Age-related small vessel disease: a potential contributor to neurodegeneration in multiple sclerosis.

Ruth Geraldes, MD, Margaret M Esiri MD, FRCPath,

Gabriele C DeLuca, MD, DPhil,

Jacqueline Palace BM, DM

Nuffield Department of Clinical Neurosciences

John Radcliffe Hospital,

Oxford, UK

Word count: 4833

Corresponding authors: ruth.geraldes@ndcn.ox.ac.uk, jacqueline.palace@ndcn.ox.ac.uk, gabriele.deluca@ndcn.ox.ac.uk

Supplemental data: Supplemental Table 1 and Figure 1

Search terms: multiple sclerosis, vascular comorbidities, cerebral small vessel disease, ageing, neurodegeneration

Disclosures, conflicts of interest: none

Acknowledgements: We acknowledge Cairns library (Oxford University), particularly, Neal Thurley for his support in the literature review. We are further indebted to Ricardo França for graphical support in Figure 2.

Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system wherein, after an initial phase of transient neurological defects, slow neurological deterioration due to progressive neuronal loss ensues. Age is a major determinant of MS progression onset and disability. Over the past years, several mechanisms have been proposed to explain the key drivers of neurodegeneration and disability accumulation in MS. However, the effect of commonly encountered age-related cerebral vessel disease, namely small vessel disease (SVD), has been largely neglected and constitutes the aim of this review.

SVD shares some features with MS, i.e. white matter demyelination and brain atrophy, and has been shown to contribute to the neuronal damage seen in vascular cognitive impairment. Several lines of evidence suggest that an interaction between MS and SVD may influence MS-related neurodegeneration. SVD may contribute to hypoperfusion, reduced vascular reactivity and tissue hypoxia, features seen in MS. Venule and endothelium abnormalities have been documented in MS but the role of arterioles and of other neurovascular unit structures, such as the pericyte, have not been explored. Vascular risk factors (VRF) have recently been associated with faster progression in MS though the mechanisms are unclear since very few studies have addressed the impact of VRF and SVD on MS imaging and pathology outcomes. Therapeutic agents targeting the microvasculature and the neurovascular unit may impact both SVD and MS and may benefit patients with dual pathology.

Word count: 235

INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with neurodegeneration contributing to long-term disability(112). After a phase of active inflammatory demyelination, translating into transient neurological worsening(37), patients enter into a progressive phase wherein accumulation of neurologic disability is driven by neuronal/axonal loss disproportionate to inflammatory activity(123) (211).

Age is one of the stronger predictors of entry into the progressive phase and accumulation of severe disability (38, 207, 178). This implies that factors known to be associated with aging, such as hypoxia(163), mitochondrial dysfunction (43), and iron accumulation (215) may be important contributors to neuronal damage, and by extension, disability in MS(53, 191, 124, 125).

Cerebral small vessel disease (SVD) is another age-related phenomenon and affects cerebral small cerebral arterioles, capillaries, and venules(152) (60). SVD associates with microinfarcts, microbleeds and with periventricular white matter (WM) pathology. Age and Vascular Risk Factors (VRF), such as hypertension, are the most important predictors of SVD (174). SVD associates with neurodegeneration in the elderly(229) and in young adults with VRFs (64,32) and may contribute to age-related neurodegenerative pathology seen in MS (Figure 1).

(Figure 1 here)

There are several reasons why the interaction between MS and age related - SVD warrants further study, including: 1) MS patients have a longer life expectancy (128) reaching 60 years of age and beyond (187) and thus susceptible to the accumulation of vascular comorbidities; 2) Vascular comorbidities contribute to MS progression(129), reduced life

expectancy(73,131, 27), increase load of WM lesions and brain atrophy(97) ; 3) In MS focal demyelination occurs in watershed areas(78), where hypoperfusion and tissue hypoxia are features (124, 53, 47) suggesting an important relationship between MS pathology and cerebral arterial perfusion; 4) The chronic inflammatory milieu in MS may predispose to SVD; and, 5) Drugs targeting the microvasculature may be beneficial not only in SVD but also in MS (135, 33, 148).

Herein we present a comprehensive review on MS and SVD pathology. Through a critical analysis of clinical, radiographic, and pathologic data, we explore the impact of these diseases on the cerebral microvasculature, their overlapping features, and the possible additive effect of SVD on MS clinical outcomes and neurodegeneration. In so doing, we hope to shed light onto the striking age-related accumulation of neurologic disability that characterises the later stages of MS.

CEREBRAL MICROCIRCULATION

The cerebral circulation can be classified according to vessel size into macro- and micro-circulation(106)(94)(98) as illustrated in Figure 2. Microvessels do not provide sufficient collateral flow to perfuse tissue when a penetrating arteriole or venule is blocked(20). This contributes to deep WM vulnerability to ischaemia because its major blood supply is via long medullary arterioles (217)(24). Communication between the brain parenchyma and microvasculature, is mediated through the neurovascular unit (NVU) that includes different structures including the pericytes that act as capillary sphincters and control blood flow(80). The normal microvasculature structural features and functions have been reviewed elsewhere (106)(182, 113, 89, 226, 156, 11) and are summarized in Table 1.

(Figure 2 here)

DIFFERENTIATING MS FROM SVD

Classical MS features detected by conventional MRI and its pathologic correlates

MS lesions occur in different areas of the CNS and can be detected *in vivo* using brain magnetic resonance imaging (MRI) and on post-mortem examination. Typical conventional MRI and pathology features are summarized on Table 2.

WM hyperintensities (bright) lesions (WMH) on T2/ fluid-attenuated inversion recovery (FLAIR) imaging sequences are key features of MS, corresponding to different degrees and types of pathology(114, 102, 51, 69, 142, 111). Myelin constituents are differentially affected in well-defined WM lesions (plaques), diffusely abnormal WM (DAWM) or areas of normal WM (NAWM) on conventional MRI. Global loss of all myelin constituents is a key feature of plaques, whereas in areas of DAWM, myelin phospholipids and certain proteins such as myelin associated glycoprotein (MAG) are reduced with relative preservation of other constituents such as proteolipid protein (PLP)(114). However, in practice, differentiating histological changes in NAWM from DAWM may be challenging. Lesions also change over time, older patients with longer disease duration having more inactive than active lesions(69).

While no area is typically spared, there are regions of predilection for WM pathology(37),(32 111,,134). The underlying substrate for this topographic predilection is not fully understood but some observations suggest that the relationship between MS lesions and the cerebral vessels is important. Persistent T2 lesions are more common in central areas of the brain relative to the peripheral regions whereas acute, transient contrast enhancing lesions are more evenly distributed throughout the cerebral WM(116). This suggests that additional factors,

such as periventricular WM susceptibility to hypoxia, may contribute to persistent tissue damage(66) in some areas. Focal WM lesions occur at sites of high venous density but also in watershed areas of low arterial blood flow(78) further supporting a dynamic interaction between MS pathology and the cerebral circulation.

While cortical and deep grey matter pathology are important features of progressive MS that associate with clinical disability, (51) sensitivity to detect lesions in these areas on conventional MRI is relatively poor. This is particularly true of the cerebral cortex. As grey matter signal change is conspicuously absent in MRI diagnostic criteria, which concentrate on brain WM lesions (periventricular, juxta-cortical) (15), we have focussed the current review on WM changes classically described radiographically in the disease. That being said, it should be acknowledged that non-conventional MRI techniques and recent pathology studies demonstrate damage in the cerebral cortex and deep grey matter including demyelination, inflammation and neuronal loss (51). The relationship between these lesions and cerebral vasculature are yet to be explored and therefore is beyond the scope of the current review.

Age-related small vessel disease

The ageing brain

Brain volume and myelin loss occur during "normal ageing" (195). At a cellular level, there is astrocyte and microglial hyperactivity, cellular senescence, stem cell exhaustion, altered intercellular communication, genomic instability, mitochondrial dysfunction and free radical generation, loss of proteostasis, and dysregulated nutrient sensing(120, 92, 153, 34, 22) to name a few. These changes also affect the cerebral vessels(223) particularly arterioles but also veins (62, 23, 95). Cerebral vasoreactivity is impaired in aged brains and changes in the nitric–oxide pathways(121), reduction of endothelin-A and beta-adrenergic receptors(181) have been reported. This age-related degeneration of brain vessels may impair local perfusion(89). With ageing, there is increased BBB permeability and BBB-pericyte injury(63,

150, 142). Other neuronal signalling changes, serotonin, acetylcholine and other vasoactive neurotransmitters, may also affect cerebral blood flow control (55). All these factors may predispose the ageing brain to increased vulnerability to ischemic/hypoxic injury. Structural age-related microvascular changes have been summarized in Table 1.

Typical clinical, conventional MRI and pathology features of age-related SVD

VRF such as hypertension, diabetes, smoking, hypercholesterolemia and obesity, associate with increased risk of cerebrovascular disease in general, even in younger populations (210). Clinical manifestations of arteriolosclerosis-related SVD are varied, including stroke, cognitive decline, urinary and walking dysfunction (153, 28, 186, 118, 189, 155, 9). SVD lesions can also be asymptomatic (217)(93). Amyloid deposition is also an important cause of age-related SVD (60)(122)(77)(31). Although this principally affects cortical arterioles, the underlying subcortical WM may be affected and show signs of damage because these arterioles supply blood to this area. It is generally accepted that the spinal cord is relatively spared in SVD, though this is based on relatively scant literature(205,67). Morphological vascular changes in age-related SVD are summarized in Table 1(186)(52)(16) and illustrated in Figure 2. Of the microvasculature, arterioles are particularly affected with vessel wall thickening and tortuosity. That being said, thickening of vein vessel walls and perivenous collagenosis have also been described (23, 95). Key imaging-pathology features SVD are leukoaraiosis(64,66,74), defined as WMH on FLAIR/T2 brain MRI images without prominent hypo-intensity on the T1 images, lacunes(217)(28)(56), microbleeds(74)(90)(41) and enlarged perivascular spaces(217)(119) as summarized in Table 2. Leukoaraiosis occurs in 5-10 % of patients aged 20-40 years(32) and in up to one third of people aged 65-84 years(22). Lesion distribution patterns differ according to the different VRF(174). A selective loss of MAG and relative preservation of PLP, is a feature of SVD WM abnormalities(12). The role of energy failure in SVD-related disease is highlighted by the observation that there is a decrease in the number of mitochondria in leukoaraiosis (193).

VRF and SVD also associate with generalised and focal brain atrophy(143, 147, 18, 82, 206, 115) and reduced perfusion in NAWM (213) (200).

Imaging discriminators between MS from SVD

Several WM lesion locations and characteristics segregate more clearly with MS (21)(14), while others more with SVD (16,21,44,166,107,13,199,71,4,15) (Table 2). The distinction between these two disease processes on imaging can be difficult in longstanding and late onset MS(184) where both conditions are more likely to coexist. Comparative quantitative measures have shown more heterogeneous lesions in MS (199)(138). Medial lemniscus T2 hyperintensity in the dorsal pons has been reported more frequently in SVD than in MS patients(79). SWI (100)(109) improves differentiation between MS and vascular lesions based on lesion location, perivascular orientation and the presence of hypointense (rims around) lesions as well as detection of central veins(136). The exact specificity of central veins needs to be assessed in larger studies, particularly taking into account vascular comorbidities. Differentiating MS lesions without a central vein from ischemic lesions still remains difficult, particularly in diffuse WM lesions. Annual rate of SVD-related lesion volume increase was similar to the rate of MS-related lesion burden increase in secondary progressive MS observed in natural history studies or the placebo arms of treatment trials (179, 180, 140) and thus not very useful in distinguishing the two disorders. Only small studies using nonconventional imaging techniques have compared MS with SVD(39)(164). In magnetization transfer studies, normal appearing WM seems to be spared in SVD(164) in contrast to MS. Diffusion coefficient measurements(150), as well as magnetic resonance spectroscopy (96)may be useful in the differential diagnosis.

MS AND SVD: EVIDENCE FOR A POSSIBLE INTERACTION

Most of the current literature has concentrated on differentiating MS white matter change from that associated with SVD (Supplemental Figure 1). In the previous section, we compared the two disorders and identified distinctive clinical and imaging features. Nevertheless, DAWM changes and brain atrophy are common in both disorders, particularly in older patients. SVD may coexist in longstanding MS and be responsible for additional brain damage and clinical disability. In this section, we will summarise the direct and indirect evidence of a possible interaction between MS and SVD. MS predominantly affects the veins and venules while SVD predominantly affects the arterioles. Despite this, factors such as ageing, VRFs and chronic inflammation could predispose to microvasculature damage, including in arterioles, that leads to hypoperfusion and tissue hypoxia that contributes to the extent and distribution of MS-related pathology.

