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Clinical application of novel marker for cerebral small vessel disease

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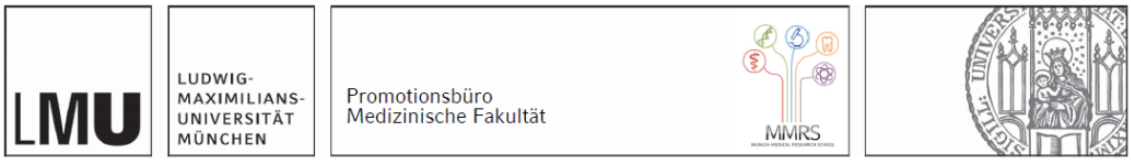

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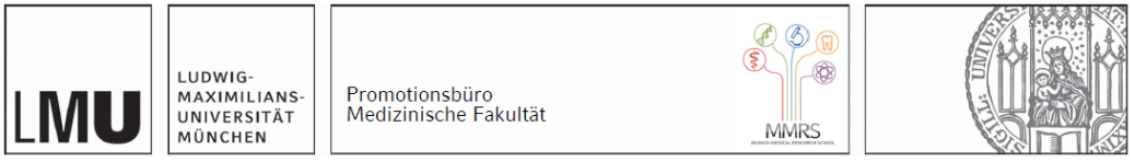

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Table of content

Affidavit **3**

Confirmation of congruency **4**

Table of content **5**

List of abbreviations **6**

List of publications **7**

1. Your contribution to the publications **8**

1.1 Contribution to paper I 8

1.2 Contribution to paper II 8

1.3 Contribution to paper III 8

2. Introductory summary **9**

2.1 Introduction 9

2.2 Neuroimaging lesions and underlying pathomechanisms 9

2.3 Types of SVD 10

2.3.1 Arteriolosclerosis-related SVD 10

2.3.2 Cerebral amyloid angiopathy 10

2.3.3 CADASIL 11

2.4 Clinical characteristics 11

2.5 Treatment 11

2.6 Disease markers 12

2.6.1 Established SVD markers 12

2.6.2 Novel SVD markers 13

2.7 Aims of the thesis 14

2.7.1 Multi-shell diffusion MRI models for white matter characterization in cerebral small vessel disease 14

2.7.2 Neurofilament light chain as serum marker for cerebral small vessel disease 15

2.7.3 Prognostic relevance of cortical superficial siderosis in cerebral amyloid angiopathy 15

2.8 Discussion 15

2.8.1 Main findings 15

2.8.2 Clinical utility of novel SVD markers 16

2.8.3 Biological underpinnings of SVD related white matter lesions 17

2.9 Conclusions and future directions 18

3. Paper I: Multi-shell diffusion imaging improves tissue characterization in cerebral small vessel disease **19**

4. Paper II: Serum Neurofilament Light Chain Levels Are Related to Small Vessel Disease Burden **20**

5. Paper III: Prognostic relevance of cortical superficial siderosis in cerebral amyloid angiopathy **21**

References **22**

Acknowledgements **27**

List of abbreviations

SVD	Small vessel disease
MRI	Magnetic resonance imaging
WMH	White matter hyperintensities
BBB	Blood-brain-barrier
CNS	Central nervous system
CAA	Cerebral amyloid angiopathy
cSS	Cortical superficial siderosis
ICH	Intracranial hemorrhages
CADASIL	Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
DW	Diffusion-weighted
DTI	Diffusion tensor imaging
FA	fractional anisotropy
MD	mean diffusivity
DKI	Diffusion kurtosis imaging
NODDI	Neurite orientation dispersion and density imaging
NfL	Neurofilament light chain
Ph.D.	Doctor of Philosophy
DW-MRI	Diffusion-weighted magnetic resonance imaging

List of publications

Konieczny M. J., Dewenter A., ter Telgte A., Gesierich B., Wiegertjes K., Finsterwalder S., Kopczak A., Hübner M., Malik R., Tuladhar A. M., Marques J. P., Norris D. G., Koch A., Dietrich O., Ewers M., Schmidt R., de Leeuw F. E., & Duering M. (2020) Multi-shell diffusion MRI models for white matter characterization in cerebral small vessel dis-ease. Neurology, in press.

Peters, N., van Leijsen, E., Tuladhar, A. M., Barro, C., **Konieczny, M. J.,** Ewers, M., Lyrer, P., Engelter, S. T., Kuhle, J., & Duering, M. (2020). Serum Neurofilament Light Chain Is Associated with Incident Lacunes in Progressive Cerebral Small Vessel Disease. Journal of Stroke, 22(3), 369–376.

Finsterwalder, S., Vlegels, N., Gesierich, B., Araque Caballero, M. Á., Weaver, N. A., Franzmeier, N., Georgakis, M. K., **Konieczny, M. J.,** Koek, H. L., & Network (DIAN), D. I. A. (2020). Small vessel disease more than Alzheimer's disease determines diffusion MRI alterations in memory clinic patients. Alzheimer's & Dementia.

Gesierich, B., Tuladhar, A. M., Ter Telgte, A., Wiegertjes, K., **Konieczny, M. J.,** Finsterwalder, S., Hübner, M., Pirpamer, L., Koini, M., & Abdulkadir, A. (2020). Alterations and test–retest reliability of functional connectivity network measures in cerebral small vessel disease. Human Brain Mapping.

Finsterwalder, S., Wuehr, M., Gesierich, B., Dietze, A., **Konieczny, M. J.,** Schmidt, R., Schniepp, R., & Duering, M. (2019). Minor gait impairment despite white matter damage in pure small vessel disease. Annals of Clinical and Translational Neurology, 6(10), 2026–2036.

Wollenweber, F. A., Opherk, C., Zedde, M., Catak, C., Malik, R., Duering, M., **Konieczny, M. J**., Pascarella, R., Samões, R., & Correia, M. (2019). Prognostic relevance of cortical superficial siderosis in cerebral amyloid angiopathy. Neurology, 92(8), e792–e801.

Duering, Marco, Finsterwalder, S., Baykara, E., Tuladhar, A. M., Gesierich, B., **Konieczny, M. J.,** Malik, R., Franzmeier, N., Ewers, M., & Jouvent, E. (2018). Free water determines diffusion alterations and clinical status in cerebral small vessel disease. Alzheimer's & Dementia, 14(6), 764–774.

Duering, Marco, **Konieczny, M. J.,** Tiedt, S., Baykara, E., Tuladhar, A. M., Van Leijsen, E., Lyrer, P., Engelter, S. T., Gesierich, B., & Achmüller, M. (2018). Serum neurofilament light chain levels are related to small vessel disease burden. Journal of Stroke, 20(2), 228.

Konieczny, M. J., Ri, S.-J., & Georgiadis, J. R. (2016).Omental approach to functional recovery after cerebrovascular disease. World Neurosurgery, 87, 406–416.

