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Grey Matter Perfusion in Clinically Isolated Syndrome and Relapsing-Remitting Multiple Sclerosis

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List of Abbreviations

AIF	arterial input function
ASL	arterial spin labelling
CBF	cerebral blood flow
CBV	cerebral blood volume
CCSVI	chronic cerebrospinal venous insufficiency
CIS	clinically isolated syndrome
CNS	central nervous system
DisDur	disease duration
DSC	dynamic susceptibility contrast
EDSS	Expanded Disability Status Scale
ET-1	endothelin-1
gCIS	subgroup of subjects diagnosed with clinically isolated syndrome
Gd-enhancing lesions	Gadolinium-enhancing lesions on T1-weighted magnetic resonance images
GM	cerebral grey matter
GMCort	region of interest comprising the cortical grey matter
gMS	subgroup of subjects diagnosed with relapsing-remitting multiple sclerosis
IQR	interquartile range
MPRAGE	magnetization-prepared rapid acquisition and multiple gradient echo technique
MRI	magnetic resonance imaging
MS	multiple sclerosis

MWU-Test	two-sided Mann-Whitney-U-Test
NAGM	normal appearing cerebral grey matter
NAWM	normal appearing cerebral white matter
NBV	normalized brain volume
NGMV	normalized cerebral grey matter volume
NWMV	normalized cerebral white matter volume
PPMS	primary progressive multiple sclerosis
PUT	region of interest comprising the putamen
rCBF	relative cerebral blood flow
rCBV	relative cerebral blood volume
ROI	region of interest
RRMS	relapsing-remitting multiple sclerosis
SPMS	secondary progressive multiple sclerosis
T2w lesions	hyperintense lesions on T2-weighted magnetic resonance images
T2wLES	region of interest comprising the hyperintense lesions on T2-weighted magnetic resonance images
THAL	region of interest comprising the thalamus
WM	cerebral white matter
WMROI	region of interest comprising cerebral white matter

1. Introduction

Multiple sclerosis (MS) is the most common acquired inflammatory demyelinating disorder of the central nervous system (Keegan and Noseworthy 2002) and it is the leading cause of non-traumatic neurological disability in young adults (Compston and Coles 2002; Dutta and Trapp 2011). The clinical presentation of MS is diverse and ranges from focal neurological deficits to cognitive impairment. Radiologically, MS also presents a wide range of affections such as focal lesions in the sense of local inflammation and changes in diffusion and perfusion characteristics. The estimated prevalence of MS is about 2.5 million people worldwide (Noseworthy et al. 2000) and there is a notable gender imbalance, as two thirds to three quarters of the patients are women (Noseworthy et al. 2000; Whitacre 2001). MS affects patients of all ages, but most patients, who present with a first manifestation of MS are aged between 20 and 50 years. MS was first described by Jean-Martin Charcot (1825 – 1893), who is often considered the founder of modern neurology. Charcot was the first to recognise MS as a distinct affliction and described it as the triad consisting of nystagmus, kinetic tremor and scanning speech. He also undertook the first neuropathological investigation of MS and linked his neuropathological findings to clinical symptoms. Even though there has been extensive research into the neurological, neuropathological and neuroradiological characteristics and mechanisms of MS since then, many aspects of MS remain elusive.

Familial genetic studies have shown that there is a genetic influence, as family members of MS patients have an up to 250-fold increased risk of developing MS compared to the average member of the public (Ebers et al. 2000). The individual genetic factor associated with the highest risk of developing MS is the HLA DRB1*15 variant of the human leukocyte antigen system, which codes for the MHC *II*-receptor (Epplen et al. 1997; Compston 1999; Lin et al. 2012). This genetic variant increases the risk of developing MS by a factor of three and has been found in the more than 70% of

MS patients (Eppelen et al. 1997; Compston 1999; Lin et al. 2012). But even though there have been attempts to uncover the genetic risk factors for MS using genome-wide association studies, each respective identified risk factor on its own only accounted for a fractionally increased risk of developing the disease (Sadovnick et al. 1988; Robertson et al. 1996). Therefore the heritability of MS is thought to be polygenic and potentially even epigenetic.

The prevalence of MS is comparatively low in equatorial regions and increases towards both poles, constituting a distinct geographical pattern (Pugliatti et al. 2006). The reasons for this are unknown, but low vitamin D levels have been found to be a risk factor for acquiring MS, both in epidemiological studies (Munger et al. 2006) and in animal models of MS (Lemire and Archer 1991; Cantorna et al. 1996; Spach and Hayes 2005).

The Epstein-Barr virus has received special attention with respect to the risk of acquiring MS. Avoiding Epstein-Barr virus infection significantly reduces the individual risk of developing MS in adults (Sumaya et al. 1985; Munch et al. 1997; Myhr et al. 1998; Ascherio and Munch 2000; Wagner et al. 2000; Ascherio et al. 2001; Haahr et al. 2004; Sundström et al. 2004; Ponsonby et al. 2005) as well as in children (Alotaibi et al. 2004; Pohl et al. 2006). Additionally, there is also a significant difference with respect to the risk of acquiring MS between seropositive individuals with and without a history of infectious mononucleosis (Thacker et al. 2006), which in the opinion of Ascherio and Munger (2007a) suggests that older age at infection further increases the odds of developing MS. The significance of infection with the Epstein-Barr virus has been controversially discussed because of the general prevalence of Epstein-Barr antibodies of 95% in adults, which makes it difficult to analyse the suspected effect to any sufficient degree of certainty. But as Ascherio and Munger (2007a) pointed out, there are other viruses such as the polio virus, which show similar behaviour: the polio virus used to be endemic in some countries, infecting virtually all children, but only causing poliomyelitis in a limited number of cases. So the high seroprevalence of the Epstein-Barr virus is not in itself a valid argument for discounting it as a potentially important player in the acquisition of MS (Ascherio and Munger 2007a).

Several other influencing factors have been identified or speculated upon. Smoking has been identified both, as a general risk factor and also as interacting negatively with the risk posed by an Epstein-Barr virus infection (Simon et al. 2010). Other virus infections

than Epstein-Barr virus have been discussed as potentially increasing or decreasing the risk of developing MS, depending on the virus (reviewed in Bach 2002). However, it should be noted that most studies used animal models of autoimmune disease. Since there is a notable gender imbalance and also a typical age range, gender and age seem to be influential factors. Mandoj et al. (2015) found a disrupted lipid homeostasis was associated with high disease activity, while Fellows et al. (2015) found high serum levels of HDL-cholesterol to be protective with respect to the integrity of the blood brain barrier in MS patients. Potentially influential factors such as hormones, especially estrogens, diet, both with respect to fatty acids and with respect to antioxidants have been discussed (see Ascherio and Munger (2007b) for a comprehensive review). General hygiene has received some attention with respect to developing autoimmune diseases in general, not only MS. But the hypothesis of Leibowitz et al. (1966), which postulates that exposure to several infectious agents early in life is protective against MS is still a topic of scientific discussion.

1.1 Responsible Mechanisms

Despite its common occurrence and years of scientific effort, the mechanisms of MS are still comparatively poorly understood. However, it is generally accepted that there are two major components in MS, namely neuroinflammation and neurodegeneration. The aspect that has long been in the focus of attention is the (focal) inflammatory component. The reasons for this central role mostly stem from history. For one thing, focal brain lesions were the first affection of MS to be detected early on in brain dissection of MS patients. White matter brain lesions were also the first MS affection, which could be detected and quantified in vivo, when magnetic resonance imaging (MRI) first became available. Additionally, there is a significant amount of pharmaceutical knowledge about targeting and regulation parts of the immune system, which has been around for a while. So researching the inflammatory component held much more promise in terms of potential drug development.

The inflammatory component of MS is thought to be mediated by autoreactive T- and B-cells, which migrate to the central nervous system causing focal demyelination, oligodendrocyte loss and also neuronal damage (Dutta and Trapp 2007; Trapp and Nave 2008; Dutta and Trapp 2011; Kipp et al. 2012). Infiltrating macrophages were found

in single large-mass (tumefactive) lesions alongside myelin loss and preserved axons in clinically isolated syndrome (CIS) patients (Miller et al. 2005b). However, it should be kept in mind that these histopathological findings are based on atypical presentations of CIS, which makes them inherently biased. Furthermore, primary mechanisms such as oligodendrocyte dysfunction have also been found to be a potential disease-triggering factor (Barnett and Prineas 2004). These changes are not limited to focal lesions, however (Bö et al. 2006). Similar changes have been found in extensive areas of seemingly normal white matter (Kutzelnigg et al. 2005; Androdias et al. 2010). Recent histopathological and MRI findings have shown that MS pathology also involves grey matter lesions and diffuse grey matter damage (Bö et al. 2006; Filippi et al. 2012; Kipp et al. 2012), where focal grey matter lesions also show demyelination and oligodendrocyte loss (Bö et al. 2006; Kipp et al. 2012). Lucchinetti et al. (2011) even found inflammatory meningeal pathology additional to widespread cortical pathology outside of lesions.

The second major component is neurodegeneration. Its pathogenesis is less well understood than that of neuroinflammation. Axonal pathology in MS lesions and its mechanisms have received much attention (Ferguson et al. 1997; Trapp et al. 1998; Kornek et al. 2000; Bjartmar et al. 2000; Kuhlmann et al. 2002). Cortical lesions are a common occurrence in MS patients and have been found in 26% of MS patients by means of in vivo MRI, as well as post mortem MRI and neuropathological analysis (Kidd et al. 1999). While these focal grey matter lesions display inflammatory features, they also display substantial amounts of axonal and neuronal damage (Bö et al. 2006; Kipp et al. 2012). Peterson et al. (2001) also found death of neuronal cell bodies early on in the disease in a post mortem tissue analysis of cortical lesions in MS patients. Sailer et al. (2003) and Inglese et al. (2004) also found widespread grey matter involvement in normal appearing cerebral grey matter (NAGM) and focal cortical thinning in an in vivo MRI study, suggesting axonal loss.

However, neuroinflammation and neurodegeneration are not two separate components, but are intrinsically linked to each other. There is evidence that the destruction in focal inflammatory MS lesions leads to Wallerian degeneration, which in turn contributes significantly to early axonal pathology in MS patients (Casanova et al. 2003; Dzedzic et al. 2010). Wallerian degeneration has also been suggested as the process responsible for diffuse tissue damage in the so called normal appearing cerebral white matter

(NAWM) outside focal lesions (Seewann et al. 2009). Trapp et al. (1998) also found that early axonal pathology correlates with immune cell infiltration. Conversely, Wallerian degeneration has also been implicated in the recruitment of inflammatory cells into the central nervous system (CNS) (Tsunoda et al. 2007).

1.2 Diagnostic Criteria and Clinical Appearance

There is no such thing as “the” MS. Rather, MS is a collection of several different subtypes, which are characterised by markedly different forms of progression. There is a wide range from very benign courses to rapidly-progressing disability. Moreover, the subtype is not definite and can change in the course of the disease. Lublin and Reingold (1996) defined the following three subtypes and revised them in 2013 (Lublin et al. 2014):

- CIS: clinical presentation of a disease which shows characteristics of inflammatory demyelination and which could be MS, but does not yet fulfil the diagnostic criteria of MS with respect to dissemination in time.
- Relapsing-remitting multiple sclerosis (RRMS): clearly defined disease relapses with full recovery or a residual neurological and/or functional deficit. The periods between relapses are characterised by a lack of disease progression.
- Primary progressive multiple sclerosis (PPMS): disease progression from onset with occasional plateaus. Temporary minor improvements are allowed, but distinct relapses are not.
- Secondary progressive multiple sclerosis (SPMS): initially shows all characteristics of RRMS, but turns into progression which might be overlaid with additional relapses. Minor remissions and plateaus are possible, but if there are still relapses, periods between those relapses are characterised by disease progression (as opposed to RRMS).