"MS Vascular theory" and microcirculation morphological changes in MS

Several vascular changes in the MS brain have been described over the years and a comprehensive review of the history of these observations has recently been published (160). Early pathology studies traced MS lesions to draining CNS veins and capillaries, surrounded by perivascular inflammatory cell infiltrates (170,2,48). Structural microvasculature changes reported in MS are summarized in Table 1 and include thickening of vessel walls, and sometimes vessel thrombosis (212,158,1,26,70). The latter findings led to disappointing trials using anticoagulants and hyperbaric oxygen as a treatment for the disease(159), that were followed by reduced interest in the role of vessels in MS pathology(160). During the last decade, the hypothesis of venous insufficiency in MS patients was raised given the claim that venous blood flow alterations were more prevalent in MS patients (224), an observation not replicated in larger, more controlled studies(204). Recently using new MRI techniques, many MS lesions have been demonstrated *in vivo* to centre around veins of RR and

PPMS(101,196,105) further supporting post-mortem observations that the inflammatory process dominantly involves the veins and venules in MS. However, it is somehow surprising that the arterioles remain intact in longstanding MS and the current published literature does not rule out that structural or functional arteriole changes play a role in MS pathology.

In MS, enlargement of the PVS, particularly at the brain convexity, associates with brain atrophy(99) and perivascular protein changes(70,139,208) further pointing to a possible relationship between vascular pathology and MS disease severity. Structural abnormalities of PVS adjacent to venules have been mentioned above but it is not clear if PVS adjacent to pial and penetrating arterioles are spared. Meningeal inflammation spreads into convexity PVS(85) but it is not always clear if this PVS inflammation occurs exclusively around veins or also surrounds penetrating arterioles, since this information is not always specified. The temporal profile of these structural vascular and perivascular changes in MS, in the context of age-related changes, requires further study. Even if not directly related to MS, arteriole changes are predictable as part of aging and increased VRF though they have not been systematically quantified so far.

NVU disintegration is increasingly recognised as a contributor to neurodegeneration (229) and has been described in MS not only in active demyelinating lesions but also in NAWM (101,3,154) (Table 1). NVU dysfunction can lead to clotting and fibrinolytic pathway abnormalities (11), may impair repair mechanisms such as angiogenesis in chronic MS(72, 83) and potentially affect CBF regulation.

Indirect evidence of vascular dysfunction in MS

Hemodynamic changes in MS

Several studies have found that patients with MS have reduced cerebral perfusion (162, 45, 76). Cerebral blood flow is reduced in non-enhancing WM lesions(188), cortical and

subcortical grey matter(108) associating with higher disability scores and T2 lesion load(151). Chronic MS plaques are more prevalent in WM regions with lower relative perfusion(84) and hypoperfusion associates with T1 hypointensities (146). The observed hypoperfusion in MS appears to be a primary phenomenon and not merely a consequence of neuronal death (176), taking into account the fact that reduced cortical and deep grey matter cerebral blood flow is present in all disease course subtypes(59) even in the absence of corresponding volume loss (151)(50). However, large longitudinal studies are still needed to confirm that hypoperfusion precedes neurodegeneration. Reduced cerebrovascular reactivity has also been reported in MS and impaired dilator capacity of cerebral arterioles to vasomotor stimulation has been proposed as a possible contributor to MS hemodynamic changes (132).

Hypoxia in MS

MS inflammation and associated NVU dysfunction(3) may lead to tissue hypoxia(45)(110). In the animal model, experimental autoimmune encephalitis (EAE), acute tissue hypoxia develops rapidly in response to inflammation, triggers enlargement of vessel lumen and increased vessel number, and is related to neurological deficits(47). Reducing tissue hypoxia may be an underestimated therapeutic target since it may reduce demyelination in animal models(53). There has been an increased interest in the relationship between angiogenesis and MS (72,117,177) but few studies have investigated the relationship between tissue hypoxia and structural microvasculature abnormalities in MS(47). MS WM lesions and classic ischaemic WM disease share some histopathological changes (110)(49). Using vascular distance maps, larger MS lesions tend to be further from vessels (104). Recently, it has been shown that MS lesions tend to accumulate not only in areas of high venule density but also in watershed areas(78) where hypoxia due to low arterial perfusion may contribute to and/or amplify mitochondrial dysfunction, previously documented in MS(125). It is not clear if only arteriolar and venule damage could explain these findings or if they relate to downstream changes at the capillary level, including pericyte and astrocyte dysfunction(49)(80). Indeed, pericytes when exposed to hypoxia and ischemia constrict and die (80). However

abnormalities at the capillary level, particularly pericyte injury, have been relatively neglected in MS and warrant investigation. Additionally, the link between vessel fibrin deposition and tissue hypoxia/ ischemia needs to be clarified. Differences between acute focal demyelinating lesions and diffuse WM changes may associate to different vascular changes, the former to perivenular inflammation and the later with arteriolar changes.

MS and VRF

Epidemiological studies

Studies have shown that MS patients have a greater prevalence of VRF, such as smoking and increased body mass index, compared to the general population while other VRF, such as hypertension, diabetes and hyperlipidaemia do not differ significantly between groups (130). However, the presence of vascular comorbidity (diabetes, hypertension, hypercholesterolemia, heart disease, and peripheral vascular disease)(198) associates with increased risk of more severe MS-related disability(129)(131)(46). Tobacco smoking has also been associated with increased disability and faster progression of clinical disability in MS in some(81) but not all studies (144). Studies on other non-classical VRF have been less explored(192). Higher homocysteine, also associated with SVD(91), has been found in MS when compared to healthy controls in most studies (198) and this could be due to Vitamin B12 deficiency (168)(167) . The presence of VRF also affects disability (e.g. gait impairment) and increases mortality in people without MS. It is not clear if the effect on MS outcome is due to the additive effect of non-MS pathology or due to worse MS pathology.

MRI studies on the effect of VRF in MS

Few MS imaging studies have taken into account the effect of VRF on lesion distribution and size and brain atrophy (Supplementary Table 1) (81,228)(219). North American MS patients with one or more vascular risk factor(s) have an increased lesion burden and more brain atrophy (97) compared to MS patients without VRF. However, how SVD contributes to these

more severe imaging features is not clear. It is possible that increased T2 lesion load in MS patients with VRF is due to additive periventricular WM vascular lesions, lacunes and microbleeds, as a mere association of two disorders (Figure 2). Similarly, vascular risk factor associated atrophy could simply be additive to that in MS patients (115) .

MS and cerebrovascular disease

Epidemiological studies

MS patients have reduced life expectancy that is estimated to be between 7-14 years compared to individuals without the disease (178). Though increased mortality can be directly related to MS, cardiovascular diseases are also important contributors (73). MS is associated with an increased rate of ischemic stroke, myocardial infarction and heart failure in the first years after diagnosis(34,35). The reported cardiovascular disease excessive risk early after MS diagnosis might be due to surveillance bias and later in the disease course may relate to venous disorders in progressive MS, suggesting that immobilization may be a predisposing factor(173). The heterogeneity among studies on the incidence and prevalence of cardiovascular disorders in MS makes it difficult to fully understand the epidemiology of vascular comorbidity in MS(127). There is scarce information of global atherosclerosis burden in MS patients assessed clinically or with vascular imaging, though an increase of subclinical markers of atherosclerosis has been reported in a small group of MS patients particularly in those with reduced physical activity(161).

SVD imaging and pathology correlates in MS

As previously mentioned, MS is associated with haemodynamic changes. These could be related to MS but the contribution of additional factors, such as concomitant SVD, have not been evaluated. Though this may not be a significant concern in young MS patients with no VRF, it is somehow surprising that the potential presence of SVD has not been considered in older patients since SVD dramatically increases with age. Most of the imaging studies have

excluded patients with previous symptomatic cerebrovascular disease (e.g. stroke or ischemic heart disease) and recording of VRF has not been documented systematically.

After an extensive search (Supplemental Figure 1) for publications assessing SVD imaging characteristics in MS only two MRI studies with conflicting results assessed the prevalence of microbleeds in MS patients (227)(58). An imaging-pathological study aiming to track iron in 2 MS brains disclosed iron precipitation in aggregates typical of microbleeds. Indeed, both cases were older than 60 and one had significant VRF and evidence of severe atherosclerosis at autopsy(10). An older pathology study also reported perivenular hemosiderin deposition related to MS plaques in 21 out of 70 MS cases, of which 4 had coexistent cardiovascular disease(1). There are no publications quantifying concomitant arteriolar SVD in MS. Differentiating venous collagenosis associated with leukoaraiosis from MS-associated venous abnormalities may be challenging due to the lack of specific markers. In longstanding MS, basal ganglia T1 hypointensities along with diffuse WM changes have been reported but no information regarding vascular comorbidities was provided(165). Since no imaging-pathological correlation had been performed, the findings could be related to MS and/or SVD pathology.

The chronic inflammatory milieu in MS could contribute to cerebral small vessel damage and vascular damage may impact brain inflammatory response

Of interest is whether there is an interaction between MS and SVD pathology, and if there is in what direction this lies, since the chronic inflammatory milieu of MS could exacerbate SVD (207) and VRF could exacerbate MS pathology. Rheumatoid Arthritis, a systemic inflammatory disorder, is associated with increased risk of cardiovascular disease(8) and accelerated atherosclerosis(225). Inflammation can *per se* be deleterious to the vessel wall and is thought to be an important risk for systemic atherosclerosis (75)(216). The relationship between inflammation and SVD has not been sufficiently explored but the following data suggest that it deserves more attention: 1) systemic inflammation measured by interleukin-6 is

associated with SVD(137); 2) genes associated with inflammatory pathways are upregulated in SVD brains(171, 209) ; and 3) prominent inflammatory infiltrates are found in some amyloid angiopathy subtypes (7). In MS, perivascular inflammation may increase cerebral vessel vulnerability to vascular risk factor-related damage, thus contributing to increase of SVD.

MS and SVD may share common genetic and/or environmental factors

It is possible that common genetic factors simultaneously affect MS and vascular disease phenotypes(214). Apolipoprotein E, an important atherosclerosis risk factor (218) does not seem to affect MS clinical course in humans(25) though it may impact the inflammatory response in MS animal models(185). Mitochondrial genetic variants have been implicated in both MS and leukoaraiosis(194). Underlying variants of fibrinolytic systems can also produce an effect on MS inflammation(57) and also on cerebral ischemia(221).

Environmental factors may also potentially trigger and/or contribute to both MS and atherosclerosis pathology. *Chlamydia pneumoniae* infection has been associated with both disorders(40) but its role in their pathogenesis has not been demonstrated. Sodium chloride intake strongly correlates with hypertension(190) and has been associated with increased clinical and radiological MS activity (61) possibly secondary to the induction of Th17 lymphocytes as demonstrated in EAE (103). Smoking, another risk factor shared by both disorders (19,133), associates with endothelial and BBB disruption (133)(145) and may cause brain damage through multiple pathways (30). The deleterious effect of smoking in cerebral vessels could explain increased lesion load in MS smokers when compared to non-smokers (supplemental table 1) but this not been investigated.

Potential interaction between MS and Arteriosclerosis therapies

Current MS treatments, such as fingolimod (148) may have an antiatherogenic effect. Also alpha-beta1 integrin (VLA-4) blockade has been shown to be effective in reducing CNS inflammation in MS(169) but may also reduce neointimal growth following vascular damage(17), since this integrin is involved in vascular remodelling and atherosclerosis(87). Fumarates may have a cardioprotective effect (6) and may improve CNS response to hypoxia (220). On the other hand statins, known to delay atheroma plaque progression and prevent ischemic cardiovascular events, may delay MS progression(33). The potential benefit and risks of aspirin in MS have been recently reviewed(203). Interestingly, a 1961 publication a trial comparing prednisolone, placebo and calcium aspirin, reported no deterioration in patients on aspirin whereas there was clear clinical worsening in the other two groups(135). Moreover, antihypertensive drugs with a protective effect on cerebrovascular disease, such as amiloride (183), may exert a neuroprotective effect in progressive MS(5). Finally, biotin, an important co-factor for many mitochondrial enzymes that protects against hypoxia associated energy failure, may reverse disability in progressive MS (201). These effects may relate to a pluripotent mechanism of these drugs that interfere with different pathophysiological cascades but could reduce the effect of one pathology on the other. Either way, to develop individualised treatments, comorbidities, such as SVD, should be taken into account and the influence of therapeutic interventions in these comorbidities should not be overlooked.

SVD AS A POSSIBLE CONTRIBUTOR TO NEURODEGENERATION IN MS

Older MS patients: less inflammation, more age-related disorders

Although the pathological hallmark of MS is the presence of multifocal areas of demyelination with relative axonal preservation, imaging and pathological studies have shown that neuronal/axonal injury can occur early and associate with active inflammation and demyelination(65,202) However, age and disease duration affect the inflammatory response

in MS lesional and non-lesional WM and GM, all of which seem to decrease in older patients with a longer disease duration(68)(67). In these patients, neurodegeneration is related, in part, to not only on-going low-grade inflammation but also several mechanisms including increased energy deficiency, oxidative injury, hypoxia and exhaustion of functional reserve capacity (124). Age-related pathology, such as Alzheimer's or vascular disease may amplify all of these mechanisms and thus contribute to increased neuronal damage (67).

"Second hit" hypothesis: could SVD contribute to hypoperfusion and brain atrophy in progressive MS?