Alberts, N.*, Groen, K.*, Klein, L.*, **Konieczny, M. J**.*, & Koopman, M.* (2014). Dorsal root ganglion neurons carrying a P301S Tau mutation: A valid in vitro model for screening drugs against tauopathies? Journal of Neuroscience, 34(14), 4757–4759.

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1. Your contribution to the publications

1.1 Contribution to paper I

The first, comprehensive diffusion MRI study using two independent samples had three parts: cross-sectional data, longitudinal data and an inter-site dataset. I participated in the cross-sectional data acquisition of CADASIL patients within the VASCAMY study, including clinical characterization, neuropsychological testing and MRI acquisition. I was also responsible for data quality control and assurance of diffusion MRI, including visual checks and quality ratings. For analysis, I established athe data processing pipeline for diffusion MRI data, in particular the implementation of advanced diffusion models to be evaluated in this project. I also contributed to the rating of conventional small vessel disease lesion markers. I conducted the statistical analysis of the project, including simple and multivariable models. Finally, I discussed results with co-authors, drafted the figures and the manuscript, and revised the manuscript after co-author feedback. The first authorship is shared with Anna Dewenter, who was involved in the analysis of the longitudinal study part and also drafted the manuscript.

1.2 Contribution to paper II

For this study, I again participated in data acquisition (MRI data, neuropsychology) of CADASIL patients within the VASCAMY study. I prepared the data sources and performed the statistical analysis in all samples. I was involved in drafting the manuscript, e.g. by drafting the methods section, tables and figures.

1.3 Contribution to paper III

In this multicenter study, I performed the collection of MRI data from all centers, as well as data organization and quality control, including visual quality checks of all MRI scans. I contributed to the statistical analysis and to the discussion of results as well as manuscript writing.

2. Introductory summary

2.1 Introduction

Cerebral small vessel disease (SVD) is highly prevalent in the aging society. Globally, it contributes to every second case of dementia – either on its own or in combination with Alzheimer's disease – and the prevalence is expected to further increase [Prince et al., 2015]. SVD is a chronic, progressive disease and the underlying cause for the majority of hemorrhagic and one quarter of ischemic strokes [Wardlaw et al., 2019]. Due to the progressive nature and the lack of a curative treatment, patients show increasing deficits in cognitive, affective, and motor domains [Ter Telgte et al., 2018]. SVD affects the microcirculation of the brain and leads to multiple neuroimaging manifestations, predominantly in the white matter. While it was originally assumed that the amount of white matter lesions as seen on neuroimaging gives an objective indication of disease severity, recent studies revealed a more heterogeneous lesion-symptom relationship [van Uden et al., 2016; Wardlaw et al., 2019]. Clinical observations and case studies reported about an enormous heterogeneity in symptom severity for patients with a similar degree of conventional neuroimaging lesions [Ter Telgte et al., 2018]. This heterogeneity can be partly explained by the inability of conventional neuroimaging markers to visualize early and subtle manifestations of the disease, such as alterations in the so-called normal appearing white matter [Nucifora et al., 2007]. Other potential sources of variability include compensatory and reserve mechanisms, which might preserve functional independence even with severe lesion load [Ter Telgte et al., 2018]. This highlights our limited understanding of the involved pathomechanisms. Clinically validated and sensitive markers are needed for unraveling these mechanisms as well as for better diagnosis and treatment of the disease.

The current thesis comprises the results of three research projects about novel disease markers for SVD and their clinical utility. The objective was to assess their potential in sporadic as well as genetic SVD samples. Another goal was the exploration of possible biological underpinnings. The results of the studies presented herein will facilitate characterization and management of the disease, treatment response monitoring in pharmacological trials, and insight into disease mechanisms. In the upcoming sections, I will summarize the current understanding of the disease pathology, followed by an overview of the different subtypes of SVD, their clinical characterization, and treatment options. Finally, an outline of existing biomarkers and their limitations will highlight the need for novel markers, as intended by the current thesis.

2.2 Neuroimaging lesions and underlying pathomechanisms

As defined by consensus criteria, typical neuroimaging features of SVD on MRI include white matter alterations with hyperintense signal on T2-weighted sequences (white matter hyperintensities), recent small subcortical infarcts, microbleeds and large intracranial hemorrhages, enlarged periventricular spaces, lacunes, and atrophy [Wardlaw et al., 2013]. Apart from visible lesions, signal alterations can also be measured in the so-called normal appearing white matter

[Baykara et al., 2016; Maillard et al., 2011]. The underlying pathology for parenchymal lesions is only incompletely understood, but recent evidence highlights the importance of blood-brain-barrier (BBB) dysfunction as a key contributor. The BBB and its cellular constituents are essential for various aspects of physiological brain functioning [Cuadrado-Godia et al., 2018]. Damage to these cells might contribute to disease manifestations in several ways including impaired vasoreactivity, inflammatory cell migration, edema formation, and demyelination [Rajani et al., 2018; Shi et al., 2020; Shoamanesh et al., 2015]. For a better understanding of the disease it is crucial to clarify which of these mechanisms (e.g. edema formation or demyelination) is predominantly responsible for SVD-related white matter damage and cognitive consequences. To explore the contribution of possible disease mechanisms, we analyzed the association between neurite specific imaging markers that can reflect different types of disease processes, e.g. vasogenic edema or demyelination and clinical symptoms.

2.3 Types of SVD

SVD is an umbrella term encompassing multiple sub-types of vasculopathies. Most prevalent are the arteriolosclerosis type and SVD due to cerebral amyloid angiopathy (CAA). The remaining SVD types are rare and include hereditary SVD, immunological-related SVD, venous collagenosis, and other causes [Pantoni, 2010]. The following description is confined to SVD types that were part of the research projects.

2.3.1 Arteriolosclerosis-related SVD

SVD with arteriolosclerosis, also referred to as sporadic SVD, is related to cardiovascular risk factors, in particular hypertension and diabetes, as well as aging. The disease mainly affects perforating arterioles. Histological features are lipohyalinosis, fibrinoid necrosis, loss of smooth muscle cells, leakage of plasma proteins, and vessel wall thickening. Although all types of conventional neuroimaging lesions can be seen on radiological examination, patients most often present with periventricular white matter hyperintensities (WMH), lacunes, and deep microbleeds [Cuadrado-Godia et al., 2018]. Sporadic SVD is the most common type of SVD and the main focus of this thesis.