McDonald et al. (2001) first developed objective criteria for definitely diagnosing MS. These criteria have since been revised by the International Panel on Diagnosis of MS (Polman et al. 2011). It should be noted that these criteria are explicitly developed

and validated for patients who present with a typical CIS suggestive of RRMS or at least with symptoms consistent with inflammatory demyelinating processes in the CNS and should not be applied to different collectives (Polman et al. 2011). The diagnosis of MS is the result of a synthesis of clinical and especially MRI-derived paraclinical information (Polman et al. 2011). Beyond symptoms of inflammatory demyelination of the CNS in the sense of hyperintense lesions on T2-weighted magnetic resonance images (T2w lesions) and Gadolinium-enhancing lesions on T1-weighted magnetic resonance images (Gd-enhancing lesions), it essentially requires the exclusion of other disorders that can mimic MS and objective evidence for a disseminated disease course both in time and in space. The dissemination can either be substantiated clinically or by means of MRI according to specific criteria (Swanton et al. 2006, 2007; Polman et al. 2011). Additionally, MRI has developed into the primary tool to distinguish CIS from early MS (McDonald et al. 2001; Rot and Mesec 2006; Swanton et al. 2007; Miller et al. 2008; Polman et al. 2011). For the sake of completeness, it should be noted that the European collaborative research network that studies magnetic resonance imaging in multiple sclerosis (MAGNIMS) has recently suggested several modifications (Filippi et al. 2016) to the 2010 revisions of the McDonald criteria (Polman et al. 2011) with regard to MRI.

The clinical appearance of MS is heterogeneous, depending on the anatomical areas targeted by intense disease activity. Patients presenting with CIS typically show symptoms linked to the optic nerve, the brain stem/cerebellum, the spinal cord, or the cerebral hemispheres (Polman et al. 2011). About 85% of patients who later develop RRMS first present with a clinically isolated syndrome (Miller et al. 2005b), but there is no pathognomonic characteristic to predict which of the CIS patients will develop definite RRMS. Generally, more than 80% of the MS patients start out with CIS or RRMS, but convert to SPMS approximately 20 years after onset (Confavreux and Vukusic 2006). In RRMS there is a good correlation between new Gd-enhancing lesions and the occurrence of relapses, which brakes down when the disease converts to a progressive form (Pittock et al. 2004). The inflammatory processes corresponding to such a Gadolinium uptake generally cause focal afflictions such as optic neuritis, locally limited dysesthesia or reduced strength in limited areas. In contrast to this, diffuse axonal damage often leads to neurological and cognitive impairments (Keegan and Noseworthy 2002; Schulz et al. 2006). Cognitive impairment is generally a common occurrence in MS and affects

43 – 70% of the patients (Rao et al. 1991; Amato et al. 2001; Chiaravalloti and DeLuca 2008). It can be detected already comparatively early in the disease course (Amato et al. 1995) and has a considerable impact on both, the private and the working life of MS patients (Rao et al. 1991; Chiaravalloti and DeLuca 2008). Additionally, MS patients show as much as a tenfold increase in frequency of epileptic seizures compared to healthy subjects (Eriksson et al. 2002).

Therapeutic options in MS are limited and directly depend on the clinical subtype as introduced above. There is a reasonable range of immunomodulatory drugs available, which target the neuroinflammatory component of RRMS. Several of these disease-modifying agents verifiably reduce the number of relapses, MRI-derived disease activity and to a lesser degree even the progression of clinical disability in RRMS (e.g. Polman et al. 2006; Mikol et al. 2008; Kappos et al. 2010; Cohen et al. 2012; Gold et al. 2012; Calabresi et al. 2014). However, therapeutic options are rare with respect to the progressive forms of MS presumably dominated by neurodegeneration. These immunomodulatory agents which are effective in RRMS only have a very limited effect in progressive forms of MS (European Study Group on Interferon β -1b in Secondary Progressive MS 1998; Leary and Thompson 2003). Mitoxantrone is used in SPMS, but its use is limited by severe cumulative side effects. There is currently no therapeutic agent approved for use in PPMS (Ransohoff et al. 2015). Treatment decisions in PPMS are made on an individual basis (Ontaneda et al. 2015). In light of this, it is even more important that several clinical trials have shown that administering disease-modifying treatment to CIS patients reduces their likelihood of developing RRMS and also reduces MRI-derived disease activity (Jacobs et al. 2000; Comi et al. 2001; Kappos et al. 2006; Comi et al. 2009). Similarly, transition to SPMS can be delayed by effective immunomodulatory medication (Tedeholm et al. 2013). Therefore timely treatment is also of prognostic importance on several counts.

1.3 Brain Atrophy as a Marker of Neurodegeneration

Conventional MRI with contrast enhancement is a routine means of diagnosing and monitoring the course of MS. But even if the current diagnostic criteria (Polman et al.

2011; Filippi et al. 2016) only consider T2w lesions and Gd-enhancing lesions, there is far more information which MRI can provide in the context of MS. This is of particular importance as the correlation between conventional radiological parameters and clinical disability is comparatively poor (Barkhof 2002).

One of the parameters on which MRI can provide information is brain atrophy. Reliable methods to estimate brain atrophy have been around for some time (Bermel and Bakshi 2006; Zipp 2009) and are constantly being refined (Smeets et al. 2016). There is a significantly increased annual brain volume loss of 0.5 – 1.0% in MS patients compared to an annual brain volume loss of 0.1 – 0.3% in healthy individuals (Simon 2006; Fotenos et al. 2008; Fisher et al. 2008; Barkhof et al. 2009; De Stefano et al. 2010; De Stefano et al. 2014; Vollmer et al. 2015). This brain volume loss occurs in all MS patients regardless of the respective subtype (Tedeschi et al. 2005; De Stefano et al. 2010). Both, demyelination due to inflammatory activity and neurodegeneration contribute to this loss (Simon 2006; Barkhof et al. 2009; Barten et al. 2010). Contrary to long-held beliefs, neurodegeneration begins early on in the disease (Silber and Sharief 1999; Dutta and Trapp 2007) and is already visible on MRI of CIS patients suggestive of MS (Chard et al. 2002; Henry et al. 2008; Chard and Miller 2009; Raz et al. 2010). There is a correlation between brain volume loss and disability/disease progression (Bermel and Bakshi 2006; Simon 2006; Amato et al. 2007; Minneboo et al. 2008; Fisniku et al. 2009; Lukas et al. 2010; Filippi and Rocca 2011; Zivadinov et al. 2013b; De Stefano et al. 2014; Jacobsen et al. 2014) and Sormani et al. (2014) showed that additionally measuring brain atrophy provides new information in comparison with just quantifying T2w lesion volume. Today, measurement of brain volume change is widely accepted as a method for quantifying neurodegeneration and is recommended for use in clinical trials to assess neurodegeneration, neuroprotection and the efficacy of tested therapies (e.g. Zivadinov and Bakshi 2004; Barkhof et al. 2009; De Stefano et al. 2014). Some of the available software tools such as SIENAX (Smith et al. 2002; Smith et al. 2004) and FreeSurfer (Fischl et al. 2002) also provide estimates of regional volumes. This addresses the fact that several studies suggest that it is mainly grey matter atrophy, which predicts disease progression, disability and cognitive impairment (De Stefano et al. 2003; Amato et al. 2004; Chard et al. 2004; Sanfilippo et al. 2005; Sanfilippo et al. 2006; Fisher et al. 2008; Zivadinov et al. 2013b; Popescu et al. 2013; Zivadinov et al. 2013a; Fisniku et al. 2008). As De Stefano et al. (2014) pointed out, this may have important implications

with respect to the recommendation to use brain atrophy as a primary end-point for measuring neuroprotection in clinical trials. Furthermore, neurodegeneration in the form of MRI-derived brain atrophy also has some prognostic value with respect to the conversion from CIS to MS (Bjartmar et al. 2000). But on a note of caution, it should be mentioned that there are several confounders such as image quality, age, life habits, genetic load and comorbidities (Enzinger et al. 2005; Zivadinov et al. 2009; De Stefano et al. 2014), which can obscure the underlying processes.

1.4 Perfusion

Changes in perfusion in MS patients have received increasing scientific attention in the last couple of years. Early on, Wuerfel et al. (2004) showed that the inflammatory processes involved in forming a new MS plaque are accompanied by altered local perfusion. This change in local perfusion can be detected by means of perfusion MRI prior to permeability of the blood brain barrier (Wuerfel et al. 2004). Wuerfel et al. (2004) concluded that elevation of perfusion must therefore be an early event in the development of a plaque. This is in line with the results of Haselhorst et al. (2000) and Ge et al. (2005b), who also found that the early stage of plaque development is characterised by inflammation and increased perfusion. Contrarily, normal to diminished blood supply has been described for fully-formed MS plaques with a tendency to decrease further with increasing axonal damage (Haselhorst et al. 2000; Law et al. 2004; Ge et al. 2005b). Several other studies suggest that overall perfusion of MS patients in lesions as well as in various parts of NAWM and NAGM is decreased compared to healthy subjects (Law et al. 2004; Ge et al. 2005b; Adhya et al. 2006; Inglese et al. 2007). It is therefore a broad consensus that MS patients display altered cerebral perfusion.

An idea which has been the center of intense and controversial discussion is the concept of venous drainage pathology termed chronic cerebrospinal venous insufficiency (CCSVI), which was reintroduced by Zamboni (2006) as a potential reason for developing MS. Professor Zamboni has suggested that endovascular therapy of the vein blocking the cranial outflow might be a means to cure MS and his group has published several studies in support of the CCSVI theory (Zamboni et al. 2007; Zamboni et al. 2009a; Zamboni et al. 2009b; Zamboni et al. 2009c; Bartolomei et al. 2010; Zamboni and Galeotti 2010). However, the study of Zamboni (2006) has been severely criticized with

respect to the methods and criteria implied, as comprehensively presented by Valdueza et al. (2013). Most other research groups could not corroborate that cerebral venous pathology occurs more often in MS than in healthy controls, neither using ultrasound nor the more objective MRI (e.g. Sundström et al. 2010; Doepp et al. 2010; Doepp et al. 2011; Wattjes et al. 2011b; Wattjes et al. 2011a; Zivadinov et al. 2011; Bourdette and Cohen 2014; Tsivgoulis et al. 2015; Krogias et al. 2016; Cardaioli et al. 2016).

But the general idea that there is some sort of MS-associated vasculopathy is still around, as small venules have been found to be affected in MS in several ways. Histopathological studies found significant wall damage and perivascular inflammation (Tanaka et al. 1975; Adams et al. 1985; Adams 1988). These findings were corroborated by MRI, which showed widespread perivascular inflammation and altered perivascular spaces (Ge et al. 2005a; Wuerfel et al. 2008). High field MRI could also show that each MS plaque is associated with a venule (Tallantyre et al. 2008; Ge et al. 2008; Kollia et al. 2009; Tallantyre et al. 2009; Tallantyre et al. 2011; Sinnecker et al. 2012a; Sinnecker et al. 2012b; Wuerfel et al. 2012). Furthermore, Sinnecker et al. (2013) found that the general density of perivascular veins is reduced in MS. They attribute this rarefaction in part to venous pathology (Sinnecker et al. 2013). The perivascular configuration of MS plaques and the rarefaction of perivascular veins has given rise to the idea that obliterating processes in the course of lesion chronification might be one of the reasons for reduced cerebral perfusion in MS patients. But D'haeseleer et al. (2015) convincingly argue against this hypothesis by pointing out that this would lead to a more patchy pattern of focal cerebral blood flow (CBF) decrease than that observed in MS patients. Similarly, there is no indication of substantial obliteration such as microvessel thrombosis and other structural abnormalities in focal MS lesions (De Keyser et al. 2008), which also argues against obliterating processes being the reason for hemodynamic changes.