As previously mentioned, VRF(18)(29) and SVD (206) associate with brain atrophy and cognitive impairment. The exact relationship between SVD and brain damage is incompletely understood(152,217,172) but factors such as hypoperfusion/ischemia due to reduced vessel lumen size, impairment of perivascular lymphatic drainage, BBB dysfunction(215) and subclinical inflammation may lead to oligodendrocyte damage and loss of myelin causing WM lesions and neuronal loss(152,217,172,86). NVU dysfunction leading to neuronal-vascular uncoupling, has been implicated in perpetuating tissue damage in ischemia(80).The presence of SVD in MS patients may represent the extra hit that hinders compensatory mechanisms. In this case, vascular dysfunction and hypoperfusion with consequent chronic hypoxia could contribute to neuronal death leading to slow neurological deterioration independent of relapses. Cerebrovascular disease has been shown to contribute to neuronal damage in neurodegenerative diseases such as Alzheimer disease(229)(54) and vascular cognitive impairment (157) promoting cycles of chronic hypoperfusion, pericyte and astrocyte dysfunction with BBB permeability changes, oxidative stress, inflammation and mitochondrial impairment(43)(124)(193). This "vasculo-neuronal-inflammatory" model of neurodegenerative diseases, centred in NVU dysfunction, could be applied to MS(229). In MS energy deficiency and tissue hypoxia due to mitochondrial dysfunction leads to ionic imbalance and axonal degeneration and this could be potentiated by concomitant SVD, in

particular in watershed areas, where MS lesions tend to accumulate (124). Not only detailed mapping of MS lesions related to arterial and venous blood supply needs to be investigated (124) but characterization and quantification of SVD, including scoring of arterial vessel wall changes, microbleeds, microinfarcts, and its relationship to energy failure in MS is warranted.

Oligodendrocyte regeneration mechanisms can also be impaired due to dual pathology affecting ventricular-subventricular zone-derived progenitor cells(126), since this area is frequently affected by SVD and MS. These mechanisms may particularly cause or potentiate hypoperfusion and brain damage in MS (49), not only affecting focal lesion topography(78) but also diffuse abnormal WM lesions where there are imaging and pathologic similarities to SVD. Additionally, chronic inflammation in MS may also predispose to microvasculature and NVU damage leading to abnormalities in fibrinolytic pathways(11,57), impairing angiogenesis(117), and repair after ischemic injury and thus perpetuating neuronal injury.

Could SVD potentiate tissue damage related to acute inflammation in MS?

The effect of the interaction between SVD and MS on acute inflammation and subsequent neuronal damage has not been sufficiently explored but may contribute to the age-related decline of recovery after a relapse(42). Vessel integrity is essential for many steps of the immune response, including leukocyte priming, activation and migration(222). The effect of cerebral age-related SVD on each of these steps is not well characterized. Taking into account the previously described structural vascular changes, it is expected that the immune responses will be compromised to some extent. Cerebral SVD-related BBB dysfunction has been shown to associate with endothelial cell and monocyte/macrophage activation(175), which could contribute to inflammation-related neuronal damage in MS. As previously mentioned, there is tissue hypoxia associated with inflammation in MS (45,110,53,117) and it is plausible that, if present in MS patients, SVD impairs compensatory mechanisms to acute inflammation-related hypoxia, potentiating tissue damage.

CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Understanding the contribution of SVD to brain damage in MS patients and in particular to its role in the neurodegenerative process is a high priority. Some common imaging and pathological features may be markers of SVD and MS interaction: diffuse periventricular WM lesions and enlargement of perivascular spaces. The challenge lies in identifying specific biomarkers that differentiate these two pathologies and ensuring that identified MS-specific features have arisen from cohorts where VRF have been excluded. VRF and comorbidities are associated with faster MS progression and increased lesion load through unclear mechanisms. Future imaging research on brain volumetrics and WM lesions should take into account VRF and comorbidities in MS patients.

In MS, there is hypoperfusion and reduced vascular reactivity. Venule and endothelium abnormalities have been described but the contribution of arterioles and the NVU to these hemodynamic changes is still to be explored. Human imaging-pathological studies would allow to better dissecting of the interplay between MS and age related vascular changes/SVD, MS animal models should be set up to look at the direct effect of vascular comorbidities on MS pathology and at potential common MS and SVD pathogenic pathways. Understanding the impact of SVD in MS is important in planning treatment trials, particularly in older progressive patients and may lead to better neuroprotective therapies in the future.

References

1.Adams CW (1988) Perivascular iron deposition and other vascular damage in multiple sclerosis. J Neurol Neurosurg Psychiatry **51(2)**:260–5.

2.Adams CW, Abdulla YH, Torres EM, Poston RN (1987) Periventricular lesions in multiple sclerosis: their perivenous origin and relationship to granular ependymitis. Neuropathol Appl Neurobiol **13(2):**141–52.

3.Alexander JS, Zivadinov R, Maghzi A-H, Ganta VC, Harris MK, Minagar A (2011) Multiple sclerosis and cerebral endothelial dysfunction: Mechanisms. Pathophysiology **18(1)**:3–12.

4.Aliaga ES, Barkhof F (2014) MRI mimics of multiple sclerosis. Handb Clin Neurol 122:291–316.

5.Arun T, Tomassini V, Sbardella E, de Ruiter MB, Matthews L, Leite MI, Gelineau-Morel R, Cavey A, Vergo S, Craner M, Fugger L, Rovira A, Jenkinson M, Palace J (2013) Targeting ASIC1 in primary progressive multiple sclerosis: evidence of neuroprotection with amiloride. Brain **136(1):**106–15.

6.Ashrafian H, Czibik G, Bellahcene M, Aksentijević D, Smith AC, Mitchell SJ, Dodd MS, Kirwan J, Byrne JJ, Ludwig C, Isackson H, Yavari A, Støttrup NB, Contractor H, Cahill TJ, Sahgal N, Ball

DR, Birkler RI, Hargreaves I, Tennant DA, Land J, Lygate CA, Johannsen M, Kharbanda RK, Neubauer S, Redwood C, de Cabo R, Ahmet I, Talan M, Günther UL, Robinson AJ, Viant MR, Pollard PJ, Tyler DJ, Watkins H (2012) Fumarate Is Cardioprotective via Activation of the Nrf2 Antioxidant Pathway. Cell Metab **15(3):**361–71.

7.Auriel E, Charidimou A, Gurol ME, Ni J, Van Etten ES, Martinez-Ramirez S, Boulis G, DiFrancesco JC, Frosch MP, Pontes-Neto OV, Shoamanesh A, Reijmer Y, Vashkevich A, Ayres AM, Schwab KM, Viswanathan A, Greenberg SM (2016) Validation of Clinicoradiological Criteria for the Diagnosis of Cerebral Amyloid Angiopathy-Related Inflammation. JAMA Neurol **73(2):**197–202.

8.Aviña-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D (2008) Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum **59(12):**1690–7.

9.Baezner H, Blahak C, Poggesi a., Pantoni L, Inzitari D, Chabriat H, Erkinjuntti T, Fazekas F, Ferro JM, lanhorne P, O´Brien J, Scheltens P, Visser MC, Wahlund LO, Waldemar G, Wallin A, Hennerici MG (2008) Association of gait and balance disorders with age-related white matter changes: The LADIS Study. Neurology **70(12):**935–42.

10.Bagnato F, Hametner S, Yao B, Van Gelderen P, Merkle H, Cantor FK, Lassmann H, Duyn JH (2011) Tracking iron in multiple sclerosis: A combined imaging and histopathological study at 7 Tesla. Brain. **134(12):**3602-15.

11.Bardehle S, Rafalski VA, Akassoglou K (2015) Breaking boundaries-coagulation and fibrinolysis at the neurovascular interface. *Front Cell Neurosci* **9**:354.

12.Barker R, Wellington D, Esiri MM, Love S (2013) Assessing white matter ischemic damage in dementia patients by measurement of myelin proteins. J Cereb Blood Flow Metab **33(7):**1050–7.

13.Barkhof F (1996) Role of MR imaging in the diagnosis of MS. Advances in MRI Contrast **4(2):** 31–8.

14.Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH, Comi G, Ader HJ, Losseff N, Valk J (1997) Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain**. 120**:2059–69.

15.Barkhof F, Rocca M, Francis G, Van Waesberghe JH, Uitdehaag BMJ, Hommes OR, Hartung HP, Durelli L, Edan G, Fernández O, Seeldrayers P, Sorensen P, Margrie S, Rovaris M, Comi G, Filippi M (2003) Validation of diagnostic magnetic resonance imaging criteria for multiple sclerosis and response to interferon β1a. Ann Neurol. **53(6):**718–24.

16.Barkhof F, Scheltens P (2002) Imaging of white matter lesions. Cerebrovasc Dis. **13** Suppl 2(suppl 2):21–30.

17.Barringhaus KG, Phillips JW, Thatte JS, Sanders JM, Czarnik AC, Bennett DK, Ley KF, Sarembock IJ (2004) Alpha4beta1 integrin (VLA-4) blockade attenuates both early and late leukocyte recruitment and neointimal growth following carotid injury in apolipoprotein E (-/-) mice. J Vasc Res **41(3):**252–60.

18.Beauchet O, Celle S, Roche F, Bartha R, Montero-Odasso M, Allali G, Annweiler C (2013) Blood pressure levels and brain volume reduction. J Hypertens **31(8)**:1502–16.

19.Belbasis L, Bellou V, Evangelou E, Ioannidis JP a, Tzoulaki I (2015) Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses. Lancet Neurol **14(3**):263–73.

20.Blinder P, Tsai PS , Kaufhold JP, Knoutsen PM, Suhl H, Klienfeld (2013) The cortical angiome:an interconnected vascular network with noncolumnar patterns of blood flow *Nat Neurosci* **16**:889–97.

21.Bot JCJ, Barkhof F, Lycklama à Nijeholt G, van Schaardenburg D, Voskuyl AE, Ader HJ, Pinjnenburg JA, Polman CH, Uidehaag BM, Vermeulen EG, Castelijns JA (2002) Differentiation of multiple sclerosis from other inflammatory disorders and cerebrovascular disease: value of spinal MR imaging. Radiology **223(1):**46–56.

22.Breteler MM, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout JH, can Harskamp F, Tanghe HL, de Jong PT, van Gijn J (1994) Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. Neurology **44(7)**:1246–52.

23.Brown WR, Moody DM, Challa VR, Thore CR, Anstrom J A (2002) Venous collagenosis and arteriolar tortuosity in leukoaraiosis. J Neurol Sci; **204**:159–63.

24.Brown WR, Thore CR (2011) Review: cerebral microvascular pathology in ageing and neurodegeneration. *Neuropathol Appl Neurobiol* **37**:56–74.

25.Burwick RM, Ramsay PP, Haines JL, Hauser SL, Oksenberg JR, Pericak-Vance MA, Schmidt S, Compston A, Sawcer S, Cittadella R, Savettieri G, Quattrone A, Polman CH, Uitdehaag BM, Zwemmer JN, Hawkins CP, Ollier WE, Weatherby S, Enzinger C, Fazekas F, Schmidt H, Schmidt R,

Hillert J, Masterman T, Hogh P, Niino M, Kikuchi S, Maciel P, Santos M, Rio ME, Kwiecinski H, Zakrzewska-Pniewska B, Evangelou N, Palace J, Barcellos LF (2006) APOE epsilon variation in multiple sclerosis susceptibility and disease severity: some answers. Neurology **66(9):**1373–83.

26.C.B. B, Beggs CB (2013) Venous hemodynamics in neurological disorders: An analytical review with hydrodynamic analysis. BMC Med **11(1):**142.

27.Capkun G, Dahlke F, Lahoz R, Nordstrom B, Tilson HH, Cutter G, Bischof D, Moore A, Simeone J, Fraeman K, Bancken F, Geissbuhler Y, Wagner M, Cohan S (2015) Mortality and comorbidities in patients with multiple sclerosis compared with a population without multiple sclerosis: An observational study using the US Department of Defense administrative claims database. *Mult Scler Relat Disord*;**4**:546–54.

28.Caplan LR (2015) Lacunar Infarction and Small Vessel Disease : Pathology and Pathophysiology **17(1):**2–6.

29.Celle S, Annweiler C, Pichot V, Bartha R, Barthelemy J-C, Roche F, Beauchet O (2012) Association Between Ambulatory 24-Hour Blood Pressure Levels and Brain Volume Reduction: A Cross-Sectional Elderly Population-Based Study. Hypertension **60(5):**1324–31.

30.Chang RC-C, Ho Y-S, Wong S, Gentleman SM, Ng H-K (2014) Neuropathology of cigarette smoking. Acta Neuropathol **127(1):**53–69.

31.Charidimou A, Hong YT, Jäger HR, Fox Z, Aigbirhio FI, Fryer TD, Menon DK, Warburton EA, Werring DJ, Baron JC (2015) White Matter Perivascular Spaces on Magnetic Resonance Imaging. Stroke **46(6):**1707–9.

32.Charil A, Yousry T a, Rovaris M, Barkhof F, De Stefano N, Fazekas F, Miller DH, Montalban X, Simon JH, Polman C, Filippi (2006) MRI and the diagnosis of multiple sclerosis: expanding the concept of 'no better explanation'. *Lancet Neurol* **5**:841–52

33.Chataway J, Schuerer N, Alsanousi A, Chan D, MacManus D, Hunter K, Anderson V, Bangham CR, Clegg S, Nielsen C, Fox NC, Wilkie D, Nicholas J, Calder V, Greenwood J, Frost C, Nicholas R (2014) Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. *Lancet* **383**:2213–21.

34.Chen R-L, Balami JS, Esiri MM, Chen L-K, Buchan AM (2015) Ischemic stroke in the elderly: an overview of evidence. Nat Rev Neurol **6(5):**256–65.