2.3.2 Cerebral amyloid angiopathy

Cerebral amyloid angiopathy (CAA) is associated with old age and often exists next to Alzheimer's disease [Arvanitakis et al., 2011; Kalaria et al., 1999]. The pathological hallmark is deposition of amyloid-beta protein in the vessel walls of pial arteries and cortical perforators. Typical MRI findings in CAA patients are lobar microbleeds, large intracranial hemorrhages (ICH), and cortical superficial siderosis (cSS) [Cuadrado-Godia et al., 2018]. Due to the high risk and the severe clinical consequences of ICH, prognostication of bleeding in CAA is of major importance. Thus, one project of this thesis validated the imaging marker cSS for the prediction of ICH in a prospective, multicenter cohort of CAA patients.

2.3.3 CADASIL

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common hereditary type of SVD and the most prevalent monogenetic stroke disorder. Similar to the arteriolosclerosis-related type of SVD, CADASIL affects penetrating arteries and arterioles. Besides the pathological features of arteriolosclerosis, a pathognomonic finding is the deposition of granular osmiophilic material [Chabriat et al., 2009] in vessel walls. The disease is caused by missense mutations in the *NOTCH3* gene, encoding for a transmembrane receptor protein. It is assumed that the mutated protein is responsible for deposition of protein aggregates and eventually dysfunction or even death of vascular cells [Joutel et al., 1996]. Neuroimaging features are mostly similar to the arteriolosclerotic SVD type, but with an earlier onset and a more severe manifestation [Chabriat et al., 2009]. Due to clinico-pathological similarities with sporadic SVD, we used CADASIL patients for independent validation of our findings in sporadic SVD patients. Importantly, the analysis of these genetically-defined patients with younger age of onset allows to conclude that findings are indeed driven by SVD, and not by age-related comorbidities.

2.4 Clinical characteristics

The clinical picture of SVD is variable. Although mild radiological manifestations of SVD can be found in almost all elderly individuals, only some will progress and develop clinical symptoms [De Leeuw et al., 2001]. Initial disease stages are characterized by subtle cognitive deficits, mainly in processing speed. During the course of the disease, gait impairment and urinary incontinence may develop and cognition as well as mood worsens gradually [Pantoni, 2010]. The progressive nature of the disease and absence of curative treatment options renders patients bedridden and demented at terminal disease stages [Wardlaw et al., 2019]. Importantly, the variability in disease severity for patients with a similar degree of white matter lesion load is only incompletely understood. This heterogeneity might be explained by the location and severity of parenchymal lesions, different vascular risk factor profiles, and compensatory mechanisms, but requires further investigation [Ter Telgte et al., 2018]. To address this topic, we evaluated the utility of novel imaging markers. By taking the complexity of brain tissue into account, these markers are supposed to better quantify the gradual nature of brain tissue damage in SVD.

2.5 Treatment

Due to the lack of curative therapies, treatment strategies in SVD are limited and mainly confined to reduction of risk factors and treatment of comorbidities [Bath et al., 2015]. Therefore, prevention programs focusing on lifestyle and vascular risk factors are highly relevant [Wardlaw et al., 2019]. A large, multidomain prevention study investigating the effects of exercise, risk factor reduction, cognitive training, and diet, showed improvements in cognitive functioning over the 2-year follow-up period [Ngandu et al., 2015]. Although this study did not specifically focus on SVD but was intended to investigate preventive approaches in patients at risk taken form the general population, it can be assumed that a considerable amount of study participants suffered from SVD due

to its high prevalence. However, as shown by another longitudinal prevention trial, there was no effect of the intervention on the progression of WMH lesions [Van Dalen et al., 2017]. As already acknowledged in interventional trials for Alzheimer's disease, targeting patients at early disease stages might enlarge the effect of interventions [Mehta et al., 2017]. A major obstacle for research on mildly affected SVD patients is the lack of markers that are sensitive for early disease manifestations. Pharmacological studies targeting platelet aggregation and cardiovascular risk factors (hypertension and hyperlipidemia) showed mixed results [Wardlaw et al., 2019]. Treatment with antiplatelet drugs and antihypertensives reduced the recurrence of ischemic and hemorrhagic stroke, respectively [Benavente et al., 2012; Kwok et al., 2015; Pearce et al., 2014]. However, inhibition of platelet aggregation increased the number of serious adverse events, including bleeding in patients with CAA [DeSimone et al., 2017; Kwok et al., 2015]. Similarly, extensive blood pressure reduction was associated with cerebral hypoperfusion especially in patients with severe lesion load [Pettersen et al., 2017]. Our current disease markers for CAA and sporadic SVD are only marginally suited to predict intracranial bleeding and to objectify white matter lesions, respectively. Therefore, prognostic and sensitive disease marker are required to support clinical decision making and weigh the risks and benefits of pharmacotherapies.

2.6 Disease markers

By definition a biomarker is an objectively measured indicator of a physiological state or process [Biomarkers Definitions Working, 2001]. Biomarker are indispensable in clinical care for accurate diagnostics and prognosis. Furthermore, biomarker play an important role in interventional and mechanistic research. Ideally, a disease marker displays excellent performance in terms of reliability and validity, is non-invasively collected and automatically analyzed [Biomarkers Definitions Working, 2001]. As interventional trials increasingly focus on pre-clinical disease stages, novel biomarkers need to be sensitive to early manifestations of the disease [Cummings et al., 2019].

2.6.1 Established SVD markers

Pathological hallmarks of SVD are intra- and perivascular changes affecting the small vessels of the brain as well as secondary parenchymal lesions [Cuadrado-Godia et al., 2018]. Due to the limited resolution of conventional MR imaging, it is almost impossible and clinically not feasible to directly visualize early and subtle pathological changes inside the vessels wall [Pantoni, 2010]. Therefore, the disease is characterized by the presence and extent of SVD-related brain parenchymal lesions which can be detected by MR imaging (i.e. conventional neuroimaging marker) [Wardlaw et al., 2013]. These markers have advanced our understanding of the disease and represent the current diagnostic gold-standard, but lack the sensitivity to visualize early and subtle disease manifestations [de Groot et al., 2013; Pantoni, 2010]. Diffusion-weighted MRI is a non-invasive and quantitative MRI method measuring the diffusion of water molecules in brain tissue [Hagmann et al., 2006]. The random motion of water molecules is influenced by the presence and density of macromolecules and (intra-)cellular structures. Measurement of these diffusion patterns provides an objective characterization of the microstructural integrity of the tissue [O'Sullivan

et al., 2004]. A straightforward model to quantify diffusion-weighted MRI is diffusion tensor imaging (DTI) [Nucifora et al., 2007]. The DTI model allows to assess the directedness (fractional anisotropy) and the extent (mean diffusivity) of water diffusion. SVD-related white matter alterations are reflected in a decrease of fractional anisotropy and an increase in mean diffusivity. Importantly, these diffusion changes were already observed in brain tissue outside of conventional neuroimaging lesions (i.e. in normal-appearing white matter) and can be regarded as early signs of the disease [de Groot et al., 2013]. Next to the higher sensitivity of DTI for subtle SVD manifestations, studies in sporadic as well as genetic SVD samples showed stronger correlations with cognitive functions and clinical deterioration compared with conventional neuroimaging markers [Holtmannspötter et al., 2005; Tuladhar et al., 2015; van Norden et al., 2012].