Another mechanism which has been suggested as a reason for reduced cerebral perfusion is an altered metabolism. Blood supply in the brain is regulated by astrocytes according to blood and oxygen demands of the local neurons (Petzold and Murthy 2011). While a decrease in cerebral perfusion in this context seems a somewhat natural consequence of axonal loss and the implied reduction in metabolism, there are several studies which point to other pathomechanisms. For one thing, there is an increase in the excitability of primary motor cortex neurons of MS patients, which suggests an increase of metabolism (Dutta et al. 2006; Conte et al. 2009; Vucic et al. 2012). Furthermore, Debernard et al.

(2014) showed that reduced grey matter perfusion is already present in RRMS patients who do not show pathological brain volume loss yet. This indicates that axonal loss is probably not the driving mechanism of cerebral hypoperfusion (D'haeseleer et al. 2015). This is consistent with the findings of Saindane et al. (2007), who simultaneously used perfusion and diffusion tensor imaging to investigate hemodynamic changes. They found the decreased CBF levels in their study to be rather consistent with primary ischemia than with hypoperfusion caused by Wallerian degeneration (Saindane et al. 2007). This is in line with the results of De Keyser et al. (2008), who found that ischemic changes are a possible reason for lesion development in certain types of lesions instead of a consequence (De Keyser et al. 2008). The notion that decreased perfusion in MS patients is not simply a direct consequence of a reduced metabolism is further supported by the spectroscopic results of Steen et al. (2013).

Beyond an altered metabolism, primary astrocyte dysfunction has been suggested as a key player in the pathomechanism of reduced perfusion De Keyser et al. (2008). Reactive astrocytes in MS plaques have also been identified as a possible source for elevated levels of the vasoconstrictive peptide endothelin-1 (ET-1) (D'haeseleer et al. 2013), which has recently become the focus of scientific attention. Several studies have found enhanced blood levels of ET-1 in peripheral venous blood as well as in cerebrospinal fluid of MS patients (Speciale et al. 2000; Haufschild et al. 2001; D'haeseleer et al. 2013). Enhanced blood levels of ET-1 have also been associated with reduced extra-ocular blood flow velocities (Pache et al. 2003). Furthermore, Marshall et al. (2014) could show that the functional response of cerebral arterioles to vasomotor stimulation is impaired in MS patients, which indicates the presence of counteracting vasoconstrictive effects. In addition to that, D'haeseleer et al. (2013) even found that reduced perfusion in MS patients could be significantly increased by administrating an ET-1 receptor antagonist. Overall, these findings provide strong evidence that reduced cerebral perfusion in MS patients is at least partly mediated by elevated ET-1 levels, suggesting a pathology of arterioles as an important player in hemodynamic change in MS patients.

Impaired perfusion in MS patients has been linked to loss to higher brain functions, such as verbal memory and executive motor task function, as well as to fatigue score (Inglese et al. 2007; Papadaki et al. 2012; Papadaki et al. 2014a; Papadaki et al. 2014b). This assumes a new significance in light of the emerging understanding that impaired perfusion in MS is not simply a consequence of inflammation and/or axonal loss, but

might independently contribute to disease progression and all the ensuing disability. This makes cerebral perfusion a valuable tool not only in assessing disease progressing, but also in assessing the efficacy of drugs undergoing clinical testing. Moreover, hemodynamic change is a process, which starts early on in the disease (e.g. Rashid et al. 2004; Papadaki et al. 2012; Papadaki et al. 2014b). Also, Varga et al. (2009) found some evidence that hemodynamic change in MS starts out in the NAWM and only spreads to the cerebral grey matter (GM) as the disease progresses. Considering this in view of the potentially reversible nature of this impaired perfusion D'haeseleer et al. (2013), restoring cerebral perfusion could become an important pharmacological target in the hitherto inadequately addressed diffuse axonal damage in MS. It is therefore crucial to further investigate the process underlying impaired cerebral perfusion in MS to better understand its (temporal) evolution and the mechanisms governing it.

2. Material and Methods

A total of 106 untreated patients aged between 18 and 65, who had been diagnosed with CIS or RRMS according to the revised McDonald criteria (Polman et al. 2011) were prospectively recruited from the Charité outpatient clinic, Department of Neurology, Charité University Medicine, Berlin, Germany. A total of 11 patients were excluded, 3 because the diagnosis was revised in the course of the study, 5 because of technical problems (contrast agent peak not properly captured, motion artefacts), 2 because of insufficient enhancement and 1 because of an unusually shaped lesion suspicious of a tumour. The remaining 95 patients were included in this study.

All patients underwent a neurological examination conducted by a board-certified neurologist and were evaluated according to the EDSS (Kurtzke 1983). Written informed consent according to the declaration of Helsinki was obtained from all patients. A positive vote of the Ethics Committee at Charité University Medicine, Berlin, Germany was obtained (*EA1/182/10*). More detailed demographic information can be found in Table 2.1.

2.1 Magnetic Resonance Imaging

All subjects underwent MRI. The following sequences were acquired in all subjects on a 3T scanner (Tim Trio, Siemens Medical Systems, Erlangen, Germany) at the Berlin Center of Advanced Neuroimaging (Charité University Medicine, Berlin, Germany):

1. Three-dimensional T1- weighted $1 \times 1 \times 1 \text{ mm}^3$ isotropic magnetization-prepared rapid acquisition and multiple gradient echo technique (MPRAGE), TE 2.55 ms, TR 1900 ms, TI 900 ms.
2. Three-dimensional T2-weighted $1 \times 1 \times 1 \text{ mm}^3$ isotropic, TE 502 ms, TR 5000 ms.

Subjects	n	95
Gender	Male	29 (31%)
	Female	66 (69%)
Age (years)	Mean \pm SD	32 ± 9
	Min – max	18 – 56
EDSS	Median	1.5
	Min – max	1 – 4
NBV (cm ³)	Mean \pm SD	1707 ± 91
	Min – max	1451 – 1973
NGMV (cm ³)	Mean \pm SD	692 ± 38
	Min – max	590 – 804
NWMV (cm ³)	Mean \pm SD	1015 ± 58
	Min – max	862 – 1168

Table 2.1: Cohort demographics. SD: standard deviation; Min: minimum value; max: maximum value.

3. Three-dimensional T2-weighted fluid-attenuated inversion recovery $1 \times 1 \times 1 \text{ mm}^3$ isotropic, TE 388 ms, TR 5000 ms, TI 1800 ms.
4. Two-dimensional dynamic susceptibility contrast (DSC) echo-planar imaging $1.8 \times 1.8 \text{ mm}^2$ with a slice thickness of 5 mm (TE 30 ms, TR 1490 ms) after intravenous injection of 7 ml Gadovist (0.1 mmol/kg body weight, Bayer Healthcare Germany, Radiology, Leverkusen) at a rate of 3 ml/s followed by 20 ml saline. Data acquisition started 10 s before the beginning of the contrast agent injection with a temporal resolution of 1.5 s and was continued for 75 s.

2.2 Image Analysis

All acquired images were stored in DICOM file format in an instance of version 1.6.5 of the open-source Extensible Neuroimaging Archive Toolkit (Marcus et al. 2007), which is available for download at <http://www.xnat.org/download>. Most steps of the image analysis was implemented as a pipeline in the framework provided by the Extensible Neuroimaging Archive Toolkit. All computations were carried out on a Mac Mini (OS X 10.9.5, Intel Core i7 2.6 GHz, 16 GB 1600 MHz DDR3).

2.2.1 Brain Volumes

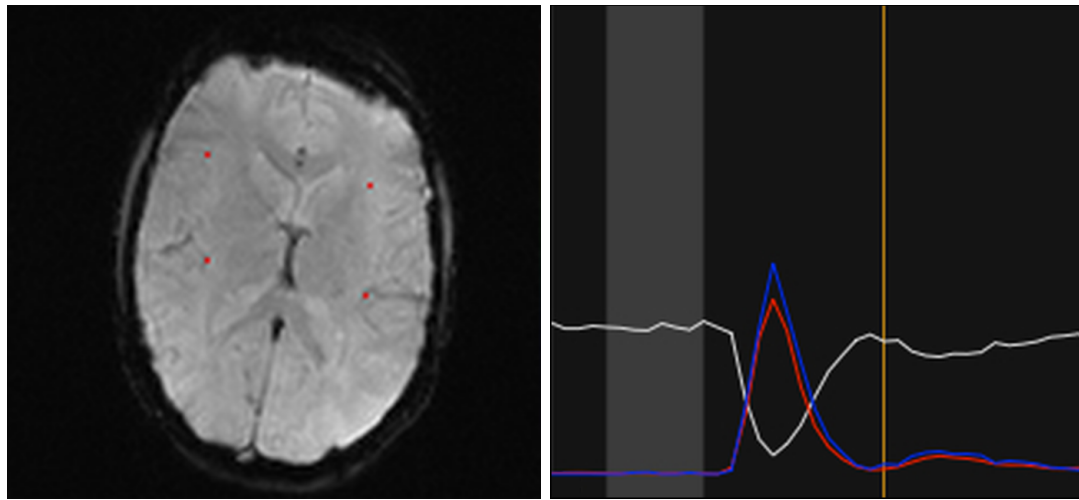
Brain tissue volume, normalised for subject head size, was estimated with version 2.6 of SIENAX (Smith et al. 2001, 2002), part of FSL (Smith et al. 2004), which is available for download at <https://fsl.fmrib.ox.ac.uk/fsldownloads/fsldownloadmain.html>. SIENAX starts by extracting brain and skull images from the single whole-head input data (Smith 2002). The brain image is then affine-registered to MNI152 space (Jenkinson and Smith 2001; Jenkinson et al. 2002), using the skull image to determine the registration scaling. The registration is primarily carried out to obtain the volumetric scaling factor, which is used as a normalisation for head size. In the next step, tissue-type segmentation with partial volume estimation is carried out (Zhang et al. 2001) in order to calculate the normalised total volume of brain tissue, including separate estimates of the volumes of grey matter and white matter. SIENAX is referenced here as requested in the documentation (SIENAX 2016).

2.2.2 White Matter Lesions

Bulk white matter T2w lesion load was manually quantified on T2-weighted sequences using version 6.0 of the OsiriX open-source software (Rosset et al. 2004), which is available for download at <http://www.osirix-viewer.com>. Binary lesion masks were created and converted to NIfTI file format. 11 patients did not show any white matter lesions.

2.2.3 Perfusion Imaging

Perfusion images were analysed version 6.0 of the OsiriX open-source software (Rosset et al. 2004) and the IB Neuro (Imaging Biometrics, LLC, <http://www.imagingbiometrics.com>, v1.2) plug-in, which offers good accuracy and consistency (Hu et al. 2015). The IB Neuro plug-in offers standardised rCBF maps and rCBV maps corrected for contrast agent leakage. Standardization is carried out by transforming rCBV values to a consistent intensity scale regardless of make, model and field strength of the scanner used (Schmainda et al. 2004; Boxerman et al. 2006; Bedekar et al. 2010). In order to define the AIF, four voxels were manually selected in distant branches of the medial cerebral artery (Ebinger et al. 2010) with early and well-defined contrast agent



(a) Defining voxels for AIFs.

(b) Defining voxels for AIFs.

Figure 2.1: (a) Exemplary defining voxels for AIF and (b) resulting curves (last manually defined AIF in white, average AIF in red, difference between last manually defined AIF and average AIF in blue). Light gray interval denotes definition of the baseline, vertical orange line denotes the end of the integration interval.

inflow, two in each hemisphere (see Figure 2.1 for an example). All created maps were converted to NIfTI file format.