35.Christiansen CF (2012) Risk of vascular disease in patients with multiple sclerosis: a review. Neurol Res **34(8):**746–53.

36.Christiansen CF, Christensen S, Farkas DK, Miret M, Sørensen HT, Pedersen L (2010) Risk of arterial cardiovascular diseases in patients with multiple sclerosis: a population-based cohort study. Neuroepidemiology **35(4)**:267–74.

37.Compston A, Coles A (2002) Multiple sclerosis. *Lancet* **359**:1221–31.

38.Confavreux C, Vukusic S (2006) Age at disability milestones in multiple sclerosis. *Brain* **129**:595–605.

39.Confort-Gouny S, Vion-Dury J, Nicoli F, Dano P, Donnet A, Grazziani N, Gastaut JL, Cozzone PJ(1993) A multiparametric data analysis showing the potential of localized proton MR spectroscopy of the brain in the metabolic characterization of neurological diseases. J Neurol Sci **118(2):**123–33.

40.Contini C, Seraceni S, Cultrera R, Castellazzi M, Granieri E, Fainardi E (2010) Chlamydophila pneumoniae Infection and Its Role in Neurological Disorders. Interdiscip Perspect Infect Dis 273573.

41.Cordonnier C, Al-Shahi Salman R, Wardlaw J (2007) Spontaneous brain microbleeds: Systematic review, subgroup analyses and standards for study design and reporting. Brain **130(8):**1988–2003.

42.Cossburn M, Ingram G, Hirst C, Ben-Shlomo Y, Pickersgill TP, Robertson NP (2012) Age at onset as a determinant of presenting phenotype and initial relapse recovery in multiple sclerosis. **18(1)**:45–54.

43.Currais A. Ageing and inflammation – A central role for mitochondria in brain health and disease. Ageing Res Rev **21**:30–42.

44.D. U, S. S, Uhlenbrock D, Sehlen S (1989) The value of T1-weighted images in the differentiation between MS, white matter lesions, and subcortical arteriosclerotic encephalopathy (SAE). Neuroradiology **31(3):**203–12.

45.D'haeseleer M, Hostenbach S, Peeters I, Sankari S El, Nagels G, De Keyser J, C´hooghe MB (2015) Cerebral hypoperfusion: a new pathophysiologic concept in multiple sclerosis? J Cereb Blood Flow Metab **35(9):**1406–10.

46.Dagan A, Gringouz I, Kliers I, Segal G (2016) Disability Progression in Multiple Sclerosis Is Affected by the Emergence of Comorbid Arterial Hypertension. J Clin Neurol. **12(3):** 345-50

47.Davies AL, Desai RA, Bloomfield PS, McIntosh PR, Chapple KJ, Linington C, Fairless R, Diem R, Kasti M, Murphy MP, Smith KJ (2013) Neurological deficits caused by tissue hypoxia in neuroinflammatory disease. Ann Neurol **74(6):**815–25.

48.Dawson JW (1916) XVIII.—The Histology of Disseminated Sclerosis. Trans R Soc Edinburgh Cambridge University Press; **50(03):**517–740.
49.De Keyser J, Steen C, Mostert JP, Koch MW (2008) Hypoperfusion of the cerebral white matter in multiple sclerosis: possible mechanisms and pathophysiological significance. J Cereb Blood Flow Metab **28(10):**1645–51.
50.Debernard L, Melzer TR, Van Stockum S, Graham C, Wheeler-Kingshott CA, Dalrymple-Alford JC, Miller DH, Mason DF (2014) Reduced grey matter perfusion without volume loss in early relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry **85(5):**544–51.
51.DeLuca GC, Yates RL, Beale H, Morrow SA (2015) Cognitive impairment in multiple sclerosis: clinical, radiologic and pathologic insights. *Brain Pathol* **25**:79–98.
52.Deramecourt V, Slade JY, Oakley AE, Perry RH, Ince PG, Maurage C, Kalaria RN (2012) Staging and natural history of cerebrovascular pathology in dementia. Neurology **78(14)**:1043–50.
53.Desai RA, Davies AL, Tachrount M, Kasti M, Laulund F, Golay X, Smith KJ (2016) Cause and prevention of demyelination in a model multiple sclerosis lesion. Ann Neurol 79(4):591-604
54.Di Marco LY, Venneri A, Farkas E, Evans PC, Marzo A, Frangi AF (2015) Vascular dysfunction in the pathogenesis of Alzheimer's disease – A review of endothelium-mediated mechanisms and ensuing vicious circles. Neurobiol Dis **82:**593-606
55.Drake CT, Iadecola C (2007) The role of neuronal signaling in controlling cerebral blood flow. Brain Lang **102(2)**:141–52.
56.Duering M, Csanadi E, Gesierich B, Jouvent E, Hervé D, Seiler S, Belaroussi B, Ropele S, Scmidat R, Chabriat H, Dichigan M (2013) Incident lacunes preferentially localize to the edge of white matter hyperintensities: insights into the pathophysiology of cerebral small vessel disease. Brain **136(9)**:2717–26.
57.East E, Baker D, Pryce G, Lijnen HR, Cuzner ML, Gverić D (2005) A role for the plasminogen activator system in inflammation and neurodegeneration in the central nervous system during experimental allergic encephalomyelitis. Am J Pathol **167(2):**545–54.
58.Eisele P, Alonso A, Griebe M, Szabo K, Hennerici MG, Gass A (2016) Investigation of cerebral microbleeds in multiple sclerosis as a potential marker of blood-brain barrier dysfunction. Mult Scler Relat Disord **7**:61–4.
59.Elsankari S, Balédent O, van Pesch V, Sindic C, de Broqueville Q, Duprez T (2013) Concomitant analysis of arterial, venous, and CSF flows using phase-contrast MRI: a quantitative comparison between MS patients and healthy controls. J Cereb Blood Flow Metab **33(9):**1314–21.
60.Esiri M, Chance S, Joachim C, Warden D, Smallwood A, Sloan C, Christie S, Wilcock G, Smith D (2015) Cerebral Amyloid Angiopathy, Subcortical White Matter Disease and Dementia: Literature Review and Study in OPTIMA. *Brain Pathol* **25**:51–62.
61.Farez MF, Fiol MP, Gaitán MI, Quintana FJ, Correale J (2015) Sodium intake is associated with increased disease activity in multiple sclerosis. **86(1):**26–31.
62.Farkas E, de Vos R a I, Donka G, Jansen Steur EN, Mihály A, Luiten PGM (2006) Age-related microvascular degeneration in the human cerebral periventricular white matter. Acta Neuropathol. 2006;**111(2):**150–7.
63.Farrall AJ, Wardlaw JM (2009) Blood-brain barrier: Ageing and microvascular disease - systematic review and meta-analysis. Neurobiol Aging. 30(3):337–52.
64.Fazekas F, Schmidt R, Kleinert R, Kapeller P, Roob G, Flooh E (1998) The spectrum of age-associated brain abnormalities: their measurement and histopathological correlates. *J Neural Transm Suppl* **53**:31–9.
65.Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997) Axonal damage in acute multiple sclerosis lesions. Brain **120:**393–9.
66.Fernando MS, Simpson JE, Matthews F, Brayne C, Lewis CE, Barber R, Kalaria RN, Forster G, Esteves F, Wharton SB, Shaw PJ, O´Brien JT, Ince PG (2006) White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. *Stroke* **37**:1391–8.
67.Fieschi C, Gottlieb A, De Carolis V (1970) Ischaemic lacunae in the spinal cord of arteriosclerotic subjects. J Neurol Neurosurg Psychiatry **33(2)**:138–46.
68.Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H (2009)The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain **132(5):**1175–89.
69.Frischer JM, Weigand SD, Guo Y, Kale N, Parisi JE, Pirko I, Mandrekar J, Brarnow S, Metz I, Bruck W, Lassmann H, Luchinetti CF (2015) Clinical and pathological insights into the dynamic nature of the white matter multiple sclerosis plaque. *Ann Neurol* **78**:710–21.
70.Gay D, Esiri M (1991) Blood-brain barrier damage in acute multiple sclerosis plaques. An

immunocytological study. Brain. **114 (1):**557–72.
71.Gelfand JM (2014) Multiple sclerosis: diagnosis, differential diagnosis, and clinical presentation. Handb Clin Neurol **122**:269–90.
72.Girolamo F, Coppola C, Ribatti D, Trojano M (2014) Angiogenesis in multiple sclerosis and experimental autoimmune encephalomyelitis. Acta Neuropathol Commun **2**:84.
73.Goodin DS, Corwin M, Kaufman D, Golub H, Reshef S, Rametta MJ, Knappertz V, Cutter G, Pleimes D (2014) Causes of Death among Commercially Insured Multiple Sclerosis Patients in the United States. *PLoS One* **9**:e105207.
74.Gouw A a, Seewann A, van der Flier WM, Barkhof F, Rozemuller AM, Scheltens P, Geurts JJ (2011) Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. J Neurol Neurosurg Psychiatry **82(2):**126–35
75.Gregersen I, Holm S, Dahl TB, Halvorsen B, Aukrust P (2016) A focus on inflammation as a major risk factor for atherosclerotic cardiovascular diseases. Expert Rev Cardiovasc Ther **14 (3)**: 391-403
76.Haeseleer M, Cambron M, Vanopdenbosch L, De Keyser J (2011) Vascular aspects of multiple sclerosis. Lancet Neurology **10(7):** 657–66.
77.Haglund M, Englund E (2002) Cerebral amyloid angiopathy, white matter lesions and Alzheimer encephalopathy - a histopathological assessment. Dement Geriatr Cogn Disord **14(3)**:161–6.
78.Haider L, Zrzavy T, Hametner S, Hoftberger R, Bagnato F, Grabner G, Trattnig S, Pfeifenbring S, Bruck W, Lassmann H (2016) The topograpy of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain* **139** (3):807-15.
79.Hakky M, Erbay SH, Brewer E, Midle J, French R, Erbay SH (2013) T2 hyperintensity of medial lemniscus: Higher threshold application to roi measurements is more accurate in predicting small vessel disease. J Neuroimaging **23(3):**345–51
80.Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O´Farrell FM, Buchan AM, Lauritzen M, Attwell D (2014) Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* **508**:55–60.
81.Healy BC, Ali EN, Guttmann CRG, Chitnis T, Glanz BI, Buckle G, Houtchen M, Stazzone L, Moodie J, Berger AM, Duan Y, Bakshi R, Khoury S, Weiner H, Ascherio A (2009) Smoking and disease progression in multiple sclerosis. Arch Neurol **66(7):**858–64
82.Heijer T Den, Launer LJ, Prins ND, Dijk EJ Van (2005) Association between blood pressure , white matter lesions, and atrophy of the medial temporal lobe **64**:263–7.
83.Hill J, Rom S, Ramirez SH, Persidsky Y (2014) Emerging roles of pericytes in the regulation of the neurovascular unit in health and disease. J Neuroimmune Pharmacol **9(5):**591–605.
84.Holland CM, Charil A, Csapo I, Liptak Z, Ichise M, Khoury SJ, Bakshi R, Weiner HL, Guttman CR (2012) The relationship between normal cerebral perfusion patterns and white matter lesion distribution in 1,249 patients with multiple sclerosis. J Neuroimaging **22(2):**129–36.
85.Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, Serafini B, Aloisi F, Roncaroli F, Magliozzi R, Reynods R (2011) Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain **134(9):**2755–71.
86.Huang YH, Zhang WW, Lin L, Feng J, Zhao XX, Guo WH, Wei W (2010) Could changes in arterioles impede the perivascular drainage of interstitial fluid from the cerebral white matter in leukoaraiosis? Neuropathol Appl Neurobiol **36(3):**237–47.
87.Huo Y, Ley K (2001) Adhesion molecules and atherogenesis. Acta Physiol Scand **173(1)**:35–43.
88.Iadecola C (2013) The pathobiology of vascular dementia. Neuron **80(4):**844–66.
89.Iadecola C, Anrather J (2012) The immunology of stroke: from mechanism to translation. *Nat Med* **17**:796–808.
90.Janaway BM, Simpson JE, Hoggard N, Highley JR, Forster G, Drew D, Gebril OH, Matthews FE, Brayne C, Wharton SB, Ince PG (2014) Brain haemosiderin in older people: Pathological evidence for an ischaemic origin of magnetic resonance imaging (MRI) microbleeds. Neuropathol Appl Neurobiol. **40(3)**:258–69.
91.Jeon S, Kang D, Kim JS, Kwon SU (2014) Homocysteine, small-vessel disease, and atherosclerosis. Neurology **83**:695–701.
92.Jiang T, Cadenas E (2014) Astrocytic metabolic and inflammatory changes as a function of age. Aging Cell **13(6):**1059–67.
93.Jokinen H, Gouw a. a., Madureira S, Ylikoski R, Van Straaten ECW, Van Der Flier WM, Barkhof F, Scheltens P, Fazekas F, Schmidt R, Verdelho A, Ferro JM, pantoni L, Inzitari D, Erkinjuntti T (2011) Incident lacunes influence cognitive decline: The LADIS study. Neurology **76(22):**1872–8.