2.6.2 Novel SVD markers

Technological advancements in MRI hard- and software improved the quantification of diffusion processes through acquisition of multiple and stronger diffusion weightings (multi-shell acquisition protocols). This enables a more detailed characterization of tissue microstructure through the application of advanced diffusion models [Alexander, 2008]. Among advanced diffusion models, diffusion kurtosis imaging (DKI) and neurite orientation dispersion and density imaging (NODDI) are most frequently used. DKI improves the characterization of non-Gaussian diffusion processes, i.e. water movement that does not adhere to a normal distribution but is best described by a leptokurtic (peaked) or platykurtic (flat) distribution [Jensen et al., 2005]. This gives a more realistic picture of diffusion in complex biological tissue by taking into account the heterogeneous composition of the brain. DKI was shown to be more sensitive for white matter alterations in patients with multiple sclerosis than DTI [Bester et al., 2015]. NODDI is a three-compartment, biophysical model which separates the diffusion signal into a free water, a restricted extracellular water, and a intracellular water compartment [Zhang et al., 2012]. Based on complex and resource demanding calculations, NODDI aims to model biophysical properties of neurites. In stroke patients NODDI metrics showed a higher sensitivity to detect diffusion alterations [Wang et al., 2019]. Importantly, the utility of advanced diffusion models as markers for SVD is unexplored.

Due to relevant limitations of MRI markers in clinical and research settings, e.g. contraindications or center-effects constituting a roadblock for multicenter studies, there is a high demand for a blood-based biomarker. Serum neurofilament light chain (NfL) has been identified as promising research tool for neurological diseases [Poggesi et al., 2016]. NfL constitutes a part of the neuroaxonal cytoskeleton and is released upon axonal damage into the extracellular space and eventually peripheral circulation. So far, serum NfL was studied in various neurological conditions, such as patients with Alzheimer's disease, motor neuron disease, and frontotemporal dementia, and showed promising results with regard to prognostication as well as quantification of disease severity [Lu et al., 2015; Mattsson et al., 2017; Rohrer et al., 2016]. Preliminary studies in SVD reported associations with lacunes and WMH lesions, but conclusive evidence is missing [Jonsson et al., 2010].

Biomarkers for CAA are highly relevant due to the high risk and the severe clinical consequences of intracranial bleeding [Arvanitakis et al., 2011]. Furthermore, reliable prognostic information is

crucially important when considering antithrombotic medication for the prevention of ischemic strokes, e.g. in the presence of atrial fibrillation [DeSimone et al., 2017]. As suggested by small, retrospective studies, cSS might be a predictor for the future occurrence of ICH, but results from prospective multicenter studies are missing [Charidimou et al., 2017].

2.7 Aims of the thesis

SVD is very common in the elderly and a significant contributor to disability, cognitive decline, dementia, and ultimately loss of independence. Conventional neuroimaging markers are the diagnostic gold standard and an important research tool despite relevant shortcomings in terms of sensitivity to early-stage alterations, prognostic abilities, and usability in research settings. Current treatment strategies, such as multimodal prevention programs and pharmacotherapy, are only moderately effective and associated with adverse effects. Relevant obstacles for a better management of the disease are the limited understanding of the pathology, challenges in identification of patients at early disease stages, and difficulties in detection of possible contraindications against pharmacotherapy. Novel and clinically validated disease markers may improve disease characterization, prognostication of complications, and facilitate large interventional trials.
Correspondingly, the overarching goal of this Ph.D. thesis was to evaluate novel SVD markers with regard to their sensitivity for early and subtle disease manifestations and their prognostic utility. An additional aim was to elucidate the biological underpinnings of white matter damage in SVD using novel approaches, including biophysical modelling of white matter microstructure and a blood-based marker for neuroaxonal damage.

2.7.1 Multi-shell diffusion MRI models for white matter characterization in cerebral small vessel disease

SVD leads to widespread microstructural changes that can be best assessed by diffusion MRI. Measures from DTI are strongly associated with clinical deficits, in particular processing speed performance. However, the simple tensor model has limitations and provides only limited insight into the underlying tissue microstructure. We therefore evaluated the utility of multishell acquisition and novel, advanced diffusion models: DKI for characterization of non-Gaussian diffusion and the NODDI three compartment model. These advanced diffusion models are potentially more sensitive to early and subtle white matter alterations and provide important insight into the disease pathology through biophysical modelling.
In the first research project, we evaluated the performance of the advanced diffusion models in comparison to the established, simpler DTI model in sporadic and genetically defined SVD samples (arteriolosclerosis-related SVD and CADASIL, respectively). We analyzed associations between diffusion markers and cognitive performance. Furthermore, to address the need for sensitive and robust markers in longitudinal multi-center trials, we evaluated the ability to monitor disease progression and determined inter-scanner reproducibility. We hypothesized that the advanced models show stronger associations with clinical deficits and disease progression.

2.7.2 Neurofilament light chain as serum marker for cerebral small vessel disease

While MRI is the method of choice for diagnosis and research of SVD, there are relevant limitations of neuroimaging-based markers, such as MRI contraindications or the susceptibility of MRI markers to center-effects. These limitations could be overcome by the implementation of a complementary, blood-based biomarker. However, so far no circulating biomarker has been identified that matches the performance of MRI markers. Serum NfL was already validated in other neurological diseases, such as Alzheimer's dementia, motor neuron disease, and frontotemporal dementia. As a marker for neuroaxonal damage, serum NfL can provide insight into underlying disease mechanisms. So far, the utility of serum NfL as marker for SVD was not explored in sufficient detail. Therefore, the aim of this study was to assess the association between serum NfL and clinical as well as neuroimaging features of SVD. Again, we used 2 samples to validate our results, a genetically-defined and a sporadic SVD sample. We hypothesized that serum NfL levels are strongly associated with clinical deficits and neuroimaging features of SVD.

2.7.3 Prognostic relevance of cortical superficial siderosis in cerebral amyloid angiopathy

CAA is the major cause for non-traumatic lobar intracranial hemorrhages (ICH) [Arvanitakis et al., 2011]. Due to high mortality rates and a significantly elevated recurrence risk of ICH, a prognostic marker for intracranial bleeding is urgently needed. Retrospective, single-center studies support the role for cSS in predicting the risk for bleeding in patients with CAA, but evidence from prospective, multicenter studies is missing. Therefore, our study investigated the prognostic relevance of cSS in a cohort of patients with possible or probable CAA from four European study sites. We hypothesized higher rates of stroke or death as well as higher ICH rates and greater worsening of disability in patients with cSS vs. those without.