2.2.4 Regions of Interest

Volumetric segmentation was performed with version 5.2.0 of FreeSurfer, which is documented and available for download at <http://surfer.nmr.mgh.harvard.edu>. Details on FreeSurfer processes are described in more detail in Dale and Sereno (1993), Dale et al. (1999), Fischl et al. (1999a), Fischl et al. (1999b), Fischl and Dale (2000), Fischl et al. (2001), Fischl et al. (2002), Fischl et al. (2004a), Fischl et al. (2004b), Ségonne et al. (2004), Han et al. (2006), Jovicich et al. (2006), Reuter et al. (2010) and Reuter et al. (2012). Briefly, this processing includes motion correction and averaging of multiple volumetric T1 weighted images, if more than one is available (Reuter et al. 2010), removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Ségonne et al. 2004), automated Talairach transformation, segmentation of the sub-cortical white matter and deep grey matter volumetric structures including thalamus, hippocampus, amygdala, caudate, putamen, ventricles (Fischl et al. 2002, 2004a), in-

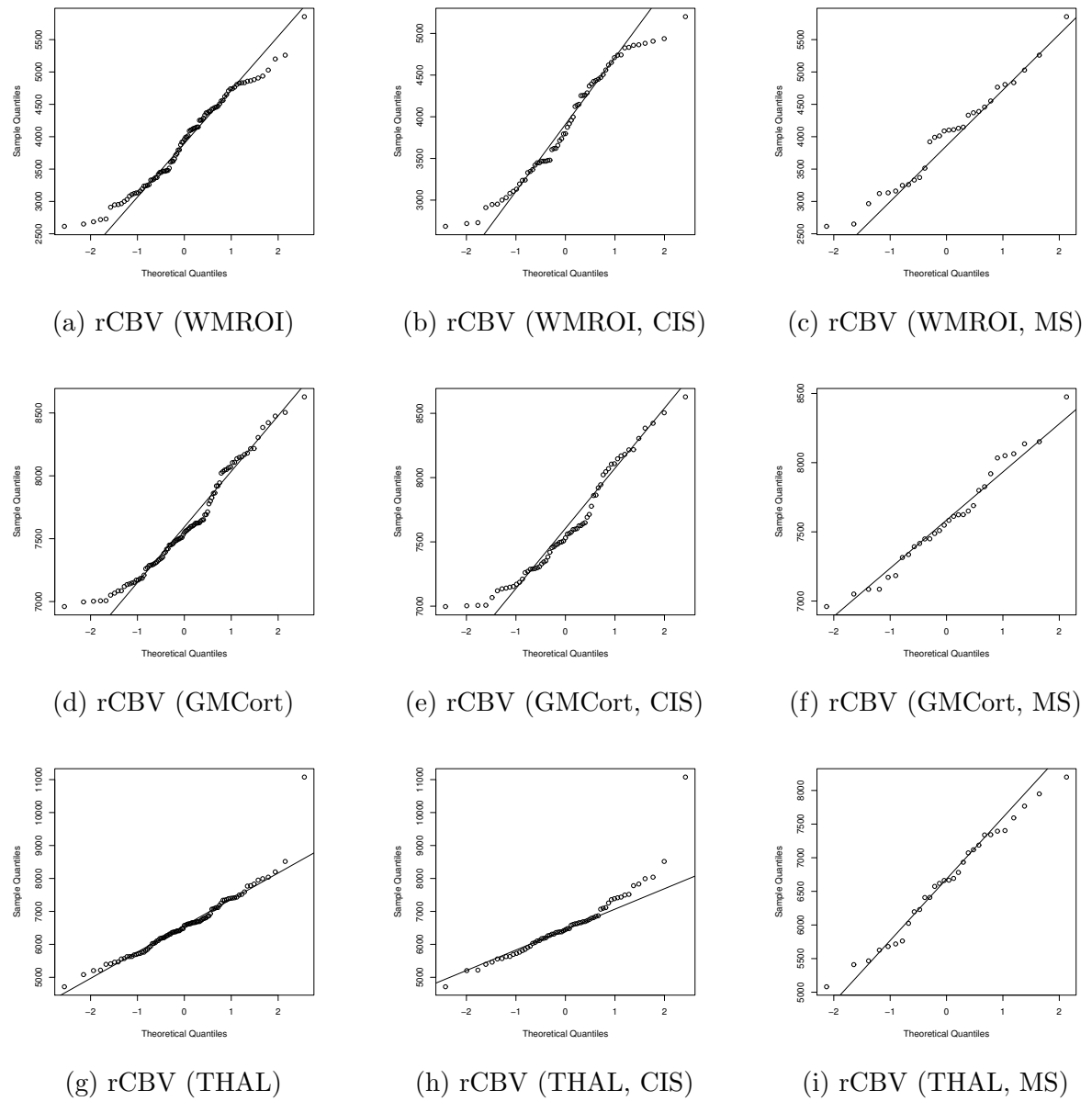


Figure 2.2: Normal plots of average rCBV values for each ROI and the respective patient groups (all, CIS, MS).

tensity normalization (Sled et al. 1998), tessellation of the grey matter white matter boundary, automated topology correction (Fischl et al. 2001; Ségonne et al. 2007) and surface deformation following intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale et al. 1999; Dale and Sereno 1993;

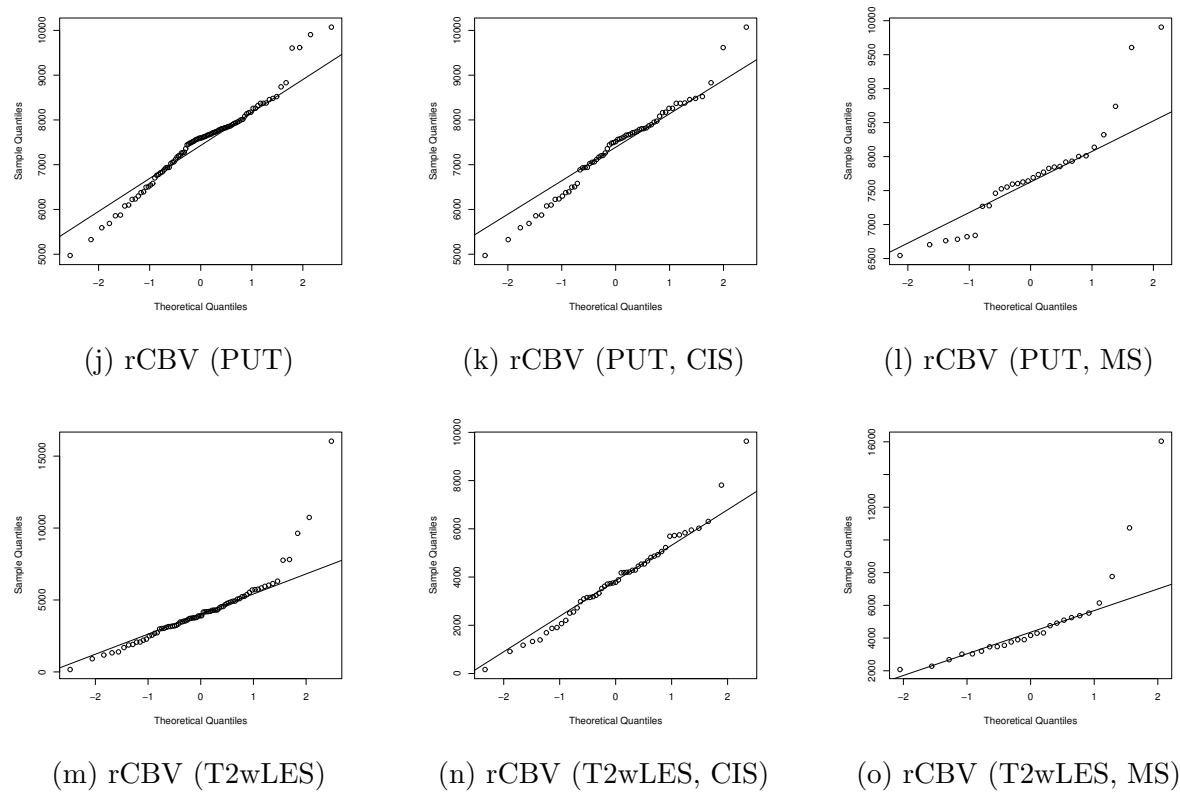


Figure 2.2: Normal plots of average rCBV values for each ROI and the respective patient groups.

Fischl and Dale 2000). FreeSurfer is referenced here as requested in the documentation (FreeSurfer 2016). FreeSurfer results were manually checked and corrected where necessary for every step as recommended by the FreeSurfer user guide.

Binary masks for the region of interest comprising the cortical grey matter (GMCort), the region of interest comprising cerebral white matter (WMROI), the region of interest comprising the thalamus (THAL) and the region of interest comprising the putamen (PUT) were extracted from the FreeSurfer output volume and converted to NIfTI file format using FMRIB's FSL software library (Jenkinson et al. 2012). T2w lesions were subsequently excluded from these ROI masks by inverting the respective T2w lesion mask and applying it to each ROI using FMRIB's FSL software library (Smith 2002). A brain-extracted version of the MPRAGE data was created using BET from FMRIB's FSL software library (Smith 2002). The first time-point of the original perfusion data was registered linearly to the brain-extracted MPRAGE data using 12 degrees

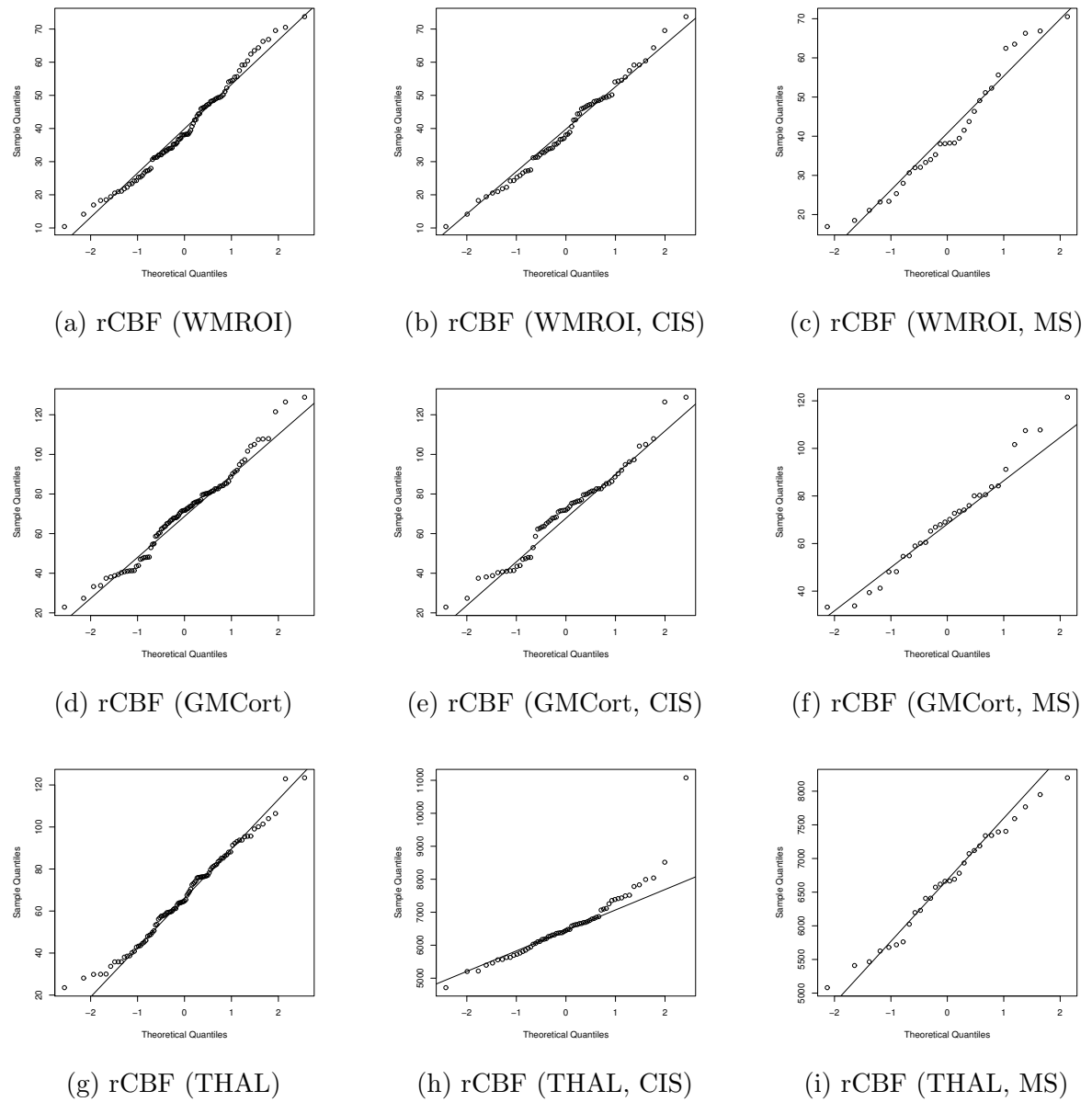


Figure 2.3: Normal plots of average rCBF values for each ROI and the respective patient groups (all, CIS, MS).