94.Jones EG (1970) On the mode of entry of blood vessels into the cerebral cortex. *J Anat* **106**:507–20.
95.Kalaria RN (1996) Cerebral vessels in ageing and Alzheimer's disease. Pharmacology and Therapeutics. p. 193–214.
96.Kapeller P, Ropele S, Enzinger C, Lahousen T, Strasser-Fuchs S, Schmidt R, Fazekas F (2005) Discrimination of white matter lesions and multiple sclerosis plaques by short echo quantitative 1H-magnetic resonance spectroscopy. J Neurol **252(10):**1229–34.
97.Kappus N, Weinstock-guttman B, Hagemeier J, Hagemeier J, Kennedy C, Melia R, Carl E, Ramasamy DP, Cherneva M, Durfee J, Bergsland N, Dwyer MG, Kolb C, Hojnacki D, Ramanathan M, Zivadinov R (2015) Cardiovascular risk factors are associated with increased lesion burden and brain atrophy in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **87**:181–7.
98.Kiliç T, Akakin A (2008) Anatomy of cerebral veins and sinuses. *Front Neurol Neurosci* **23**:4–15
99.Kilsdonk I, Steenwijk M, Pouwels P, Zwanenburg J, Visser F, Luijten P, Geurts, Barkhof F, Wattjes MP (2015) Perivascular spaces in MS patients at 7 Tesla MRI: A marker of neurodegeneration? Mult Scler **21(2):**155–62.
100.Kilsdonk ID, Wattjes MP, Lopez-Soriano A, Kuijer JP a, de Jong MC, de Graaf WL, Conijn MM, Polman CH, Luijten PR, Geurts JJ, Geerlings MI, Barkhof F (2014) Improved differentiation between MS and vascular brain lesions using FLAIR* at 7 Tesla. Eur Radiol **24(4):**841–9.
101.Kirk J, Plumb J, Mirakhur M, McQuaid S (2003) Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood-brain barrier leakage and active demyelination. J Pathol **201(2):**319–27.
102.Klawiter EC. (2013)Current and new directions in MRI in multiple sclerosis. *Contin Lifelong Learn Neurol* **19**:1058–73.
103.Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, Linker RA, Muller DN, Hafler DA (2013) Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. Nature **496(7446):**518–22.
104.Kozinska D, Holland CM, Krissian K, Westin C-F, Guttmann CR (2004) A method for the analysis of the geometrical relationship between white matter pathology and the vascular architecture of the brain. Neuroimage **22(4):**1671–8
105.Kuchling J, Ramien C, Bozin I, Dörr J, Harms L, Rosche B, Niendorf T, Paul F, Sinnecker T, Wuerfel J (2014) Identical lesion morphology in primary progressive and relapsing-remitting MS -an ultrahigh field MRI study. Mult Scler J **20(14):**1866–71.
106.Kulik T, Kusano Y, Aronhime S, Sandler AL, Winn HR (2008) Regulation of cerebral vasculature in normal and ischemic brain. *Neuropharmacology* **55**:281–8.
107.Kwa VI, Stam J, Blok LM, Verbeeten B (1997) T2-weighted hyperintense MRI lesions in the pons in patients with atherosclerosis. Amsterdam Vascular Medicine Group. Stroke **28(7)**:1357–60.
108.L. F, J.A. L, P.A. N, J.S. W. Freeman L, Lincoln JA, Narayana PA Wolinsky JS (2015) Evidence of widespread cortical hypoperfusion in MS: a surface-based MRI study with pseudo-continuous arterial spin labeling Multiple Sclerosis. **23(1):**794
109.Lane J, Bolster B, Campeau N, Welker K, Gilbertson JR (2015) Characterization of multiple sclerosis plaques using susceptibility-weighted imaging at 1.5 T: Can perivenular localization improve specificity of imaging criteria? J Comput Assist Tomogr **39(3):**317–20.
110.Lassmann H (2003) Hypoxia-like tissue injury as a component of multiple sclerosis lesions. J Neurol Sci **206(2):**187–91.
111.Lassmann H (2010) Review: The architecture of inflammatory demyelinating lesions: Implications for studies on pathogenesis. Neuropathol. Appl. Neurobiol **37**:698–710.
112.Lassmann H, Brück W, Lucchinetti CF (2007) The Immunopathology of Multiple Sclerosis: An Overview. *Brain Pathol* **17**:210–8.
113.Lau LW, Cua R, Keough MB, Haylock-Jacobs S, Yong VW (2013) Pathophysiology of the brain extracellular matrix: a new target for remyelination. *Nat Rev Neurosci* **14**:722–9.
114.Laule C, Pavlova V, Leung E, Zhao G, MacKay AL, Kozlowski P, Traboulsee AL, Moore GR (2013) Diffusely abnormal white matter in multiple sclerosis: further histologic studies provide evidence for a primary lipid abnormality with neurodegeneration. *J Neuropathol Exp Neurol* **72**:42–52
115.Launer LJ, Lewis CE, Schreiner PJ, Sidney S, Battapady H, Jacobs DR, Lim KO, DÉsposito M, Zhang Q, reis J, Davatzikos C, Bryan RN (2015) Vascular Factors and Multiple Measures of Early Brain Health: CARDIA Brain MRI Study. PLoS One **10(3):**e0122138.
116.Lee M a., Smith S, Palace J, Narayanan S, Silver N, Minicucci L, Filippi M, Arnold DL, Matthews PM (1999) Spatial mapping of T2 and gadolinium-enhancing T1 lesion volumes in multiple sclerosis: Evidence for distinct mechanisms of lesion genesis? *Brain* **122**:1261–70.

117.Lengfeld J, Cutforth T, Agalliu D (2014) The role of angiogenesis in the pathology of multiple sclerosis. Vasc Cell **6(1):**23
118.Liu X (2014). A Meta-Analysis of Association between Cerebral Microbleeds and Cognitive Impairment. Med Sci Monit [Internet]. **20**:2189–98.
119.Loos CMJ, Klarenbeek P, van Oostenbrugge RJ, Staals J (2015) Association between Perivascular Spaces and Progression of White Matter Hyperintensities in Lacunar Stroke Patients. PLoS One **10(9)**:e0137323.
120.López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell **153(6):**1194–217.
121.Lourenço CF, Ledo A, Dias C, Barbosa RM, Laranjinha J (2016) Neurovascular and neurometabolic derailment in aging and Alzheimer's disease. Front Aging Neurosci;7:103.
122.Love S, Chalmers K, Ince P, Esiri M, Attems J, Jellinger K, Yamada M, McCarron M, Minett T, Matthews F, Greenberg S, Mann D, Kehoe PG (2014) Development, appraisal, validation and implementation of a consensus protocol for the assessment of cerebral amyloid angiopathy in post-mortem brain tissue. Am J Neurodegener Dis **3(1)**:19–32.
123.Lublin FD (2014) New multiple sclerosis phenotypic classification. *Eur Neurol* **72 Suppl 1**:1–5.
124.Mahad DH, Trapp BD, Lassmann H (2015) Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol **14(2):**183–93.
125.Mahad DJ, Ziabreva I, Campbell G, Lax N, White K, Hanson PS, Lassmann H, Turnbull DM (2009) Mitochondrial changes within axons in multiple sclerosis. Brain **132(5):**1161–74.
126.Maki T, Liang AC, Miyamoto N, Lo EH, Arai K (2013) Mechanisms of oligodendrocyte regeneration from ventricular-subventricular zone-derived progenitor cells in white matter diseases. **7:** 1–11.
127.Marrie RA, Cohen J, Stuve O, Trojano M, Sørensen PS, Reingold S, Cutter G, Reider N (2015) A systematic review of the incidence and prevalence of comorbidity in multiple sclerosis: Overview. Mult Scler **21(3):**1–19.
128.Marrie RA, Elliott L, Marriott J, Cossoy M, Blanchard J, Leung S, Yu N (2015) Effect of comorbidity on mortality in multiple sclerosis. *Neurology* **85**:240–7.
129.Marrie RA, Horwitz RI (2010) Emerging effects of comorbidities on multiple sclerosis. Lancet Neurol. **9**:820–8.
130.Marrie RA, Reider N, Cohen J, Stuve O, Trojano M, Cutter G, Reingold S, Sorensen PS (2015) A systematic review of the incidence and prevalence of cardiac, cerebrovascular, and peripheral vascular disease in multiple sclerosis. Mult Scler 21(3):318–31
131.Marrie RA, Rudick R, Horwitz R, Cutter G, Tyry T, Campagnolo D, Vollmer T (2010) Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. *Neurology* **74**:1041–7.
132.Marshall O, Lu H, Brisset J-C, Xu F, Liu P, Herbert J, Grossman RI, Ge Y (2014) Impaired cerebrovascular reactivity in multiple sclerosis. JAMA Neurol **71(10):**1275–81.
133.Messner B, Bernhard D (2014) Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. Arterioscler Thromb Vasc Biol **34(3):**509–15.
134.Miki Y, Grossman RI, Udupa JK, Wei L. Kolson DL, Mannon LJ, Grossman M (1998) Isolated U-fiber involvement in MS: preliminary observations. *Neurology*;**50**:1301–6.
135.Miller H, Newell DJ, Ridley A (1961) Multiple sclerosis. Trials of maintenance treatment with prednisolone and soluble aspirin. *Lancet (London, England)* **1**:127–9.
136.Mistry N, Abdel-Fahim R, Samaraweera A, Mougin O, Tallantyre E, Tench C, Jaspan T, Morris P, Morgan PS, Evangelou N (2015) Imaging central veins in brain lesions with 3-T T2*-weighted magnetic resonance imaging differentiates multiple sclerosis from microangiopathic brain lesions. Mult Scler J;1–8. (EPub ahead of Print)
137.Miwa K, Okazaki S, Sakaguchi M, Mochizuki H, Kitagawa K (2016) Interleukin-6, interleukin-6 receptor gene variant, small-vessel disease and incident dementia. Eur J Neurol **23 (3):**656-63
138.Mohamed FB, Vinitski S, Gonzalez CF, Faro SH, Lublin FA, Knobler R, Gutierrez JE (2001) Increased differentiation of intracranial white matter lesions by multispectral 3D-tissue segmentation: preliminary results. Magn Reson Imaging **19(2)**:207–18.
139. Mohan H, Krumbholz M, Sharma R, Eisele S, Junker A, Sixt M, Newcombe J, Wekerle H, Lassmann H, Meinl E (2010) Extracellular matrix in multiple sclerosis lesions: Fibrillar collagens, biglycan and decorin are upregulated and associated with infiltrating immune cells. Brain Pathol **20(5)**:966–75.
140.Molyneux PD, Miller DH, Filippi M, Yousry T, Kappos L, Gasperini C, Adèr HJ, Barkhof F (2000) The use of magnetic resonance imaging in multiple sclerosis treatment trials: power calculations for annual lesion load measurement. J Neurol **247(1):**34–40.

