligodendrocytes and several kinds of microglial responses; and 3) lipohyalinosis, arteriosclerosis, leakage of the vessel walls and venous collagenosis are recognized microvascular changes. Suggested pathogenetic mechanisms are ischemia/ hypoxia, general hypoperfusion due to dysfunction of blood flow autoregulation, blood-brain barrier leakage and clasmatodendritic astrogliosis due to serum protein uptake, inflammatory responses, degeneration and congophilic amyloid angiopathy. In contrast, relatively few postmortem MRI studies have been published with regard to lacunes and microbleeds. Many questions about their etiology therefore remain to be answered. Recently, clinico-pathological studies have suggested that SVD features, 'invisible' on T2-weighted MRI (i.e. cortical micro-infarcts and changes in the normal appearing WM), also contribute to clinical symptoms.

Chapter 4.2

We then investigated whether postmortem QMRI and pathological measures show differences between WMH of Alzheimer's Disease (AD) patients and of non-demented controls. We further aimed to investigate whether QMRI reflects underlying pathological substrates of WMH. Brain slices of AD patients and non-demented age-matched controls were selected and scanned using postmortem MRI. Regions of interest were defined on FLAIR images in WMH and NAWM. Patient groups (AD versus controls) and tissue types (WMH versus NAWM) were compared using QMRI techniques (DTI, T1-relaxation time mapping) and neuropathological assessment (axonal density, myelin density,

astrogliosis and microglial activation). We first confirmed earlier studies that QMRI and neuropathological assessment distinguishes WMH from normal appearing WM.[28,33] Results further indicated that QMRI reveals differences in WMH between AD and non-demented elderly, with respect to more microglial activation in WMH of AD patients than in WMH of controls. This patient difference was also reflected by a higher mean T1-relaxation time in WMH of AD patients, specifically. QMRI reflected the severity of the neuropathological changes involved: axonal density was an independent determinant of DTI (fractional anisotropy), whereas T1-relaxation time was independently determined by axonal and myelin density and microglial activation. Moreover, our correlation data between QMRI and neuropathology suggested that a continuum exist from neuropathologically normal to abnormal white matter. We therefore postulate that a clear distinction between WMH and NAWM is arbitrary and can not be made on the basis of neuropathology and QMRI data.

Overall, the postmortem MRI studies showed that an explanation for the weak clinical-radiological associations need to be sought in the underlying pathology of SVD: pathological substrates of SVD expressions are heterogeneous in nature and differ in severity. QMRI revealed differences between WMH of demented and WMH of non-demented subjects, and reflected the severity of the underlying neuropathological changes. Moreover, tissue changes that are 'invisible' on MRI probably also contributes to the clinical impact of SVD.

Methodological considerations

Study population

The LADIS-study benefits from the large group of subjects and the broad range of WMH that is represented. This cohort was stratified for WMH severity and therefore consists of relatively many subjects with severe WMH. Unlike most (population based) studies, this design provided statistical power to find subtle associations with clinical parameters and specific characteristics of SVD on MRI. Because of the longitudinal design with repeated MRI scans, we were also able to investigate the change of SVD over time. However, the stratification by WMH may hamper the generalizability of the results to the general population as the sample represents an elderly patient population with WMH, seen in clinical practice, rather than a random population based sample.

An other potential limitation may be the multi-center design of the study. The recruitment of subjects in several centers with different MRI scanners could have led to clinical heterogeneity in the study sample. Moreover, although imaging guidelines were distributed among all centers and repeated MRI scans were performed using the same scan protocol, heterogeneity of MRI scans between centers was considerable. Our statistical analyses were therefore corrected for the effect of center.

Longitudinal design

The longitudinal design of the LADIS-study with repeated MRI scans at baseline and at three-year follow-up, enabled us to investigate the natural course of SVD over time. However, there are also disadvantages of longitudinal studies. The attrition rate for follow-up MRI scanning was rather high, which could be a limitation of our study because it could have led to a survival bias. Although patients without follow-up MRI had less favourable values for some baseline risk factors than our study population, the baseline MRI variables were comparable. The multi-center design made it also more difficult to implement automated methods for measuring WMH progression. Although WMH volumetry was reliable cross-sectionally, assessment of change in WMH volume involved the subtraction of baseline data from the follow-up data, thereby including the measurement error twice. The dedicated progression scales turned out to be more suitable for this sample than volumetric measurements of WMH change, possibly because they provide direct change scores by side-by-side assessment. On the other hand, scoring in a side-by-side fashion could be less ideal because of impossibility of blinding of the data.