of freedom. The resulting transformation matrix was then inverted and applied to the MPRAGE data, thus effectively registering the MPRAGE data to the baseline perfusion image. All ROI masks and the lesion mask were also registered to perfusion resolution using this inverted transformation.

This registration comprises a change in resolution from the original MPRAGE space

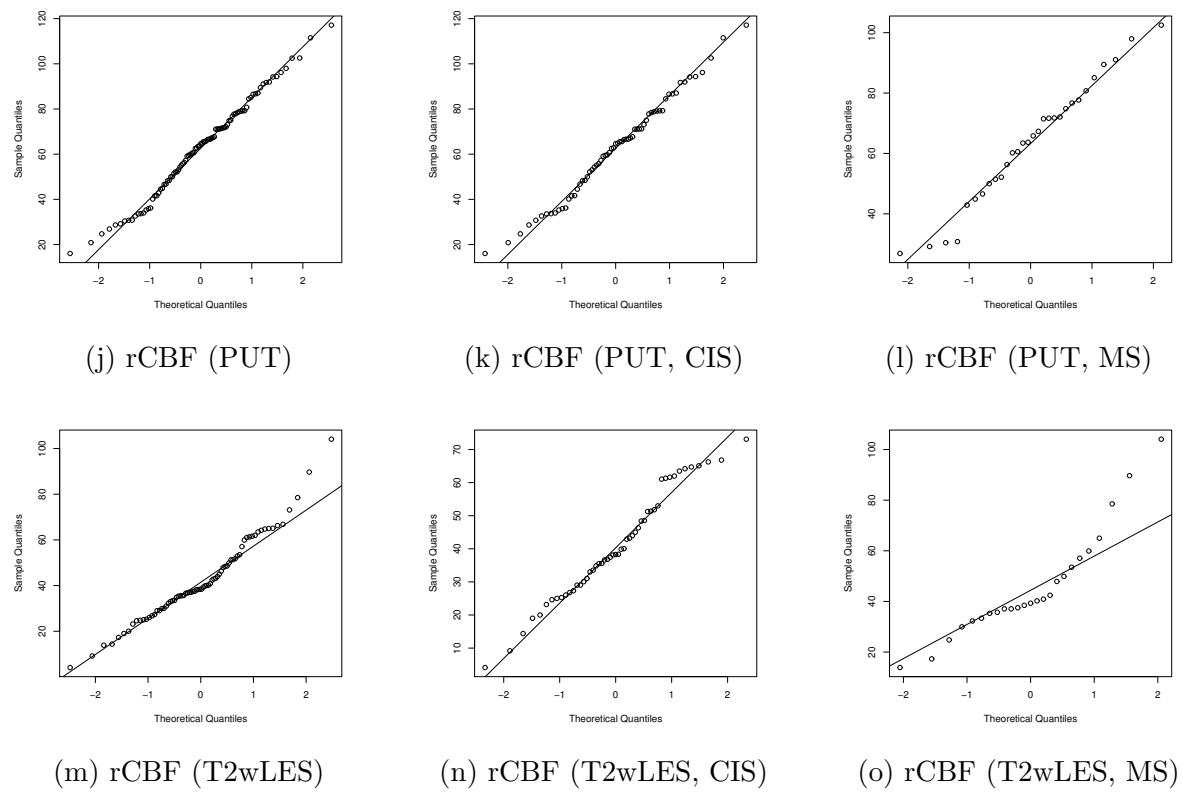


Figure 2.3: Normal plots of average rCBF values for each ROI and the respective patient groups.

($1 \times 1 \times 1 \text{ mm}^3$) to the resolution of the perfusion data ($1.8 \times 1.8 \times 5 \text{ mm}^3$). In the course of this downsampling, new voxels integrate information from several original voxels, which creates significant partial volume effects. To account for this change in resolution, a threshold was applied to all ROI masks. For all grey matter-derived ROI masks (GMCort, THAL, PUT) only those new voxels were accepted, of which at least a fraction of 80% was constructed from voxels originally belonging to the respective ROI. This aims to minimise partial volume effects while still keeping most of the original form and size of the ROI. For region of interest comprising the hyperintense lesions on T2-weighted magnetic resonance images (T2wLES) only new voxels were accepted, of which at least a fraction of 70% was constructed from original T2w lesion voxels. A slightly larger partial volume effect was accepted for T2w lesions in favour of more lesions surviving the downsampling. This is effectively in order to make sure the ROIs only contain normal appearing matter. Still 8 lesions masks were levelled by the registration.

ROI analysis was carried out using FMRIB's FSL software library (Jenkinson et al. 2012). All respective ROI masks (GMCort, WMROI, PUT, THAL, T2wLES) were applied to all respective perfusion masks (rCBF, rCBV) and calculated the mean of all non-zero voxels.

2.3 Statistics

Subjects were dichotomised according to their diagnosis in a subgroup of subjects diagnosed with clinically isolated syndrome (gCIS) and a subgroup of subjects diagnosed with relapsing-remitting multiple sclerosis (gMS). All statistical work was carried out using version 2.15.3 of R (<http://www.R-project.org>). Since all derived perfusion parameters are not normally distributed (cf. Figures 2.3 and 2.2), non-parametric methods were used: median and interquartile range (IQR) to describe the data, two-sided Mann-Whitney-U-Tests (MWU-Tests) to compare the distribution of different groups. IQRs were calculated as recommended by Hyndman and Fan (1996). A multiple regression model was used to evaluate relations between the various parameters and possible interactions simultaneously. Results were corrected for multiple testing where appropriate using the Bonferroni-Holm-correction (Holm 1979).

3. Results

The two subject subgroups (gCIS and gMS) are similar with respect to demographic characteristics, though not in size, cf. Table 3.1. gCIS is twice the size of gMS, but the gender ratios are similar for both groups. The gender ratios of approximately $F : M = 2 : 1$ are also characteristic of a population of MS patients. There is no significant difference between the two groups with respect to age, EDSS and brain volumes (compare Table 3.1). There is a significant difference between both groups with respect to disease duration (DisDur), compare Table 3.1.

3.1 Volumes

An overview of derived ROI volumes can be found in Table 3.2. Median and IQR are similar for gCIS and gMS. The results of the MWU-Tests implicate that both samples, gCIS and gMS, originate from the same statistical population for each respective ROIs. In particular, this comprises that there is no significant difference between the medians of both subject subgroups for each ROI.

3.2 Perfusion parameters

An overview of derived perfusion parameters can be found in Table 3.3, Table 3.4, Figure 3.1, Figure 3.2 and Figure 3.3. Median and IQR are similar for both groups in each ROI for both, rCBV and rCBF, respectively, cf. Figure 3.4, Figure 3.5, Table 3.3 and Table 3.4.

	gCIS	gMS	MWU-Test (p-values)
Subjects	65	30	
Gender	Male: 21 (32%) Female: 44 (68%)	Male: 8 (27%) Female: 22 (73%)	
Age (years)	33 ± 8 (20 – 56)	32 ± 9 (18 – 52)	0.39
EDSS	1.5 (0 – 3.5)	1.5 (0 – 4)	0.18
Disease duration (months)	4 (1 – 36)	7.5 (10 – 38)	1.60 × 10⁻³
NBV (cm ³)	1711 ± 93 (1451 – 1973)	1698 ± 86 (1588 – 1915)	0.26
NGMV (cm ³)	694 ± 40 (590 – 804)	689 ± 35 (624 – 757)	0.52
NWMV (cm ³)	1018 ± 59 (862 – 1168)	1009 ± 56 (919 – 1158)	0.27

Table 3.1: Comparison of the two different groups with respect to demographics. Median given for EDSS and disease duration, mean ± standard deviation given for all other numeric parameters. Ranges give minimal and maximal values. Significant p-values are given in bold print.

	All (<i>n</i> = 95)	CIS (<i>n</i> = 65)	MS (<i>n</i> = 30)	MWU-Test (p-values)
Cortex (cm ³)	M: 465 IQR: 64	M: 465 IQR: 76	M: 463 IQR: 50	0.9
THAL (cm ³)	M: 15 IQR: 3	M: 15 IQR: 3	M: 14 IQR: 2	0.6
PUT (cm ³)	M: 11 IQR: 2	M: 11 IQR: 2	M: 11 IQR: 2	0.6

Table 3.2: Descriptive statistics for the different FreeSurfer-derived volumes; M: median.

	All ($n = 95$)	CIS ($n = 65$)	MS ($n = 30$)	MWU-Test (p-values)
GMCort	M: 7548 IQR: 617	M: 7531 IQR: 640	M: 7566 IQR: 500	0.99
THAL	M: 6574 IQR: 1087	M: 6444 IQR: 932	M: 6664 IQR: 1337	0.43
PUT	M: 7595 IQR: 1000	M: 7515 IQR: 1129	M: 7666 IQR: 662	0.15
WMROI	M: 3957 IQR: 1123	M: 3797 IQR: 1098	M: 4095 IQR: 1206	0.72
T2wLES*	M: 3900 IQR: 1947	M: 3782 IQR: 2092	M: 4158 IQR: 1913	0.21

Table 3.3: Descriptive statistics for rCBV. *Number of observations is different for T2wLES (gCIS: $n = 51$, gMS: $n = 25$, All: $n = 76$), see Section 2.2.4; M: median.

3.2.1 Group Comparisons

There are several ways to look at group differences for the derived perfusion metrics and the results from the different comparisons are heterogeneous:

Comparing gCIS and gMS with respect to rCBV. The results of the MWU-Tests implicate that both samples, gCIS and gMS, originate from the same statistical population for each respective ROIs (compare Table 3.3). In particular, this comprises that there is no significant difference between the medians of each subject group for the respective ROI.

Comparing gCIS and gMS with respect to rCBF. The results of the MWU-Tests implicate that both samples, gCIS and gMS, originate from the same statistical population for each respective ROIs (compare Table 3.4). In particular, this comprises that there is no significant difference between the medians of each subject subgroup for the respective ROI.