141.Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, Harrington MG, Chui HC, Law M, Zlokovic BV (2015) Blood-Brain Barrier Breakdown in the Aging Human Hippocampus. Neuron **85(2):**296–302.
142.Moore GRW, Laule C, MacKay A, Leung E, Li DK, Zhao G, Traboulsee AL, Paty DW (2008) Dirty-appearing white matter in multiple sclerosis. *J Neurol* **255**:1802–11.
143.Moulton CD, Costafreda SG, Horton P, Ismail K, Fu CH (2015) Meta-analyses of structural regional cerebral effects in type 1 and type 2 diabetes. Brain Imaging Behav **9(4**):651-62
144.Munger KL, Fitzgerald KC, Freedman MS, Miller DH, Montalbán X, Edan G, Barkhof F, Suarez G, Radue EW, Sandbrimk R, Kappos L, Pohl C, Ascherio A (2015) No association of multiple sclerosis activity and progression with EBV or tobacco use in BENEFIT. **85(19):**1694–701.
145.Naik P, Fofaria N, Prasad S, Sajja RK, Weksler B, Couraud P-O, Romero IA, Cucullo L (2014) Oxidative and pro-inflammatory impact of regular and denicotinized cigarettes on blood brain barrier endothelial cells: is smoking reduced or nicotine-free products really safe? BMC Neurosci **15:**51.
146.Narayana PA, Zhou Y, Hasan KM, Datta S, Sun X, Wolinsky JS (2014) Hypoperfusion and T1-hypointense lesions in white matter in multiple sclerosis. Mult Scler **20(3):**365–73.
147.Nitkunan A, Lanfranconi S, Charlton R a., Barrick TR, Markus HS (2011) Brain atrophy and cerebral small vessel disease a prospective follow-up study. Stroke. **42(1)**:133–8.
148.Nofer J-R, Bot M, Brodde M, Taylor PJ, Salm P, Brinkmann V, van Berkel T, Biessen EA (2007) FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* **115**:501–8.
149.Oakley R, Tharakan B (2014) Vascular Hyperpermeability and Aging **5(2**):114–25.
150.Öztoprak B, Öztoprak İ, Topalkara K, Erkoç MF, Şalk İ (2014) Role of thalamic diffusion for disease differentiation between multiple sclerosis and ischemic cerebral small vessel disease. Neuroradiology **57(4):**339–47.
151.Paling D, Thade Petersen E, Tozer DJ, Altmann DR, Wheeler-Kingshott CA, Kapoor R, Miller DH, Golay X (2014) Cerebral arterial bolus arrival time is prolonged in multiple sclerosis and associated with disability. J Cereb Blood Flow Metab **34(1):**34–42
152.Pantoni L (2010) Cerebral small vessel disease : from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* **9**:689–701
153.Pfefferbaum A, Mathalon DH, Sullivan E V, Rawles JM, Zipursky RB, Lim KO (2012)A Quantitative Magnetic Resonance Imaging From Infancy to Late Adulthood. Arch Neurol. **51**:874–87.
154.Plumb J, McQuaid S, Mirakhur M, Kirk J (2002) Abnormal endothelial tight junctions in active lesions and normal-appearing white matter in multiple sclerosis. Brain Pathol. **12:**154–69.
155.Poggesi A, Pracucci G, Chabriat H, Erkinjuntti T, Fazekas F, Verdelho A, Hennerici M, Langhorne P, O´Brien J, Scheltens P, Visser MC, Crisby M, Waldemar G, Wallin A, Inzitari D, Pantoni L (2008) Urinary complaints in nondisabled elderly people with age-related white matter changes: The Leukoaraiosis And DISability (LADIS) Study. J Am Geriatr Soc. **56(9)**:1638–43.
156.Prakash R, Carmichael ST (2015) Blood−brain barrier breakdown and neovascularization processes after stroke and traumatic brain injury. *Curr Opin Neurol* **28**:556–64.
157.Prins ND, Scheltens P (2015) White matter hyperintensities, cognitive impairment and dementia: an update. Nat Rev Neurol **11(3):**157–65.
158.Putman T, Adler A (1937) Vascular architecture of the lesions of multiple sclerosis. Arch Neurol Psychiatry **38(1):**1–15.
159.Putnam T, Chiavacci L, Hoff H, Weizen H (1947) Results of treatment of multiple sclerosis with dicoumarin. Arch Neurol Psychiatry **57(1):**1–13.
160.Rae-grant AD, Wong C, Bernatowicz R, Fox RJ (2014) Observations on the brain vasculature in multiple sclerosis : A historical perspective. Mult Scler Relat Disord [Internet]. Elsevier; **3(2):**156–62.
161.Ranadive SM, Yan H, Weikert M, Lane AD, Linden MA, Baynard T, Moti RW, Fernhall B (2012) Vascular dysfunction and physical activity in multiple sclerosis. Med Sci Sports Exerc **44(2):**238–43.
162.Rashid W, Parkes LM, Ingle GT, Chard DT, Toosy AT, Altmann DR, Symms MR, Tofs PS, Thompson AJ, Miller DH (2004) Abnormalities of cerebral perfusion in multiple sclerosis. J Neurol Neurosurg Psychiatry **75(9):**1288–93.
163.Raz L, Knoefel J, Bhaskar K (2015) The neuropathology and cerebrovascular mechanisms of dementia. *J Cereb Blood Flow Metab* 36(1):172-86
164.Reidel MA, Stippich C, Heiland S, Storch-Hagenlocher B, Jansen O, Hahnel S (2003) Differentiation of multiple sclerosis plaques, subacute cerebral ischaemic infarcts, focal vasogenic oedema and lesions of subcortical arteriosclerotic encephalopathy using magnetisation transfer

measurements. Neuroradiology **45(5)**:289–94.

165.Renard D, Castelnovo G, Bousquet PJ, de Champfleur N, de Seze J, Vermersch P, Labauge P (2010) Brain MRI findings in long-standing and disabling multiple sclerosis in 84 patients. Clin Neurol Neurosurg **112(4):**286–90.

166.Revesz T, Hawkins CP, du Boulay EP, Barnard RO, McDonald WI (1989) Pathological findings correlated with magnetic resonance imaging in subcortical arteriosclerotic encephalopathy (Binswanger's disease). J Neurol Neurosurg Psychiatry **52(12)**:1337–44.

167.Reynolds E (2006) Vitamin B12, folic acid, and the nervous system. Lancet Neurol. **5(11):**949–60.

168.Reynolds EH (1992) Multiple sclerosis and vitamin B12 metabolism. J Neuroimmunol **40(2-3)**:225–30.

169.Rice GPA, Hartung H, Calabresi PA (2005) Anti- alpha4 integrin therapy for multiple sclerosis Mechanisms and rationale. Neurology. **64**:1336–42.

170.Rindfleisch E (1863)Histologisches Detail zu der grauen Degeneration von Gehirn und Rückenmark. (Zugleich ein Beitrag zu der Lehre von der Entstehung und Verwandlung der Zelle.). Arch für Pathol Anat und Physiol und für Klin Med **26(5-6):**474–83.

171.Ritz M-F, Grond-Ginsbach C, Kloss M, Tolnay M, Fluri F, Bonati LH, Traenka C, Zeis T, Schaeren-Weinmens N, Peters N, Engelter ST, Lyrer PA (2016) Identification of inflammatory, metabolic, and cell survival pathways contributing to cerebral small vessel disease by postmortem gene expression microarray. Curr Neurovasc Res **13(1):**58–67.

172.Rosenberg GA, Wallin A, Wardlaw JM, Markus HS, Montaner J, Wolfson L, Iadecola C, Zlokovic BV, Joutel A, Dichgans M, Duering M, Schmidt R, Korczyn AD, Grinberg LT, Chui HC, Hachinski V (2016) Consensus statement for diagnosis of subcortical small vessel disease. J Cereb Blood Flow Metab **36(1):**6–25.

173.Roshanisefat H, Bahmanyar S, Hillert J, Olsson T, Montgomery S (2014) Multiple sclerosis clinical course and cardiovascular disease risk - Swedish cohort study. Eur J Neurol **21(11):**1353–e88.

174.Rostrup E, Gouw AA, Vrenken H, van Straaten EC, Ropele S, Pantoni L, Inzitari D, Barkkof F, Waldemar G (2012) The spatial distribution of age-related white matter changes as a function of vascular risk factors-Results from the LADIS study. *Neuroimage* **60**:1597–607.

175.Rouhl RPW, Damoiseaux JGMC, Lodder J, Theunissen R, Knottnerus IL, Staals J, Henskens LH, Kroon AA, de Leeuw PW, Tervaert JW, van Oostenbrugge RJ (2012)Vascular inflammation in cerebral small vessel disease. Neurobiol Aging **33(8):**1800–6.

176.Saindane a. M, Law M, Ge Y, Johnson G, Babb JS, Grossman RI (2007) Correlation of diffusion tensor and dynamic perfusion MR imaging metrics in normal-appearing corpus callosum: Support for primary hypoperfusion in multiple sclerosis. Am J Neuroradiol. **28(4):**767–72.

177.Salvetti M, Landsman D, Schwarz-Lam P, Comi G, Thompson AJ, Fox RJ (2015) Progressive MS: from pathophysiology to drug discovery. Mult Scler **21(11)**:1376–84

178.Scalfari A, Neuhaus A, Daumer M, Muraro PA, Ebers GC (2014) Onset of secondary progressive phase and long-term evolution of multiple sclerosis. *J Neurol Neurosurg Psychiatry* **85**:67–75.

179.Schmidt R, Berghold a., Jokinen H, Gouw a. a., van der Flier WM, Barkhof F, Scheltens P, Petrovic K, Madureira S, verdelho A, ferro JM, Waldemar G, Wallin A, Wahlund LO, Poggesi A, Pantoni L, Inzitari D, Fazekas F, erkinjuntti T (2012) White Matter Lesion Progression in LADIS: Frequency, Clinical Effects, and Sample Size Calculations. Stroke. **43(10)**:2643–7.

180.Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F (2006) Subcortical vascular cognitive impairment: Similarities and differences with multiple sclerosis. J Neurol Sci. **245(1**):3–7.

181.Schutzer WE, Xue H, Reed J, Oyama T, Beard DR, Anderson S, Mader SL. Age-related β-adrenergic receptor-mediated vasorelaxation is changed by altering G protein receptor kinase 2 expression (2015) Vascul Pharmacol **55(5-6):**178–88.

182.Segarra M, Kirchmaier BC, Acker-Palmer A (2015). A vascular perspective on neuronal migration. *Mech Dev* 138 (1):17-25

183.Sepehrdad R, Chander PN, Oruene A, Rosenfeld L, Levine S, Stier CT (2003) Amiloride reduces stroke and renalinjury in stroke-prone hypertensive rats. Am J Hypertens **16(4):**312–8.

184.Seze J De, Delalande S, Michelin E, Gauvrit JY, Mackowiak MA, Ferriby D (2005) Brain MRI in late-onset multiple sclerosis **12(4):** 241–4.

185.Shin S, Walz K a, Archambault AS, Sim J, Bollman BP, Koenigsknecht-Talboo J, Cross AH, Holtz DM, Wu GF (2014) Apolipoprotein E mediation of neuro-inflammation in a murine model of multiple sclerosis. J Neuroimmunol **271(1-2):**8–17.

186.Smallwood A, Oulhaj A, Joachim C, Christie S, Sloan C, Smith AD, Esiri M (2012) Cerebral

subcortical small vessel disease and its relation to cognition in elderly subjects: a pathological study in the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort. Neuropathol Appl Neurobiol **38(4):**337–43.
187.Solaro C, Ponzio M, Moran E, Tanganelli P, Pizio R, Ribizzi G, Venturi S, Mancardi GL, Battaglia MA *(2015)* The changing face of multiple sclerosis: Prevalence and incidence in an aging population. *Mult Scler* **21**:1244–50.
188.Sowa P, Bjørnerud A, Nygaard GO, Damangir S, Spulber G, Celius EG, Due-Tønnessen P, Harbo HF, Beyer MK (2015) Reduced perfusion in white matter lesions in multiple sclerosis. Eur J Radiol **84(12):**2605–12
189.Staals J, Booth T, Morris Z, Bastin ME, Gow AJ, Corley J, Redmond P, Starr JM, deary IJ, Wardlaw JM (2015) Total MRI load of cerebral small vessel disease and cognitive ability in older people. Neurobiol Aging **36(10):**2806–11.
190.Staessen JA, Wang J, Bianchi G, Birkenhäger WH (2003) Essential hypertension. Lancet **361(9369)**:1629–41.
191.Stephenson E, Nathoo N, Mahjoub Y, Dunn JF, Yong VW (2014) Iron in multiple sclerosis: roles in neurodegeneration and repair. *Nat Rev Neurol* **10**:459–68.
192.Sternberg Z, Leung C, Sternberg D, Li F, Karmon Y, Chadha K, Levy E (2013) The prevalence of the classical and non-classical cardiovascular risk factors in multiple sclerosis patients. CNS Neurol Disord Drug Targets **12(1)**:104–11.
193.Szolnoki Z, Szekeres M, Szaniszlo I, Balda G, Bodor A, Kondacs A, Mandi Y, Somogyvari F (2015) Decreased Number of Mitochondria in Leukoaraiosis. Arch Med **46(8):**604–8.
194.Szolnoki Z, Szolnoki Z (2010) Common genetic variants of the mitochondrial trafficking system and mitochondrial uncoupling proteins affect the development of two slowly developing demyelinating disorders, leukoaraiosis and multiple sclerosis. Curr Med Chem **17(30):**3583–90.
195.Taki Y, Thyreau B, Kinomura S, Sato K, Goto R, Wu K, Kawashima R, Fukuda H (2013) A longitudinal study of age- and gender-related annual rate of volume changes in regional gray matter in healthy adults. Hum Brain Mapp] **34(9):**2292–301.
196.Tallantyre EC, Dixon JE, Donaldson I, Owens T, Morgan PS, Morris PG, Evangelou N (2011) Ultra-high-field imaging distinguishes MS lesions from asymptomatic white matter lesions. Neurology **76(6)**: 534–9
197.Tettey P, Simpson S, Taylor B V, van der Mei I AF (2014) Vascular comorbidities in the onset and progression of multiple sclerosis. J Neurol Sci **347(1-2):**23–33.
198.Teunissen CE, Killestein J, Kragt JJ, Polman CH, Dijkstra CD, Blom HJ (2008) Serum homocysteine levels in relation to clinical progression in multiple sclerosis. J Neurol Neurosurg Psychiatry **79(12):**1349–53.
199.Theocharakis P, Glotsos D, Kalatzis I, Kostopoulos S, Georgiadis P, Sifaki K, Tsakouridou K, Malarnas M, Delibasis G, Cavouras D, Nikiforidis G (2009) Pattern recognition system for the discrimination of multiple sclerosis from cerebral microangiopathy lesions based on texture analysis of magnetic resonance images. Magn Reson Imaging **27(3)**:417–22.
200.Thomas T, Miners S, Love S (2015) Post-mortem assessment of hypoperfusion of cerebral cortex in Alzheimer's disease and vascular dementia. Brain **138(4):**1059–69.
201.Tourbah A, Frenay-Lebrun C, Edan G, Clanet M, Papeix C, Vukusic S, De Sèze J, Debouverie M, Gout O, Clavelou P, Defer G, Laplaud DA, Moreau T, Labauge P, Brochet B, Sedel F, Pelletier J (2016) MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis:a randomized, double-blind, placebo-controlled study. MSJ (Epub ahead of print)
202.Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med **338(5):**278–85.
203.Tsau S, Emerson M, Lynch S, LeVine S (2015) Aspirin and multiple sclerosis. BMC Med **13(1):**1–16.
204.Tsivgoulis G, Sergentanis TN, Chan A, Voumvourakis K, Triantafyllou N, Psaltopoulou T, Gold R, Krogias C (2014) Chronic cerebrospinal venous insufficiency and multiple sclerosis: a comprehensive meta-analysis of case-control studies. Ther Adv Neurol Disord **7(2):**114–36.
205.Tubbs S, Blouir MC, Singh R, Lachman N, D'Antoni A V, Loukas M, Hattab E, Oskouian RJ (2015) Relationship Between Regional Atherosclerosis and Adjacent Spinal Cord Histology **7(9)**:e329
206.Tuladhar AM, Reid AT, Shumskaya E (2015) Relationship Between White Matter Hyperintensities, Cortical Thickness, and Cognition. Stroke. **46(2)**:425–32.
207.Tutuncu M, Tang J, Zeid NA, Kale N, Crusan DJ, Atkinson E, Pittock S, Pirko I, Keegan BM, Luchinetti CF, Noseworthy JH, Rodriguez M, Weinshenker B, Kantarci OH (2013). Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. *Mult Scler* **19**:188–98.