2.8 Discussion

2.8.1 Main findings

The current thesis summarizes the results from three research projects focused on novel markers for cerebral SVD. Our goal was to validate their potential for clinical application and elucidate possible pathomechanisms of SVD. In the first project, we evaluated the utility of multi-shell diffusion imaging and advanced diffusion models (DKI and NODDI). We found a benefit for both advanced models in explaining cognitive deficits in comparison to established SVD markers. The benefit was most pronounced in early disease stages. The reproducibility analysis showed excellent robustness for all diffusion metrics, except those from the NODDI model. Regarding possible pathomechanisms of SVD, the associations between biophysical metrics of the NODDI model and cognitive deficits indicate that edema may contribute to clinical manifestations of the disease. The second project established serum NfL as marker for disease burden in SVD. We found strong associations with clinical features and with established MRI markers. As marker for neuroaxonal damage, serum NfL is a highly valuable tool for mechanistic research. Due to relevant advantages

in comparison to MRI-based markers, serum NfL is an important alternative marker in clinical care and research settings. In the third project, we validated cSS as prognostic marker for intracranial bleeding in patients with CAA. Our prospective multicenter study showed that the presence of cSS is a strong predictor for stroke and death, ICH, and disability. In the upcoming sections, I will elaborate on the findings of the three research projects with regard to my aims and highlight the need for further investigations.

2.8.2 Clinical utility of novel SVD markers

In the first project, we evaluated the utility of multi-shell diffusion imaging and advanced diffusion models. Our study showed a benefit for markers from DKI and NODDI in explaining cognitive deficits in comparison to established SVD markers. The benefit of advanced diffusion models for explaining clinical symptoms was strongest in sporadic SVD patients and in genetically-defined SVD patients (CADASIL) with lower disease burden, indicating a benefit particularly for patients at early SVD stages. This finding is highly relevant for effective prevention programs of SVD, including lifestyle changes and pharmacological risk factor management at mild disease stages [Cummings et al., 2019; Wardlaw et al., 2019]. Importantly, we showed that markers from DKI and NODDI are more sensitive to subtle white matter alterations (i.e. alteration in normal-appearing white matter) than the simpler DTI model. As discussed in the introduction, the inability of MRI markers to detect subtle disease manifestations makes it difficult to quantify the underlying disease burden and may contribute to the heterogeneity between clinical symptoms and imaging findings. The superior sensitivity of DKI and NODDI metrics might help to reduce this variability [Ter Telgte et al., 2018]. Lastly, in our longitudinal analysis we showed that metrics from the simple DTI model perform best in tracking of disease progression. While the performance of the DKI metric radial kurtosis was comparable to DTI, the high variability over time of the NODDI model impedes its application in longitudinal settings. It is conceivable that the complex modeling approach of advanced diffusion models, in particular NODDI, results in less repeatable measures. A reduced robustness was again confirmed in our inter-scanner study, showing the lowest reproducibility for NODDI metrics. The overall best performance was seen for metrics of the DKI model: These metrics combine a high sensitivity for early and subtle SVD lesions with a stable and robust longitudinal and inter-scanner performance. DKI metrics therefore represent excellent candidate markers for future use in clinical and research settings.

The second project established serum NfL as a blood marker for SVD burden. We showed significant differences in serum NfL levels between patients and controls and strong associations with disease severity. Importantly, serum NfL outperformed all conventional MRI markers in terms of associations with cognitive deficits. Serum NfL is of high clinical utility by complementing or even replacing radiological or clinical assessment in certain scenarios. Furthermore, by overcoming relevant limitations of MRI-based markers, such as multicenter-effects and selection bias by MRI contraindications, serum NfL is a promising research tool in large, interventional trials. However, as serum NfL captures neuroaxonal damage without specificity for the underlying disease, a multi-factorial origin of increased NfL levels always needs to be kept in mind. Along the same

line of thinking, SVD needs always to be considered as a potential confounder when measuring serum NfL in elderly subjects, especially in the presence of vascular risk factors.

The third project, confirmed the high prognostic relevance of cSS for future ICH in patients with CAA, as suggested in prior retrospective studies. Using a large prospective cohort of CAA patients from four European study centers, we showed that cSS is a strong predictor of future bleeding and disability. The high predictive value of cSS can also be seen in recent work on a CAA-focused SVD score, which measures in addition to cSS the presence and severity of WMH, periventricular spaces and microbleeds [Charidimou et al., 2016]. In comparison with our study, the summary score did not show a better prediction performance, suggesting that cSS is the main driver within the score. However, calculation of this CAA summary score requires more elaborative assessment and evaluation of multiple neuroimaging features of SVD. Our work suggests that assessment of cSS alone in clinical routine is sufficient to serve as an important predictor for future events and clinical worsening. Lastly, cSS might be particularly relevant for guiding treatment decisions with respect to antithrombotic therapy. While certain cardiovascular comorbidities (e.g. atrial fibrillation) are impotant indications for antithrombotic medication [DeSimone et al., 2017], clinicians have to weigh the benefits against the risk of a major bleeding event. Assessment of cSS might be a way to identify the patient group which should avoid antithromotics even when indicated due to cardiac comorbidity [Wilson et al., 2018]. This could be tested in a future randomized controlled trial.

2.8.3 Biological underpinnings of SVD related white matter lesions

The disease mechanisms by which vessel wall damage leads to parenchymal brain lesions and finally to clinical symptoms is largely unknown [Wardlaw et al., 2019]. Research into the biological underpinnings of SVD is needed for a better understanding of the disease and for the development of targeted treatment strategies. Thus, an additional aim of this Ph.D. thesis was to examine the underlying biological changes that are reflected in the novel markers, such as metrics from the NODDI model and serum NfL.

NODDI is a biophysical diffusion model that aims to characterize specific neurite structures based on their water diffusion signature. In the first research project, we found strong associations between the NODDI metric for extracellular water and cognitive deficits, while there was no association between cognition and the NODDI metric orientation dispersion, a measure supposed to reflect demyelination [Luo et al., 2019]. Changes in extracellular water are in line with previous findings from a two-compartment diffusion model, i.e. free water imaging [Duering et al., 2018]. The free water model was introduced to separate an extracellular free water compartment from the tissue compartment, i.e. intracellular water and water with hindered diffusion by fiber structures. In SVD, elevated free water was strongly correlated with clinical symptoms, whereas no correlation was found between changes in the tissue compartment and clinical symptoms. Together with the current results, this suggests a role for edema formation without substantial loss of white matter structure in the pathophysiological cascade of SVD. In the second project, we established serum NfL as a novel blood marker for disease severity, showing strong associations

with clinical deficits and imaging markers for SVD. Due to the neuroaxonal origin of serum NfL, this result suggests that structural fiber damage plays a role in the disease mechanism. While this is at first glance in contrast with the imaging findings as discussed above, one can speculate that edema in SVD causes white matter compaction and possibly destruction of fiber tracts. Relevant white matter lesions at periventricular and subcortical locations due to excess extracellular fluid were already found in experimental and human post-mortem studies of SVD [Fernando et al., 2006]. In other conditions it has been shown that edema induced neuroaxonal injury is responsible for elevation of neurofilament levels through the accumulation of neurofilament proteins along axonal varicosities and in terminal bulbs [Siedler et al., 2014].