Postmortem MRI studies

Methodological issues of our postmortem MRI study include: the use of formalin fixed brain specimens and the relatively basic neuropathological assessment. In this study, we only found pathological correlations with fractional anisotropy (FA, spatial directionality of water diffusion), but not with apparent diffusion coefficient (ADC, extent of water diffusivity). This may explained by the fact that death and formalin fixation causes a substantial decrease in ADC, leading to the lack of correlations, whereas the FA is less dependent on these factors.[34] For post-mortem QMRI scanning, the use of fresh brain tissue would be recommended. This design, however, is challenging as it warrants a team of investigators to be on call for 24 hours a day and the availability of an MRI scanner at any time a death occurs.[26] Furthermore, we performed relatively basic neuropathological assessment (only four relatively common neuropathological stainings), because we chose to focus on the correlations with QMRI measures. We were not able to draw specific conclusions on pathogenetic mechanisms of WMH, because it would warrant extensive and detailed correlations with a larger battery of specific (immuno-)histochemical stainings.

Implications and future research

Radiology, pathology and clinical impact

Chapters 2 and 3 of this thesis focused on the relationships between radiology and clinical symptoms, whereas chapter 4 dealt with the correlation between radiology and pathology. However, as illustrated in the figure, radiology, pathology and clinical impact are interrelated and the current focus in research should aim to integrate all three fields to be able to obtain the full picture of SVD. Moreover,

longitudinal studies are important to investigate temporal relationships and therefore, causality. A way to achieve this is to follow a large group of subjects with neuropsychological testing, physical examinations and MRI scans (including QMRI) and to eventually combine these longitudinal data with postmortem assessment (with MRI scanning and pathological evaluation). Although this design is challenging, such studies are being performed in the general population and in a cohort of nuns and will increase our knowledge of SVD.[35, 36] On the other hand, population based studies may lack statistical power because of their heterogeneity and may therefore be less suitable to investigate specific differences between patient groups. Studies with a case-control design using well characterized subjects are warranted for the elucidation of pathophysiological mechanisms.

The whole spectrum of the aging brain

Furthermore, it has been shown that not only WMH and lacunes, but also microbleeds, cortical microinfarcts and changes in the NAWM are clinically relevant.[37,38] However, most studies focused on associations with SVD features separately. Considering the whole range of SVD features together may better predict the clinical consequences. Other (degenerative) brain changes, such as AD pathology, congophilic amyloid angiopathy or cortical Lewy bodies, may also have an additive effect on cognitive decline.[39,40] These complex relationships of pathological changes suggest that all aspects in the aging brain should be taken into account, in order to understand the influence of SVD on the clinical consequences of an elderly subject.

In vivo 'pathology-specific' tools

Finally, we showed that novel QMRI techniques (DTI and T1-relaxation time mapping) are correlated to distinct pathological substrates. QMRI, therefore, has potential to bridge the gap between MRI and pathology and may be regarded as 'pathology-specific' tools in vivo, when intervention is still possible. The advantages of novel quantitative MRI techniques over conventional MRI are twofold: 1) they reflect the severity of underlying pathological changes (in diffuse changes such as WMH), and 2) they show tissue changes in areas that appear normal on conventional MRI (NAWM). Studies should be directed to establish the relationship between QMRI parameters and clinical symptoms. In this thesis, we only investigated the pathological correlates of DTI and T1-relaxation time mapping, but other QMRI techniques are available, such as Magnetization Transfer Imaging, T2-relaxation time mapping and Magnetic Resonance Spectroscopy. These other techniques may have additional value in the specificity for pathological changes and a combination in QMRI techniques may contribute to predict the clinical symptoms in the individual subject.

Conclusions

The following conclusions can be drawn from the studies presented in this thesis:

1. WMH severity is associated with gait and balance disorder and cognitive dysfunction. As simple and more complex methods yield equal correlations with clinical measures, a simple visual rating scale for white matter hyperintensities is sufficiently informative for clinical practice.
2. The location of lacunes within subcortical grey matter is a determinant of cognitive impairment, independent of WMH severity. The impact of lacunes on cognitive dysfunction can therefore be more specifically predicted, when strategic locations are taken into consideration.
3. Subjects with small vessel disease and vascular risk factors at baseline are at risk for progression of WMH and lacunes over time.
4. Differences in risk factor profiles and relationship with WMH exist between subcortical and basal ganglia/ infratentorial lacunes. The pathogenesis of lacunes may therefore involve multiple processes and differ between brain regions.
5. Pathological substrates underlying SVD expressions are heterogeneous in nature and severity. Besides MRI-'visible' SVD, there are also pathological changes that are 'invisible' to conventional T2-weighted MRI. An explanation for the weak clinical-radiological associations of SVD should be sought in the heterogeneity in underlying pathology.
6. QMRI reveals differences between WMH of demented and WMH of non-demented subjects, and reflects (the severity of) the underlying neuropathological changes. QMRI can therefore be regarded as 'pathology-specific' tools in vivo.

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