208.van Horssen J, Bö L, Vos CMP, Virtanen I, de Vries HE (2005) Basement membrane proteins in multiple sclerosis-associated inflammatory cuffs: potential role in influx and transport of leukocytes. J Neuropathol Exp Neurol **64(8):**722–9.

209.Verhaaren BF, Debette S, Bis JC, Smith JA, Ikram MK, Adams HH, Beecham AH, Rajan KB, Lopez LM, Barral S, van Buchem MA, van der Grond J, Smith AV, Hegenscheid K, Aggarwal NT, de Andrade M, Atkinson EJ, Beekman M, Beiser AS, Blanton SH, Boerwinkle E, Brickman AM, Bryan RN, Chauhan G, Chen CP, Chouraki V, de Craen AJ, Crivello F, Deary IJ, Deelen J, De Jager PL, Dufouil C, Elkind MS, Evans DA, Freudenberger P, Gottesman RF, Guðnason V, Habes M, Heckbert SR, Heiss G, Hilal S, Hofer E, Hofman A, Ibrahim-Verbaas CA, Knopman DS, Lewis CE, Liao J, Liewald DC, Luciano M, van der Lugt A, Martinez OO, Mayeux R, Mazoyer B, Nalls M, Nauck M, Niessen WJ, Oostra BA, Psaty BM, Rice KM, Rotter JI, von Sarnowski B, Schmidt H, Schreiner PJ, Schuur M, Sidney SS, Sigurdsson S, Slagboom PE, Stott DJ, van Swieten JC, Teumer A, Töglhofer AM, Traylor M, Trompet S, Turner ST, Tzourio C, Uh HW, Uitterlinden AG, Vernooij MW, Wang JJ, Wong TY, Wardlaw JM, Windham BG, Wittfeld K, Wolf C, Wright CB, Yang Q, Zhao W, Zijdenbos A, Jukema JW, Sacco RL, Kardia SL, Amouyel P, Mosley TH, Longstreth WT Jr, DeCarli CC, van Duijn CM, Schmidt R, Launer LJ, Grabe HJ, Seshadri SS, Ikram MA, Fornage M (2015) Multi-Ethnic Genome-Wide Association Study of Cerebral White Matter Hyperintensities on MRI. Circ Cardiovasc Genet **8(2):**398-409

210.Von Sarnowski B, Putaala J, Grittner U, Gaertner B, Schminke U, Curtze S, Huber R, Tanislav Cm Lichy C, Demarin V, Basic-Kes V, Ringelstein EB, Neumann-Haefelin T, Enzinger C, Fazekas F, Rothwell PM, Dichigans M, Jungehulsing GJ, Heuschmann PU, Kaps M, Norrving B, Rolfs A, Kessler C, Tatlisumak T (2013) Lifestyle risk factors for ischemic stroke and transient ischemic attack in young adults in the stroke in young fabry patients study. Stroke. **44(1)**:119–25.

211.Vukusic S, Confavreux C. Natural history of multiple sclerosis: risk factors and prognostic indicators (2007) *Curr Opin Neurol* **20**:269–74.

212.Wakefield AJ, More LJ, Difford J, McLaughlin JE (1994) Immunohistochemical study of vascular injury in acute multiple sclerosis. J Clin Pathol **47(2):**129–33.

213.Wang T, Li Y, Guo X, Huang D, Ma L, Wang DJ, Lou X (2016) Reduced perfusion in normal-appearing white matter in mild to moderate hypertension as revealed by 3D pseudocontinuous arterial spin labeling. J Magn Reson Imaging **43(3):**635-43

214.Wang Y, Bos SD, Harbo HF, Thompson WK, Schork AJ, Bettella F, Witoelar A, Lie W, McEvoy LK, Djorovic S, Desikan RS, Dale AM, Andreassen OA (2016) Genetic overlap between multiple sclerosis and several cardiovascular disease risk factors. Mult Scler J (E Pub ahead of print)

215.Ward RJ, Zucca F a, Duyn JH, Crichton RR, Zecca L (2014) The role of iron in brain ageing and neurodegenerative disorders. *Lancet Neurol* **13**:1045–60.

216.Wardlaw JM (2010) Journal of the Neurological Sciences Blood-brain barrier and cerebral small vessel disease.J Neurol Sci **299(1-2):**66–71.

217.Wardlaw JM, Smith C, Dichgans M (2013) Mechanisms of sporadic cerebral small vessel disease : insights from neuroimaging. *Lancet Neurol* **12**:483–97.

218.Weber C, Noels H (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 17(11):1410–22.

219.Weinstock-Guttman B, Zivadinov R, Mahfooz N, Carl E, Drake A, Schneider J, Teter B, Hussein S, Metha B, Weiskopf M, Durfee J, Bergsland N, Ramanathan M (2011) Serum lipid profiles are associated with disability and MRI outcomes in multiple sclerosis. J Neuroinflammation **8(1):**127.

220.Wiesner D, Merdian I, Lewerenz J, Ludolph AC, Dupuis L, Witting A (2013) Fumaric Acid Esters Stimulate Astrocytic VEGF Expression through HIF-1α and Nrf2. **8(10):**1–10.

221.Wiseman SJ, Doubal FN, Chappell FM, Valdés-Hernández MC, Wang X, Rumley A, Lowe GD, Dennis MS, Wardlaw JM (2015) Plasma Biomarkers of Inflammation, Endothelial Function and Hemostasis in Cerebral Small Vessel Disease. Cerebrovasc Dis **40(3-4):**157–64.

222.Wong CHY, Heit B, Kubes P (2010) Molecular regulators of leucocyte chemotaxis during inflammation. Cardiovasc Res **86(2)**:183–91.

223.Wu H, Roks AJM (2014) Genomic instability and vascular aging: a focus on nucleotide excision repair. Trends Cardiovasc Med **24(2)**:61–8.

224.Zamboni P, Galeotti R, Menegatti E, Malagoni a M, Tacconi G, Dall'Ara S, Bartolomei I, Salvi F (2009) Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry **80(4):**392–9.

225.Zha AM, Di Napoli M, Behrouz R (2015) Prevention of Stroke in Rheumatoid Arthritis. Curr Neurol Neurosci Rep **15(12):**77.

226.Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV (2015) Establishment and Dysfunction of the Blood-Brain Barrier. *Cell* **163**:1064–78

227.Zivadinov R, Ramasamy DP, Benedict RR, Polak P, Hagemeier J, Magnano C, Dwyer M, Bergsland N, Bertolino N, Weinstock-Guttman B, Kolb C, Hojnacki D, Utrainen D, Haacke EM, Schweser F (2016) Cerebral microbleeds in multiple sclerosis evaluated on susceptibility-weighted images and quantitative susceptibility maps : A case-control study. Radiology (Epub ahed of print)
228.Zivadinov R, Weinstock-Guttman B, Hashmi K, Abdelrahman N, Stosic M, Dwyer M, Hussein S, Durfee J, Ramanathan M (2009) Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis. Neurology **73(7):**504–10.
229.Zlokovic B V (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* **12**:723–39.

Figure legends

Figure 1. **Aging with Multiple Sclerosis (MS): review outline.**

BBB – blood brain barrier, RR – relapsing – remitting MS, P progressive MS, ECM extracellular matrix changes

Figure 2.

A – Cerebral circulation is divided in macrocirculation (**a**) that includes the internal carotid and vertebral arterial systems and in microcirculation. The microcirculation is composed of pial and perforating arterioles, capillaries and venules. **Pial arterioles** give rise to **superficial perforating arterioles** that course centripetally entering the brain at right angles to its surface, branching and terminating as end arteries. **Perforating arterioles** also arise from the main arteries branches and irrigate the basal ganglia, deep WM and brainstem. Perforating arterioles lie within the Perivascular Spaces (PVS), where vessel wall components are in close contact with the astrocyte endfeet, and terminate as end arteries (i.e. without shunts) in capillary beds. The capillary plexus drains into venules (not shown).

B - Potential interaction between Small Vessel Disease (SVD) and Multiple Sclerosis (MS).

Longstanding MS Brain MRI (FLAIR coronal section), showing focal and diffuse periventricular white matter hyperintensities adjacent to the lateral ventricles. A perforating artery affected by SVD is illustrated where enlargement of the perivascular space, thickening of the vessel wall with significant reduction of the vessel lumen is present. These vessel changes are associated with subsequent vessel occlusion leading to lacunes, vessel rupture causing microbleeds, hypoperfusion, blood brain barrier damage and subsequent myelin break down. This could cause additional white matter damage and neuronal loss in MS.

Supplemental Figure 1. **SVD and MS interaction review**

Search strategy: arterioscleros*, basal, basal ganglia hemorrhage, bleed*, brain*, cereb*, cerebral small vessel diseases, crani*disease*,disseminated, ganglia, haemorrhag*, hemorrhag*, intra-crani*, intracrani*, intracranial, intracranial arteriosclerosis, intracranial hemorrhage, hypertensive, lacun*, leukoaraios*, leukoaraiosis, micro-bleed*, microangiopath*, microbleed, ms, multiple, multiple sclerosis, multiple sclerosis, chronic progressive, multiple sclerosis, relapsing-remitting, scleros*, small, stroke*, stroke, lacunar, vessel

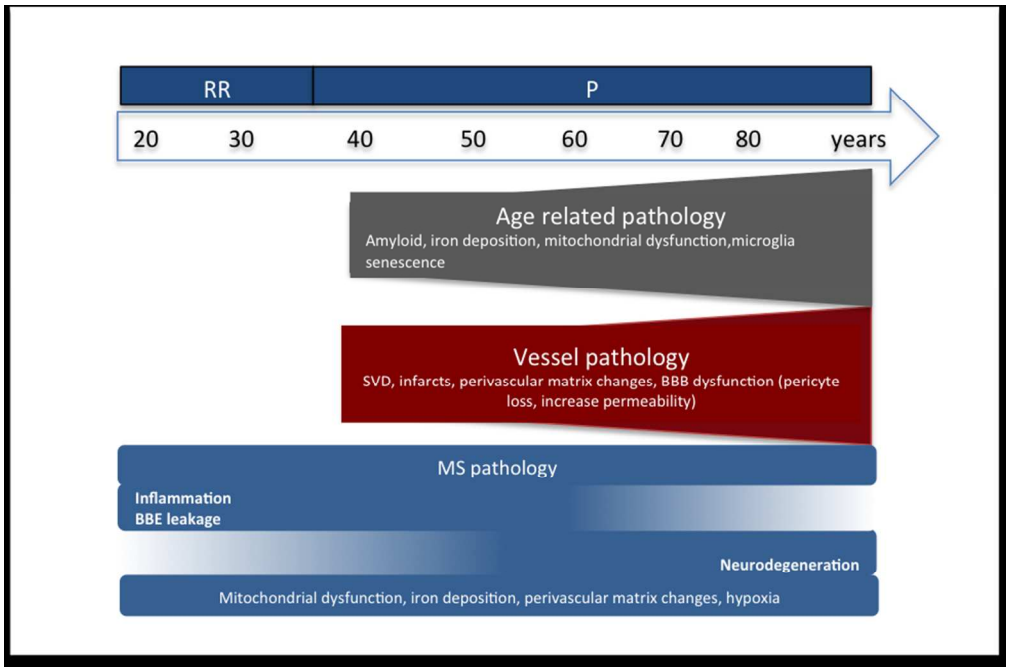


Figure 1. Aging with Multiple Sclerosis (MS): review outline.
BBB – blood brain barrier, RR – relapsing – remitting MS, P progressive MS, ECM extracellular matrix changes