2.9 Conclusions and future directions

A major roadblock for the progress in clinical care and research of SVD is the lack of accurate and valid biomarkers. In the current thesis, I addressed this issue by exploring the utility of novel blood- and imaging-based markers for the disease. The results obtained in this thesis encourage the implementation of novel SVD markers. Advanced diffusion models are highly sensitive for early and subtle white matter alterations, serum NfL is a complementary marker with considerable advantages in clinical and research settings, and cSS is a strong predictor for severe bleeding events.

Future studies on advanced diffusion models should continue exploring the sensitivity for early stage SVD patients and mild disease manifestations by studying the temporal dynamics over a longer follow-up period and encourage the implementation of diffusion MRI markers by clinical validation studies. By prospectively studying correlations between changes in the candidate marker and disease severity, treatment response and clinical outcome, a crucial step will be made for advancing biomarker development from discovery to clinical application.

Additional insight into the biological underpinnings of SVD related white matter lesions can be obtained through experimental animal studies [Joutel et al., 2014]. While there are a variety of mouse models available for SVD, the short life span of the animals makes it difficult to study the effects of an old age disease like SVD [Joutel et al., 2010]. Furthermore, the small amount of white matter in lissencephalic brains and differences in vascular anatomy compared with gyrencephalic brains represent an important translational gap. As such, neuroimaging manifestations of the disease are absent in most SVD mouse models [Chabriat et al., 2009; Joutel, 2011].

More work is needed to evaluate the safety of antithrombotic treatment in patients with cSS or to evaluate cSS as a reason to withhold antithrombotics. Since a randomized clinical trial will be difficult to perform, additional insights on the risk of bleeding and the relevance of cSS in relation to antithrombotiuc medication could also be obtained from large population-based samples [An et al., 2017].

3. Paper I: Multi-shell diffusion imaging improves tissue characterization in cerebral small vessel disease

Konieczny MJ, Dewenter A, Ter Telgte A, Gesierich B, Wiegertjes K, Finsterwalder S, Kopczak A, Hübner M, Malik R, Tuladhar AM, Marques JP, Norris DG, Koch A, Dietrich O, Ewers M, Schmidt R, de Leeuw FE, Duering M. Multi-shell Diffusion MRI Models for White Matter Characterization in Cerebral Small Vessel Disease. Neurology. 2021 Feb 2;96(5):e698-e708. doi: 10.1212/WNL.0000000000011213. Epub 2020 Nov 16. PMID: 33199431.

4. Paper II: Serum Neurofilament Light Chain Levels Are Related to Small Vessel Disease Burden

Duering M, Konieczny MJ, Tiedt S, Baykara E, Tuladhar AM, Leijsen EV, Lyrer P, Engelter ST, Gesierich B, Achmüller M, Barro C, Adam R, Ewers M, Dichgans M, Kuhle J, de Leeuw FE, Peters N. Serum Neurofilament Light Chain Levels Are Related to Small Vessel Disease Burden. J Stroke. 2018 May;20(2):228-238. doi: 10.5853/jos.2017.02565. Epub 2018 May 31. PMID: 29886723; PMCID: PMC6007291.

5. Paper III: Prognostic relevance of cortical superficial siderosis in cerebral amyloid angiopathy

Wollenweber FA, Opherk C, Zedde M, Catak C, Malik R, Duering M, Konieczny MJ, Pascarella R, Samões R, Correia M, Martí-Fàbregas J, Linn J, Dichgans M. Prognostic relevance of cortical superficial siderosis in cerebral amyloid angiopathy. Neurology. 2019 Feb 19;92(8):e792-e801. doi: 10.1212/WNL.0000000000006956. Epub 2019 Jan 23. PMID: 30674596.

References

Alexander, D.C., A general framework for experiment design in diffusion MRI and its application in measuring direct tissue-microstructure features.Magn Reson Med. 60 (2008) 439-448

An, S.J., Kim, T.J., Yoon, B.W., Epidemiology, Risk Factors, and Clinical Features of Intracerebral Hemorrhage: An Update.J Stroke. 19 (2017) 3-10

Arvanitakis, Z., Leurgans, S.E., Wang, Z., Wilson, R.S., Bennett, D.A., Schneider, J.A., Cerebral amyloid angiopathy pathology and cognitive domains in older persons.Ann Neurol. 69 (2011) 320-327

Bath, P.M., Wardlaw, J.M., Pharmacological treatment and prevention of cerebral small vessel disease: a review of potential interventions.Int J Stroke. 10 (2015) 469-478

Baykara, E., Gesierich, B., Adam, R., Tuladhar, A.M., Biesbroek, J.M., Koek, H.L., Ropele, S., Jouvent, E., Initiative, A.s.D.N., Chabriat, H., A novel imaging marker for small vessel disease based on skeletonization of white matter tracts and diffusion histograms.Annals of neurology. 80 (2016) 581-592

Benavente, O.R., Hart, R.G., McClure, L.A., Szychowski, J.M., Coffey, C.S., Pearce, L.A., Effects of clopidogrel added to aspirin in patients with recent lacunar stroke.N Engl J Med. 367 (2012) 817-825

Bester, M., Jensen, J.H., Babb, J.S., Tabesh, A., Miles, L., Herbert, J., Grossman, R.I., Inglese, M., Non-Gaussian diffusion MRI of gray matter is associated with cognitive impairment in multiple sclerosis.Mult Scler. 21 (2015) 935-944

Biomarkers Definitions Working, G., Biomarkers and surrogate endpoints: preferred definitions and conceptual framework.Clin Pharmacol Ther. 69 (2001) 89-95

Chabriat, H., Joutel, A., Dichgans, M., Tournier-Lasserve, E., Bousser, M.G., Cadasil.Lancet Neurol. 8 (2009) 643-653

Charidimou, A., Boulouis, G., Greenberg, S.M., Viswanathan, A., Cortical superficial siderosis and bleeding risk in cerebral amyloid angiopathy: A meta-analysis.Neurology. 93 (2019) e2192-e2202

Charidimou, A., Boulouis, G., Xiong, L., Jessel, M.J., Roongpiboonsopit, D., Ayres, A., Schwab, K.M., Rosand, J., Gurol, M.E., Greenberg, S.M., Viswanathan, A., Cortical superficial siderosis and first-ever cerebral hemorrhage in cerebral amyloid angiopathy.Neurology. 88 (2017) 1607-1614