Comparing tuples of ROI within gCIS with respect to rCBV. For every tuple that consists of one grey matter ROI (GMCort, THAL, PUT) and one white matter ROI (WMROI, T2wLES) each, the results of the MWU-Tests implicate that rCBV of the two ROI do not originate from the same statistical population. In particular, this implicates that the medians of both respective ROIs also differ (see superdiagonal half of Table 3.5). Additionally, all possible tuples of grey matter ROIs differ in distribution and median,

	All ($n = 95$)	CIS ($n = 65$)	MS ($n = 30$)	MWU-Test (p-values)
GMCort	M: 72 IQR: 28	M: 72 IQR: 31	M: 70 IQR: 26	0.80
THAL	M: 65 IQR: 32	M: 65 IQR: 32	M: 65 IQR: 35	0.94
PUT	M: 65 IQR: 31	M: 65 IQR: 33	M: 65 IQR: 27	0.78
WMROI	M: 38 IQR: 18	M: 38 IQR: 19	M: 38 IQR: 21	0.86
T2wLES*	M: 38 IQR: 22	M: 38 IQR: 23	M: 39 IQR: 20	0.49

Table 3.4: Descriptive statistics for rCBF. *Number of observations is different for T2wLES (CIS: $n = 51$, MS: $n = 25$, All: $n = 76$), see Section 2.2.4; M: median.

except for the tuple consisting of (GMCort,PUT) (see superdiagonal half of Table 3.5). Conversely, the results of the MWU-Test indicate that two sets of rCBV values do not differ in distribution or median for WMROI and T2wLES (compare superdiagonal half of Table 3.5).

Comparing tuples of ROI within gCIS with respect to rCBF. For every tuple that consists of one grey matter ROI (GMCort, THAL, PUT) and one white matter ROI (WMROI, T2wLES) each, the results of the MWU-Tests implicate that rCBV of the two ROI do not originate from the same statistical population. In particular, this implicates that the medians of both respective ROIs also differ (see superdiagonal half of Table 3.6). Additionally, all possible tuples of grey matter ROIs differ in distribution and median (see superdiagonal half of Table 3.6). Conversely, the results of the MWU-Test indicate that two sets of rCBV values do not differ in distribution or median for WMROI and T2wLES (compare superdiagonal half of Table 3.6).

Comparing tuples of ROI within gMS with respect to rCBV. For every tuple that consists of one grey matter ROI (GMCort, THAL, PUT) and one white matter ROI (WMROI, T2wLES) each, the results of the MWU-Tests implicate that rCBV of the two ROI do not originate from the same statistical population. In particular, this implicates that the medians of both respective ROIs also differ (see subdiagonal half of Table 3.5). Additionally, all possible tuples of grey matter ROIs differ in distribution and median,

except for the tuple consisting of (GMCort,PUT) (see subdiagonal half of Table 3.5). Conversely, the results of the MWU-Test indicate that two sets of rCBV values do not differ in distribution or median for WMROI and T2wLES (compare subdiagonal half of Table 3.5).

Comparing tuples of ROI within gMS with respect to rCBF. For every tuple that consists of one grey matter ROI (GMCort, THAL, PUT) and one white matter ROI (WMROI, T2wLES) each, the results of the MWU-Tests implicate that rCBV of the two ROI do not originate from the same statistical population. In particular, this implicates that the medians of both respective ROIs also differ (see superdiagonal half of Table 3.6). Additionally, all possible tuples of grey matter ROIs differ in distribution and median, except for the two tuples consisting of (THAL,GMCort) and (THAL,PUT) (see superdiagonal half of Table 3.6). Conversely, the results of the MWU-Test indicate that two sets of rCBV values do not differ in distribution or median for WMROI and T2wLES (compare superdiagonal half of Table 3.6).

3.2.2 Correlations

Correlations were investigated from several angles and always in relation to NBV and EDSS, respectively.

For all data with respect to rCBV. A linear model containing mean rCBV for all ROI, age, gender and DisDur contributes significantly to explaining the systematic variation of NBV. Individually, mean rCBV for GMCort, THAL, WMROI, age, gender and DisDur are significant predictors for NBV, but mean rCBV for PUT is not. Conversely, a linear model containing mean rCBV for all ROI, age, gender and DisDur does not contribute significantly to explaining the systematic variation of EDSS (compare Table 3.7).

For all data with respect to rCBF. A linear model containing mean rCBF for all ROI, age, gender and DisDur contributes significantly to explaining the systematic variation of NBV. Individually, mean rCBF for THAL, WMROI, age and DisDur are significant predictors for NBV, but mean rCBF for GMCort, PUT and gender are not. Conversely, a linear model containing mean rCBF for all ROI, age, gender and DisDur does not contribute significantly to explaining the systematic variation of EDSS (compare Table 3.7).

For gCIS with respect to rCBV. A linear model containing mean rCBV for all ROI, age, gender and DisDur significantly contributes to explaining the systematic variation of NBV. Individually, mean rCBV for GMCort, THAL, WMROI and gender are significant predictors for NBV, but PUT, age and DisDur are not. Conversely, a linear model containing mean rCBV for all ROI, age, gender and DisDur does not contribute significantly to explaining the systematic variation of EDSS, see Table 3.8.

For gCIS with respect to rCBF. A linear model containing mean rCBF for all ROI, age, gender and DisDur significantly contributes to explaining the systematic variation of NBV. Individually, mean rCBF for THAL, WMROI and age are significant predictors for NBV, but GMCort, PUT, gender and DisDur are not. Conversely, a linear model containing mean rCBF for all ROI, age, gender and DisDur does not contribute significantly to explaining the systematic variation of EDSS (compare Table 3.8).

For gMS with respect to rCBV. For gMS a linear model containing mean rCBV for all ROI, age, gender and DisDur does not contribute significantly to explaining the systematic variation of either NBV or EDSS, see Table 3.9.

For gMS with respect to rCBF. For gMS a linear model containing mean rCBF for all ROI, age, gender and DisDur does not contribute significantly to explaining the systematic variation of either NBV or EDSS. More detailed statistical results can be found in Table 3.9.

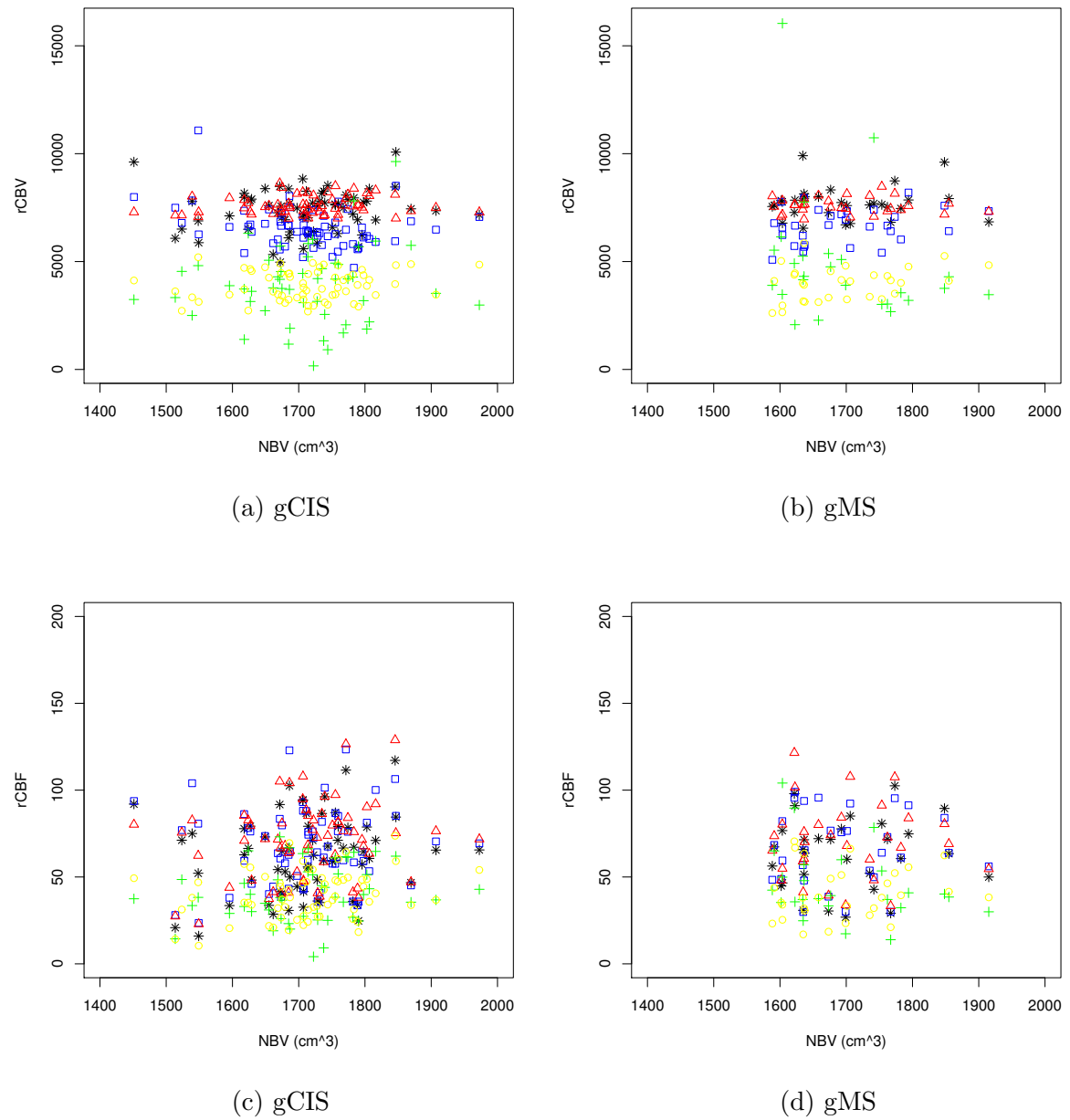


Figure 3.1: Average rCBV ((a),(b)) and rCBF ((c),(d)) against NBV for all ROIs. GMCort: red triangles, THAL: blue squares, PUT: black asterisks, WMROI: yellow circles, T2wLES: green crosses.