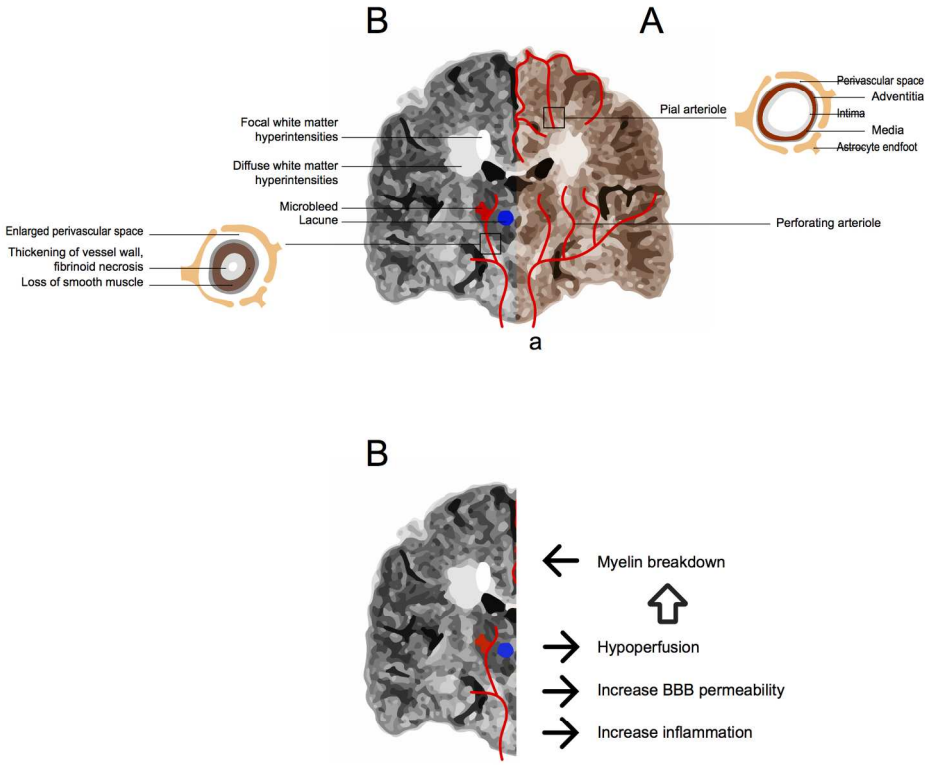


Figure 2.
A – Cerebral circulation is divided in macrocirculation that includes the internal carotid and vertebral arterial systems (a). The microcirculation is composed of pial and perforating arterioles, capillaries and venules Pial arterioles give rise to superficial perforating arterioles that course centripetally entering the brain at right angles to its surface, branching and terminating as end arteries. Perforating arterioles also arise from the main arteries branches and irrigate the basal ganglia, deep WM and brainstem. Perforating arterioles lie within the Perivascular Spaces (PVS), where vessel wall components are in close contact with the astrocyte endfeet, and terminate as end arteries (i.e. without shunts) in capillary beds. A density gradient of brain capillaries, from high to low, is observed between grey and white matter, respectively. The capillary plexus drains into venules (not shown).

B - Potential interaction between Small Vessel Disease (SVD) and Multiple Sclerosis (MS).
Longstanding MS Brain MRI (FLAIR coronal section), showing focal and diffuse periventricular white matter hyperintensities adjacent to the lateral ventricles. A perforating artery affected by SVD is illustrated where enlargement of the perivascular space, thickening of the vessel wall with significant reduction of the vessel lumen is present. These vessel changes are associated with subsequent vessel occlusion leading to lacunes, vessel rupture causing microbleeds, hypoperfusion, blood brain barrier damage and subsequent myelin break down. This could cause additional white matter damage and neuronal loss in MS.

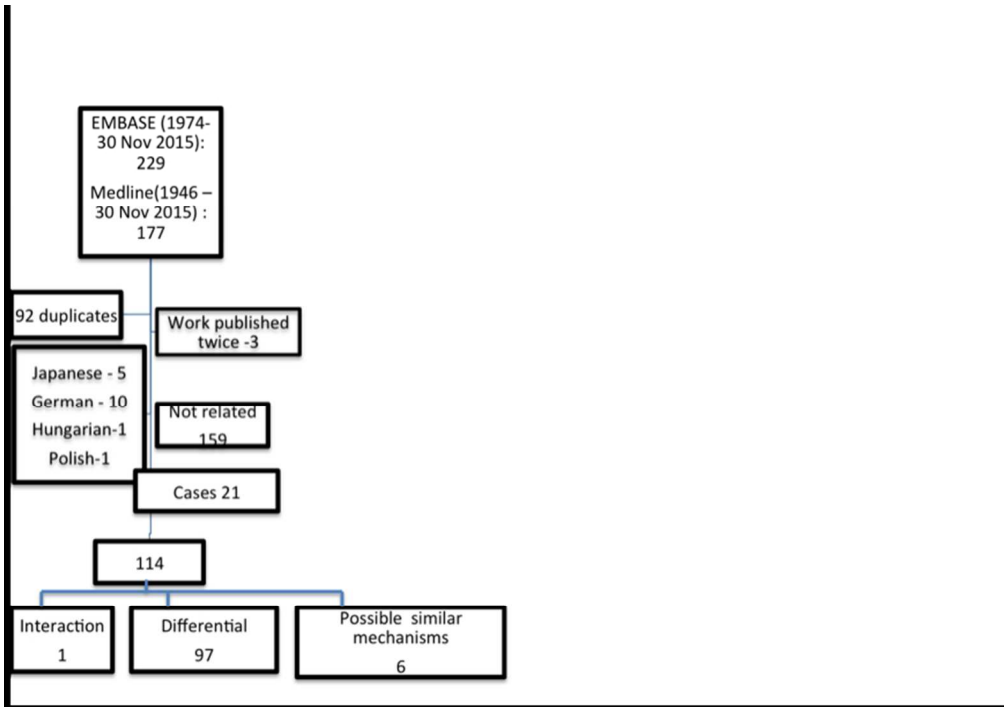
Figure 2

Microcirculation	Structure	Function	Aging	SVD	MS
Pial arteries **Perforating arteries**	Surrounded by CSF and pia-arachnoid and glia limitans. Three vessel wall layers: 1. *tunica adventitia* (mostly collagen and fibroblasts) 2. a *tunica media* (smooth muscle) 3. a *tunica intima* (single layer of endothelial cells and internal elastic lamina)	1.Nutrition 2.Cerebral blood flow regulation 3.Vasophylic neuroblast migration	1.Tortuousity 2.Thickened walls 3.Loss of elastin and smooth muscle 4. Reduced coverage by pericytes and perivascular nerve plexus	1.Severe thickening vessel wall 2.Arteriosclerosis/ fibrinoid necrosis 3. Enlarged PVS 4. Vascular ectasia 5.Amyloid wall infiltration 6.PVS Hemosiderin deposition	1.Enlarged PVS Scarce information
Capillary	Single layer of endothelial cells	1.Nutrition 2.Cerebral blood flow regulation 3. Immune response	1.Increased diameter 2. Basal membrane thickening, reduplication and vacuolization 3. Increased vessel wall hyalinosis and fibrosis 4. Reduced coverage by pericytes	1.Perivascular rarefaction 2. Increased endothelial permeability	1. Increase endothelial permeability, with protein leakage, red blood cell extravasation 2. Perivascular inflammation
Venules	Thin-walled vessels: 1. *tunica adventitia* (mostly collagen and fibroblasts) 2. a *tunica media* (thin layer of smooth muscle) 3. a *tunica intima* (single layer of endothelial cells) No valves.	1. Metabolite clearance 2.Cerebral blood flow regulation	1.PVS collagenosis	1. Vessel wall and PVS collagenosis	1.Thickening of vessel wall, vessel tortuosity 2.Intramural fibrinoid deposition, wall reduplication 3. Iron deposition 4. Enlarged PVS 5. PVS collagenosis 6. Perivascular inflamation 7. Thrombosis
Neurovascular Unit	1.Endothelial cells 2.Pericytes 3.Vascular smooth muscle cells 4.Astrocytes 5. Basal membrane 6.Neurons 7. Perivascular macrophages	1.Immune response regulation (adaptive and innate immune responses regulation of leukocyte transfer) 2. Transport of substances 3.Blood flow 4.Angiogenesis regulation 5. Coagulation and fibrinolysis	1.Endothelial cell increased permeability 2. Reduced pericytes	1. Endothelial cell increased permeability	1.Changes in endothelial cell tight and adherens junction expression (occludine, claudins, caderins, zonula-ocludens), with increase permeability 2.BM/extracellular matrix abnormalities

Table 1. Microcirculation: normal structure and changes with aging, Small Vessel Disease (SVD) and in Multiple Sclerosis (MS)

	SVD		
ntional Brain MRI	**Histopathology**	**Conventional Brain MRI**	**Histopathology**
esions	Myelin loss (all constituents)	**Focal lesions**	Myelin loss
y defined	Different degrees of axonal loss	Watershed regions	Axonal loss
S-shaped juxtacortical	**At high venule density and arterial watershed areas**	!central pons	
ndicular to the lateral ventricles (Dawson	**Active** - rich in macrophages and lymphocytes	! sparing of the spinal cord	
)		! less frequent in the corpus callosum	
s callosum	**Inactive** - minimal macrophage infiltration	! sparing U fibres	
nerve			
cord			
periphery	Myelin loss with selective reduction of phospholipids	**Diffuse WM lesions:**	
e **WM lesions** periventricular		Symmetrical*	
		Mild periventricular WM	Selective loss of phospholipids and MAG preservation Loosening of the fibre network around "tortuo Minor arteriosclerotic vessel changes
		Irregular periventricular WM	Severe myelin loss and reactive gliosis Incomp Fibrohyalinotic and arteriosclerotic vessels
		Punctate deep WM	Mild tissue changes surrounding dilated PVS Myelin loss and atrophic neuropil around fib arterioles
		Deep partial confluent/confluent WM	Axonal loss and astrogliosis. Myelin, oligodendrocyte loss, focal transitions t infarcts.
ent hypointensities	Increase extracellular space due to oedema	Areas >2 mm and <15 mm in the perforating arteries territory more in BG, pons (central), internal capsule and corona radiate	Irregular cavitations with scattered fat-laden m reactive gliosis and myelin loss
read WM			
ent hypointensities	Tissue loss		
ntricular >juxtacortical			
hyposignal	Perivascular iron deposition	Small rounded hypointensities visualized on brain MRI, 2-10mm not well visualized on T2.	
	R* signal changes do not always correspond to iron deposition	Artheriosclerosis SVD - Deep WM, BG and brainstem in arteriosclerosis-related SVD	Microscopic bleeds, small lacunes or to containing macrophages in PVS
	Iron in activated macrophages/microglia at the edges of WM lesions		Microscopic and macroscopic bleeds; □-amy wall deposition
	Iron precipitation in aggregates typical of microbleeds	Cerebral Amyloid SVD – cortex, convexity/sulcus hypointensities	
convexity perforating medullary arteries	Perivascular collagenosis and inflammatory cuffs within these enlarged spaces	Arteriosclerosis SVD - lenticulostriate arteries entering the BG through the anterior perforated substance	Enlarged PVS Perivascular collagenosis
		Cerebral Amyloid SVD – pial and superficial perforating arteries	Enlarged PVS
read in WM	Macrophage/microglia,lymphocytic infiltrates	Only in the context of acute stroke	Acute ischemic changes – red hypoxi
nt patterns: nodular, punctate, ring or ng	BBB disruption		inflammatory infiltrate

mary of core brain MRI and Pathology features in MS and SVD ! Differentiating features between Multiple Sclerosis and Small Vessel Disease, WMH- White Matter Hyperintensities, FI version recovery, PVS – perivascular spaces, SWI – susceptibility weighted imaging, CEL – contrast enhancing lesions; MAG –myelin associated glycoprotein, PLP –proteolipid protein



Author. year	Study information	Number of clinical definite MS patients/controls	Vascular Risk factor	MRI outcomes	Main MRI findings
Healy BC et al. 2009	Single centre, cross sectional survey and longitudinal follow up (3.2 years)	1045 (first MRI)/0	Tobacco Smoking	Whole brain volume T2 LV BPF	Current smokers had a lower BPF compared with never-smokers. T2 LV was higher in current smokers than in never smokers. No differences between Ex-smokers and never smokers.
Zivadinov R. (2009)	Single centre cross-sectional	368/0	Tobacco Smoking	T1 LV T2 LV CEL number and volume -BPF	Ever Smokers with higher number of CEL, T1 and T2 LV when compared with non smokers Lower BPF in smokers
Weinstock-Guttman B (2011)	Single centre, retrospective, cohort	210/0	Fasting lipid profile (HDL, LDL, triglycerides, total cholesterol, cholesterol/HDL ratio) BMI	CEL T2 LV T1 LV BPF	Higher HDL associated with a low CEL LV Higher triglyceride levels associated to higher CEL LV No associations with T2-LV and T1-LV with any of the lipid profile variables
Farez M et al.	Single centre cohort (prospective)	First group – 70 Replication - 52	Sodium intake (low, medium, high)	T2 LV	Patients with higher sodium intake had a greater chance of developing a new T2 lesions and had increased T2 LV
Kappus et al. (2015)	Single centre cohort (prospective)	326 RR 163 Progressive /175 HC	HT, heart disease, smoking, overweigh/obesity, type 1 DM	T1 and T2 LV - Normalised brain parenchyma volume, GM, WM and lateral ventricle volume	In MS patients HT and heart disease associated with decrease GM and cortical volumes Overweight/obesity associated with increase T1-LV and smoking with decrease whole brain volume.

Supplemental Table 1. Brain MRI studies on the impact of vascular risk factors on MS brain lesions

BPF - Brain parenchymal fraction; BMI – body mass index; LV – lesion volume; CEL – contrast enhancing lesions, GM – grey matter, WM – white matter, HT – hypertension, DM- diabetes mellitus