Charidimou, A., Martinez-Ramirez, S., Reijmer, Y.D., Oliveira-Filho, J., Lauer, A., Roongpiboonsopit, D., Frosch, M., Vashkevich, A., Ayres, A., Rosand, J., Gurol, M.E., Greenberg, S.M., Viswanathan, A., Total Magnetic Resonance Imaging Burden of Small Vessel Disease in Cerebral Amyloid Angiopathy: An Imaging-Pathologic Study of Concept Validation.JAMA Neurol. 73 (2016) 994-1001

Cuadrado-Godia, E., Dwivedi, P., Sharma, S., Ois Santiago, A., Roquer Gonzalez, J., Balcells, M., Laird, J., Turk, M., Suri, H.S., Nicolaides, A., Saba, L., Khanna, N.N., Suri, J.S., Cerebral Small Vessel Disease: A Review Focusing on Pathophysiology, Biomarkers, and Machine Learning Strategies.J Stroke. 20 (2018) 302-320

Cummings, J., Lee, G., Ritter, A., Sabbagh, M., Zhong, K., Alzheimer's disease drug development pipeline: 2019.Alzheimers Dement (N Y). 5 (2019) 272-293

de Groot, M., Verhaaren, B.F., de Boer, R., Klein, S., Hofman, A., van der Lugt, A., Ikram, M.A., Niessen, W.J., Vernooij, M.W., Changes in normal-appearing white matter precede development of white matter lesions.Stroke. 44 (2013) 1037-1042

De Leeuw, F., de Groot, J.C., Achten, E., Oudkerk, M., Ramos, L., Heijboer, R., Hofman, A., Jolles, J., Van Gijn, J., Breteler, M., Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study.Journal of Neurology, Neurosurgery & Psychiatry. 70 (2001) 9-14

DeSimone, C.V., Graff-Radford, J., El-Harasis, M.A., Rabinstein, A.A., Asirvatham, S.J., Holmes, D.R., Jr., Cerebral amyloid angiopathy and implications for atrial fibrillation management.Lancet. 390 (2017) 9-11

Duering, M., Finsterwalder, S., Baykara, E., Tuladhar, A.M., Gesierich, B., Konieczny, M.J., Malik, R., Franzmeier, N., Ewers, M., Jouvent, E., Biessels, G.J., Schmidt, R., de Leeuw, F.E., Pasternak, O., Dichgans, M., Free water determines diffusion alterations and clinical status in cerebral small vessel disease.Alzheimers Dement. 14 (2018) 764-774

Fernando, M.S., Simpson, J.E., Matthews, F., Brayne, C., Lewis, C.E., Barber, R., Kalaria, R.N., Forster, G., Esteves, F., Wharton, S.B., Shaw, P.J., O'Brien, J.T., Ince, P.G., Function, M.R.C.C., Ageing Neuropathology Study, G., White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury.Stroke. 37 (2006) 1391-1398

Hagmann, P., Jonasson, L., Maeder, P., Thiran, J.P., Wedeen, V.J., Meuli, R., Understanding diffusion MR imaging techniques: from scalar diffusion-weighted imaging to diffusion tensor imaging and beyond.Radiographics. 26 Suppl 1 (2006) S205-223

Holtmannspötter, M., Peters, N., Opherk, C., Martin, D., Herzog, J., Brückmann, H., Sämann, P., Gschwendtner, A., Dichgans, M., Diffusion Magnetic Resonance Histograms as a Surrogate Marker and Predictor of Disease Progression in CADASIL.Stroke. 36 (2005) 2559-2565

Jensen, J.H., Helpern, J.A., Ramani, A., Lu, H., Kaczynski, K., Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging.Magn Reson Med. 53 (2005) 1432-1440

Jonsson, M., Zetterberg, H., van Straaten, E., Lind, K., Syversen, S., Edman, A., Blennow, K., Rosengren, L., Pantoni, L., Inzitari, D., Wallin, A., Cerebrospinal fluid biomarkers of white matter lesions - cross-sectional results from the LADIS study.Eur J Neurol. 17 (2010) 377-382

Joutel, A., Pathogenesis of CADASIL: transgenic and knock-out mice to probe function and dysfunction of the mutated gene, Notch3, in the cerebrovasculature.Bioessays. 33 (2011) 73-80

Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., Alamowitch, S., Domenga, V., Cecillion, M., Marechal, E., Maciazek, J., Vayssiere, C., Cruaud, C., Cabanis, E.A., Ruchoux, M.M., Weissenbach, J., Bach, J.F., Bousser, M.G., Tournier-Lasserve, E., Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia.Nature. 383 (1996) 707-710

Joutel, A., Faraci, F.M., Cerebral small vessel disease: insights and opportunities from mouse models of collagen IV-related small vessel disease and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.Stroke. 45 (2014) 1215-1221

Joutel, A., Monet-Lepretre, M., Gosele, C., Baron-Menguy, C., Hammes, A., Schmidt, S., Lemaire-Carrette, B., Domenga, V., Schedl, A., Lacombe, P., Hubner, N., Cerebrovascular dysfunction and microcirculation rarefaction precede white matter lesions in a mouse genetic model of cerebral ischemic small vessel disease.J Clin Invest. 120 (2010) 433-445

Kalaria, R.N., Ballard, C., Overlap between pathology of Alzheimer disease and vascular dementia.Alzheimer disease and associated disorders. (1999)

Kwok, C.S., Shoamanesh, A., Copley, H.C., Myint, P.K., Loke, Y.K., Benavente, O.R., Efficacy of antiplatelet therapy in secondary prevention following lacunar stroke: pooled analysis of randomized trials.Stroke. 46 (2015) 1014-1023

Lu, C.-H., Macdonald-Wallis, C., Gray, E., Pearce, N., Petzold, A., Norgren, N., Giovannoni, G., Fratta, P., Sidle, K., Fish, M., Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis.Neurology. 84 (2015) 2247-2257

Luo, T., Oladosu, O., Rawji, K.S., Zhai, P., Pridham, G., Hossain, S., Zhang, Y., Characterizing Structural Changes With Evolving Remyelination Following Experimental Demyelination Using High Angular Resolution Diffusion MRI and Texture Analysis.Journal of Magnetic Resonance Imaging. 49 (2019) 1750-1759

Maillard, P., Fletcher, E., Harvey, D., Carmichael, O., Reed, B., Mungas, D., DeCarli, C., White matter hyperintensity penumbra.Stroke. 42 (2011) 1917-1922

Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K., Alzheimer's Disease Neuroimaging, I., Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease.JAMA Neurol. 74 (2017) 557-566