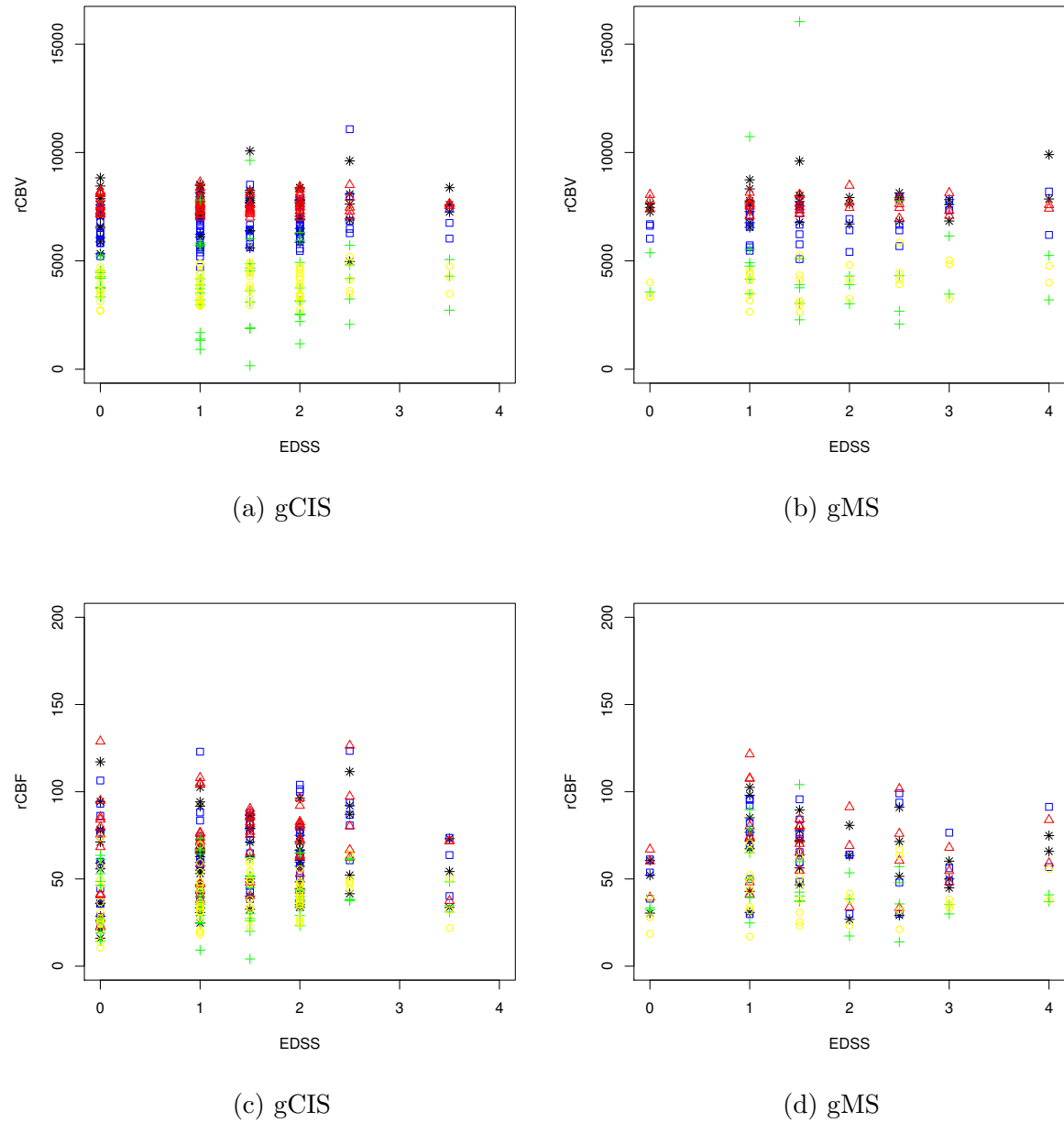


Figure 3.2: Average rCBV ((a),(b)) and rCBF ((c),(d)) against EDSS for all ROIs. GMCort: red triangles, THAL: blue squares, PUT: black asterisks, WMROI: yellow circles, T2wLES: green crosses.

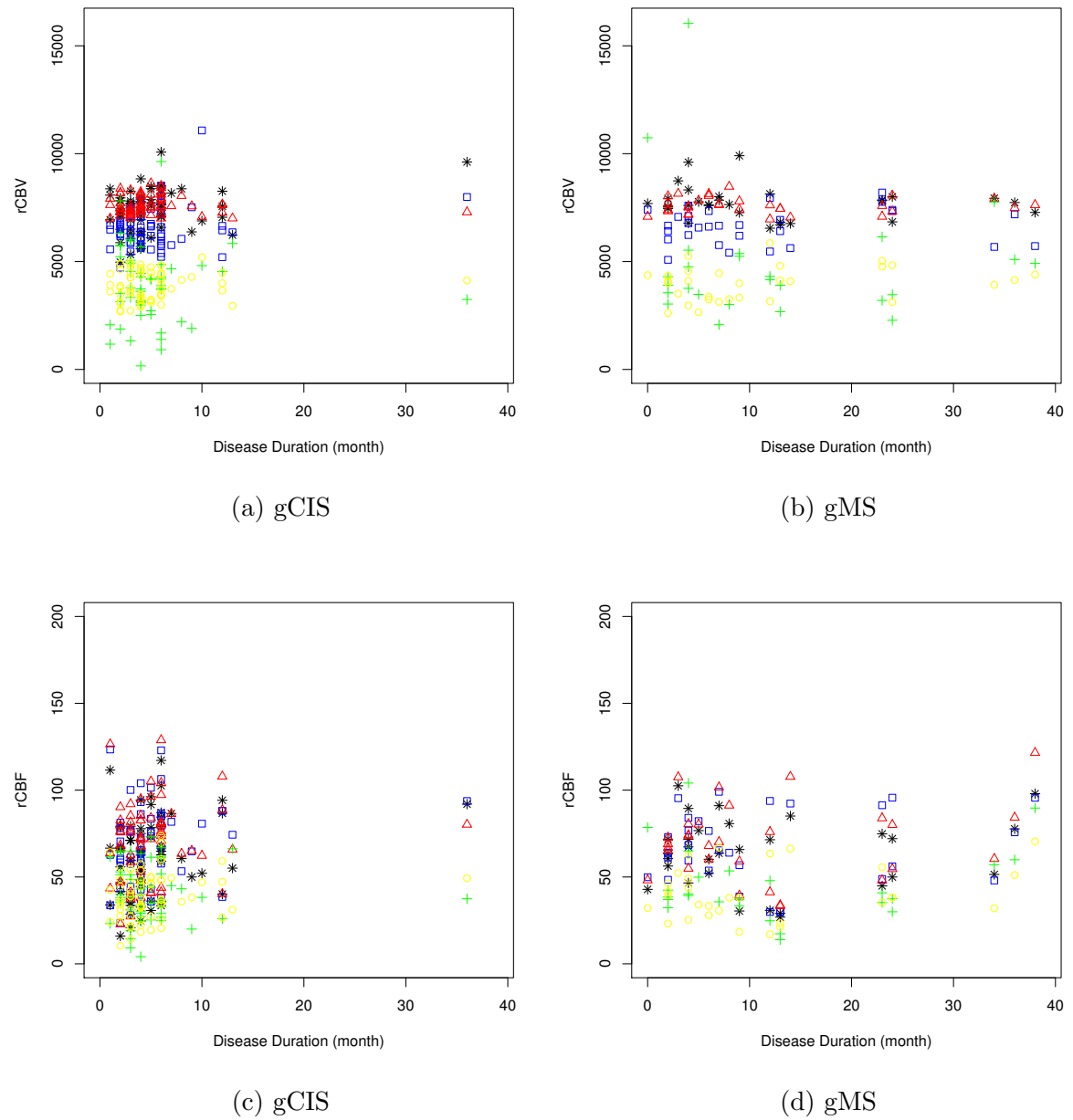


Figure 3.3: Average rCBV ((a),(b)) and rCBF ((c),(d)) against disease duration for all ROIs. GMCort: red triangles, THAL: blue squares, PUT: black asterisks, WMROI: yellow circles, T2wLES: green crosses.

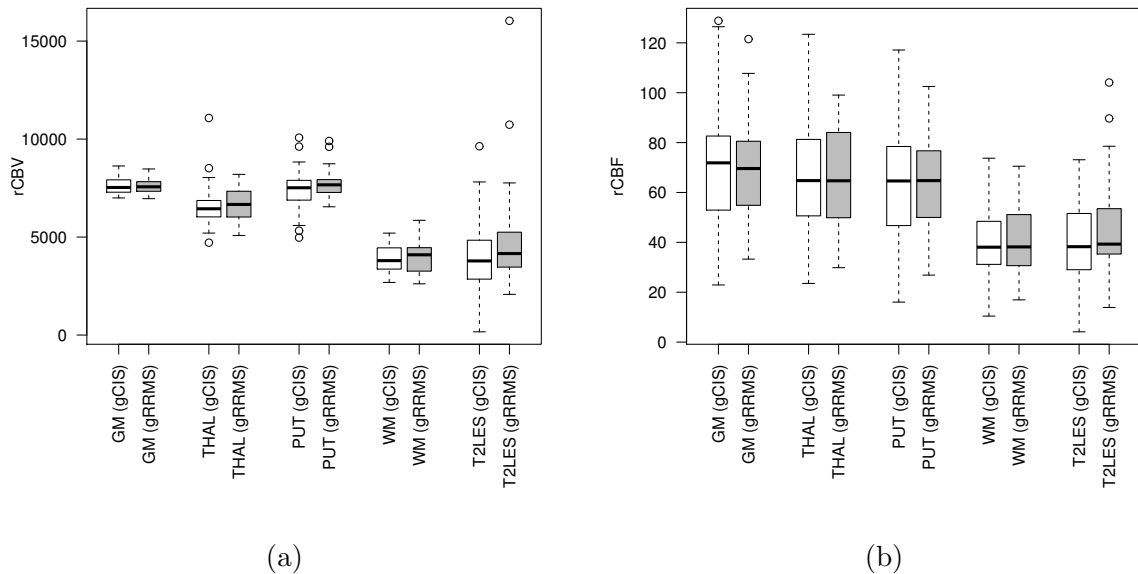


Figure 3.4: Boxplot for all ROIs and both patient groups (gCIS, gMS) for (a) rCBV and (b) rCBF.

		gCIS				
		GMCort	THAL	PUT	WMROI	T2wLES
gMS	GMCort		1.69 × 10⁻⁸	7.91 × 10 ⁻²	1.72 × 10⁻¹¹	8.02 × 10⁻¹⁷
	THAL	5.47 × 10⁻⁵		5.17 × 10⁻⁶	1.72 × 10⁻¹¹	1.81 × 10⁻¹⁴
	PUT	9.19 × 10 ⁻¹	1.93 × 10⁻⁶		1.72 × 10⁻¹¹	4.89 × 10⁻¹⁶
	WMROI	1.86 × 10⁻⁸	1.86 × 10⁻⁸	1.86 × 10⁻⁸		8.85 × 10 ⁻¹
	T2wLES	4.47 × 10⁻⁷	3.20 × 10⁻⁶	3.10 × 10⁻⁷	9.10 × 10 ⁻¹	

Table 3.5: Group comparisons with respect to rCBV. Table states corrected p-values of MWU-Tests for the tuple of ROIs defined by row/column. The superdiagonal half represents gCIS, the subdiagonal half represents gMS. Significant p-values are given in bold print.