Mehta, D., Jackson, R., Paul, G., Shi, J., Sabbagh, M., Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015.Expert opinion on investigational drugs. 26 (2017) 735-739

Ngandu, T., Lehtisalo, J., Solomon, A., Levalahti, E., Ahtiluoto, S., Antikainen, R., Backman, L., Hanninen, T., Jula, A., Laatikainen, T., Lindstrom, J., Mangialasche, F., Paajanen, T., Pajala, S., Peltonen, M., Rauramaa, R., Stigsdotter-Neely, A., Strandberg, T., Tuomilehto, J., Soininen, H., Kivipelto, M., A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial.Lancet. 385 (2015) 2255-2263

Nucifora, Verma, Lee, Melhem, Diffusion-tensor MR imaging and tractography: exploring brain microstructure and connectivity.Radiology. 245 (2007) 367-384

O'Sullivan, M., Morris, R.G., Huckstep, B., Jones, D.K., Williams, S.C., Markus, H.S., Diffusion tensor MRI correlates with executive dysfunction in patients with ischaemic leukoaraiosis.J Neurol Neurosurg Psychiatry. 75 (2004) 441-447

Pantoni, L., Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges.Lancet Neurol. 9 (2010) 689-701

Pearce, L.A., McClure, L.A., Anderson, D.C., Jacova, C., Sharma, M., Hart, R.G., Benavente, O.R., Investigators, S., Effects of long-term blood pressure lowering and dual antiplatelet treatment on cognitive function in patients with recent lacunar stroke: a secondary analysis from the SPS3 randomised trial.The Lancet Neurology. 13 (2014) 1177-1185

Pettersen, J.A., Keith, J., Gao, F., Spence, J.D., Black, S.E., CADASIL accelerated by acute hypotension: Arterial and venous contribution to leukoaraiosis.Neurology. 88 (2017) 1077-1080

Poggesi, A., Pasi, M., Pescini, F., Pantoni, L., Inzitari, D., Circulating biologic markers of endothelial dysfunction in cerebral small vessel disease: a review.Journal of Cerebral Blood Flow & Metabolism. 36 (2016) 72-94

Prince, M., Wimo, A., Guerchet, M., Ali, G., Wu, Y., Prina, M., The global impact of dementia.World Alzheimer Report. (2015) 1-82

Rajani, R.M., Quick, S., Ruigrok, S.R., Graham, D., Harris, S.E., Verhaaren, B.F., Fornage, M., Seshadri, S., Atanur, S.S., Dominiczak, A.F., Reversal of endothelial dysfunction reduces white matter vulnerability in cerebral small vessel disease in rats.Science translational medicine. 10 (2018) eaam9507

Rohrer, J.D., Woollacott, I.O., Dick, K.M., Brotherhood, E., Gordon, E., Fellows, A., Toombs, J., Druyeh, R., Cardoso, M.J., Ourselin, S., Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia.Neurology. 87 (2016) 1329-1336

Shi, Y., Thrippleton, M.J., Blair, G.W., Dickie, D.A., Marshall, I., Hamilton, I., Doubal, F.N., Chappell, F., Wardlaw, J.M., Small vessel disease is associated with altered cerebrovascular pulsatility but not resting cerebral blood flow.Journal of Cerebral Blood Flow & Metabolism. 40 (2020) 85-99

Shoamanesh, A., Preis, S.R., Beiser, A.S., Vasan, R.S., Benjamin, E.J., Kase, C.S., Wolf, P.A., DeCarli, C., Romero, J.R., Seshadri, S., Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: Framingham Heart Study.Neurology. 84 (2015) 825-832

Siedler, D.G., Chuah, M.I., Kirkcaldie, M.T., Vickers, J.C., King, A.E., Diffuse axonal injury in brain trauma: insights from alterations in neurofilaments.Front Cell Neurosci. 8 (2014) 429

Ter Telgte, A., van Leijsen, E.M.C., Wiegertjes, K., Klijn, C.J.M., Tuladhar, A.M., de Leeuw, F.E., Cerebral small vessel disease: from a focal to a global perspective.Nat Rev Neurol. 14 (2018) 387-398

Tuladhar, A.M., van Norden, A.G., de Laat, K.F., Zwiers, M.P., van Dijk, E.J., Norris, D.G., de Leeuw, F.-E., White matter integrity in small vessel disease is related to cognition.NeuroImage: Clinical. 7 (2015) 518-524

Van Dalen, J.W., Moll van Charante, E.P., Caan, M.W., Scheltens, P., Majoie, C.B., Nederveen, A.J., Van Gool, W.A., Richard, E., Effect of long-term vascular care on progression of cerebrovascular lesions: magnetic resonance imaging substudy of the PreDIVA trial (prevention of dementia by intensive vascular care).Stroke. 48 (2017) 1842-1848

van Norden, A.G., de Laat, K.F., van Dijk, E.J., van Uden, I.W., van Oudheusden, L.J., Gons, R.A., Norris, D.G., Zwiers, M.P., de Leeuw, F.E., Diffusion tensor imaging and cognition in cerebral small vessel disease: the RUN DMC study.Biochim Biophys Acta. 1822 (2012) 401-407

van Uden, I.W., van der Holst, H.M., Tuladhar, A.M., van Norden, A.G., de Laat, K.F., Rutten-Jacobs, L.C., Norris, D.G., Claassen, J.A., van Dijk, E.J., Kessels, R.P., de Leeuw, F.E., White Matter and Hippocampal Volume Predict the Risk of Dementia in Patients with Cerebral Small Vessel Disease: The RUN DMC Study.J Alzheimers Dis. 49 (2016) 863-873

Wang, Z., Zhang, S., Liu, C., Yao, Y., Shi, J., Zhang, J., Qin, Y., Zhu, W., A study of neurite orientation dispersion and density imaging in ischemic stroke.Magn Reson Imaging. 57 (2019) 28-33

Wardlaw, Smith, Dichgans, Small vessel disease: mechanisms and clinical implications.Lancet Neurol. 18 (2019) 684-696

Wardlaw, J.M., Smith, E.E., Biessels, G.J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R.I., T O'Brien, J., Barkhof, F., Benavente, O.R., Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration.The Lancet Neurology. 12 (2013) 822-838

Wilson, D., Ambler, G., Shakeshaft, C., Brown, M.M., Charidimou, A., Salman, R.A.-S., Lip, G.Y., Cohen, H., Banerjee, G., Houlden, H., Cerebral microbleeds and intracranial haemorrhage risk in patients anticoagulated for atrial fibrillation after acute ischaemic stroke or transient ischaemic attack (CROMIS-2): a multicentre observational cohort study.The Lancet Neurology. 17 (2018) 539-547

Zhang, H., Schneider, T., Wheeler-Kingshott, C.A., Alexander, D.C., NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain.Neuroimage. 61 (2012) 1000-1016

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