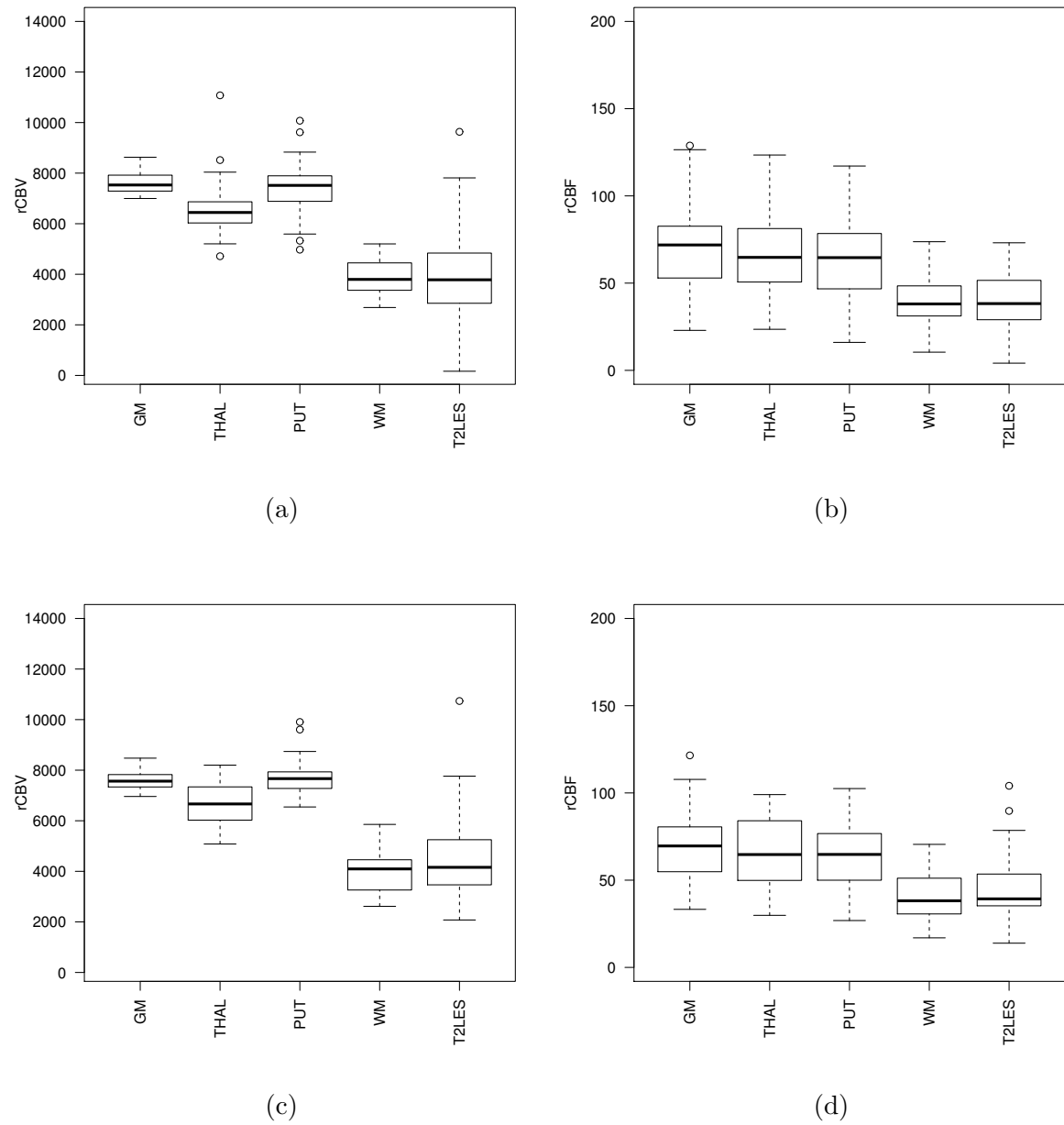


Figure 3.5: Boxplot for intragroup comparison for all ROIs and both, rCBV and rCBF, for gCIS ((a),(b)) and gMS ((c),(d)), respectively.

	gCIS					
gMS		GMCort	THAL	PUT	WMROI	T2wLES
GMCort			1.60×10^{-3}	2.96×10^{-9}	2.46×10^{-11}	1.28×10^{-10}
THAL		1.91×10^{-1}		8.39×10^{-4}	2.46×10^{-11}	1.24×10^{-8}
PUT		7.43×10^{-5}	1.91×10^{-1}		2.46×10^{-11}	1.52×10^{-6}
WMROI		1.86×10^{-8}	1.86×10^{-8}	1.86×10^{-8}		6.60×10^{-1}
T2wLES		2.34×10^{-4}	2.50×10^{-3}	6.60×10^{-3}	4.35×10^{-1}	

Table 3.6: Group comparisons with respect to rCBF. Table states corrected p-values of MWU-Tests for the tuple of ROIs defined by row/column. The superdiagonal half represents gCIS, the subdiagonal half represents gMS. Significant p-values are given in bold print.

Metric: Target:	rCBV		rCBF	
	NBV	EDSS	NBV	EDSS
F-Test	6.34×10^{-6}	5.67×10^{-2}	2.89×10^{-4}	5.99×10^{-2}
adj. R ²	2.80×10^{-1}	7.30×10^{-2}	2.04×10^{-1}	7.14×10^{-2}
GMCort	5.63×10^{-3}	4.43×10^{-1}	5.11×10^{-1}	1.75×10^{-1}
THAL	3.50×10^{-2}	5.15×10^{-1}	2.418×10^{-2}	6.31×10^{-2}
PUT	7.75×10^{-1}	6.05×10^{-1}	9.83×10^{-1}	6.45×10^{-1}
WMROI	1.09×10^{-5}	1.22×10^{-1}	3.09×10^{-3}	3.22×10^{-1}
Age	8.73×10^{-3}	4.47×10^{-1}	8.39×10^{-3}	4.26×10^{-1}
Gender	1.49×10^{-2}	8.41×10^{-1}	2.47×10^{-1}	7.32×10^{-1}
DisDur	3.95×10^{-2}	9.26×10^{-2}	4.40×10^{-2}	5.40×10^{-2}
Intercept	7.09×10^{-07}	2.75×10^{-1}	2.00×10^{-16}	1.22×10^{-1}

Table 3.7: Multiple regression results for pooled data. Table states p-values of the respective t-test for each ROI. Significant p-values are given in bold print.

Metric: Target:	rCBV		rCBF	
	NBV	EDSS	NBV	EDSS
F-Test	5.25×10^{-5}	5.33×10^{-1}	3.65×10^{-4}	3.37×10^{-1}
adj. R ²	3.38×10^{-1}	0	2.85×10^{-1}	1.77×10^{-2}
GMCort	4.16×10^{-2}	4.16×10^{-1}	2.29×10^{-1}	8.87×10^{-1}
THAL	1.26×10^{-2}	9.12×10^{-1}	1.61×10^{-2}	1.27×10^{-1}
PUT	3.14×10^{-1}	9.83×10^{-1}	9.51×10^{-1}	2.63×10^{-1}
WMROI	7.01×10^{-4}	2.68×10^{-1}	1.34×10^{-2}	4.77×10^{-1}
Age	5.88×10^{-2}	3.01×10^{-1}	3.73×10^{-2}	2.18×10^{-1}
Gender	1.82×10^{-2}	3.86×10^{-1}	3.34×10^{-1}	9.77×10^{-1}
DisDur	7.96×10^{-2}	7.09×10^{-1}	7.42×10^{-2}	8.13×10^{-1}
Intercept	3.91×10^{-6}	4.16×10^{-1}	1.10×10^{-37}	6.85×10^{-1}

Table 3.8: Multiple regression results for gCIS. Table states p-values of the respective t-test for each ROI. Significant p-values are given in bold print.

Metric: Target:	rCBV		rCBF	
	NBV	EDSS	NBV	EDSS
F-Test	2.38×10^{-1}	3.70×10^{-1}	4.64×10^{-1}	2.75×10^{-1}
adj. R ²	9.69×10^{-2}	3.49×10^{-2}	0	1.77×10^{-2}
GMCort	2.19×10^{-1}	6.62×10^{-1}	1.74×10^{-1}	4.35×10^{-2}
THAL	5.08×10^{-1}	6.26×10^{-1}	6.26×10^{-1}	6.74×10^{-1}
PUT	6.15×10^{-1}	4.46×10^{-1}	3.30×10^{-1}	1.88×10^{-1}
WMROI	9.78×10^{-2}	4.07×10^{-1}	2.29×10^{-1}	5.33×10^{-1}
Age	2.64×10^{-1}	7.22×10^{-1}	3.45×10^{-1}	6.71×10^{-1}
Gender	3.60×10^{-1}	4.74×10^{-1}	2.09×10^{-1}	6.61×10^{-1}
DisDur	3.77×10^{-1}	2.87×10^{-1}	9.68×10^{-1}	8.12×10^{-2}
Intercept	6.64×10^{-2}	3.93×10^{-1}	7.23×10^{-17}	1.90×10^{-1}

Table 3.9: Multiple regression results for gMS. Table states p-values of the respective t-test for each ROI. Significant p-values are given in bold print.

4. Discussion

Even though the two main groups (gCIS and gMS) are not comparable in size, they do not differ significantly in demographic characteristics, such as gender ratios and age, or measures of disease severity, such as EDSS and brain volumes. But there is a significant difference in disease duration. This can hardly be avoided, however, when comparing CIS, which is by definition a single incident, to RRMS, which comprises several incidents of which each would constitute a CIS. It is also possible that gCIS contains some subjects who really suffer from RRMS, but do not yet fulfil the dissemination criteria with respect to time (Polman et al. 2011). But since this study is part of a longitudinal setup, subjects were followed up for 24 – 48 months and diagnoses were updated where necessary. Thus the number of gCIS patients which really should be in gMS is likely comparatively small. Overall, the results presented in Chapter 3 are therefore likely to represent real effects rather than simply a biased group selection.

4.1 Volumes

There are no significant group differences for regional FreeSurfer-derived volumes (cf. Table 3.2). Loss of brain volume has been observed in early RRMS patients as well as in CIS patients (De Stefano et al. 2010), so generally this finding is consistent with the accepted understanding of the pathophysiology of MS. However, it should be noted that there is a small difference between gCIS and gMS with respect to disease duration. Therefore one might expect a small group difference with respect to regional volumes as well, simply because the underlying processes leading to neurodegeneration and brain volume loss have been going on for a longer period of time. But considering that FreeSurfer's sensitivity for correctly classifying a grey matter voxel was found to be only approximately 83 – 85% in ideal situations (Klauschen et al. 2009; Eggert et al.

2012), such a small difference is likely to be obscured by FreeSurfer's sensitivity. Moreover, the transformation of ROIs from the high-resolution MPRAGE image space into the space of the echo-planar perfusion image, which is also known to be geometrically distorted (Jezzard and Balaban 1995), presents another source of inaccuracy on two counts. Firstly, parts of several voxels are integrated into one new voxel, as the resolution significantly differs between the MPRAGE and the perfusion image. This causes a partial volume effect, which is accounted for by the threshold used in binarising the resulting mask (compare Section 2.2.4). The only way to be certain of not capturing any voxels outside of the original ROI would be to use a threshold of 100%. However, because of the difference in resolution between the MPRAGE and the perfusion image, this would also exclude quite a number of voxels which largely consist of ROI tissue. Especially in non-uniform ROIs, this would influence the derived mean values. This problem was addressed by using a conservative threshold on all ROI masks during downsampling, in order to make sure that the registered ROI contains most of what was originally included in the ROI. Additionally, a more liberal threshold was chosen for T2wLES, in order to make sure that all other registered ROI masks do not contain lesional tissue.

Secondly, the echo-planar perfusion image is geometrically distorted (Jezzard and Balaban 1995) in comparison with the MPRAGE, on which the ROI masks are initially defined. This is in part accounted for by using a linear transformation with 12 degrees of freedom to register the echo-planar perfusion image to the MPRAGE. But there are some problems with this method. The perfusion image displays rather insufficient grey/white contrast, so the alignment is based on other landmarks. So even with a good alignment of the brain outlines, there is not necessarily a good alignment of the (deep) grey matter ROIs. Additionally, a linear transformation only allows for scaling but not for local distortion of the images via the registration, which does not completely capture the reality. The obvious solution to this problem would be to use a non-linear transformation for the registration process. However, there is probably not much to be gained by this approach. Using a non-linear transformation allows for local distortion, but there simply is not enough information in the echo-planar perfusion image (grey/white contrast, regional landmarks) to generate good local registration results with respect to the anatomical regions used here. Therefore using a non-linear transformation introduces a lot of uncontrollable distortion of the respective ROI, which is likely to cause

a loss in registration quality compared to a linear transformation as it is likely to cause an improvement in registration quality in this particular setting. So, in summary, even though the results of this study are consistent with what we know about MS pathology, these findings should not be interpreted as hard evidence that there is no difference in (regional) volumes between both groups.

4.2 Perfusion parameters

When interpreting results from perfusion studies, it should be kept in mind that the different processing methods might be accountable for some of the differences. DSC imaging only provides relative data and inherently depends on the application of the contrast agent bolus. Data is standardised and/or normalised according to different protocols, if at all. Especially in MS patients there can be substantial leaking of the contrast agent. Again there are several methods to correct for contrast agent leakage, but leakage correction is not always performed, making it even harder to assess the comparability of existing results. In this study, the leakage correction offered by the IB Neuro plug-in was used. There are no existing studies with respect to MS and the IB Neuro plug-in, but Boxerman et al. (2006) could show that leakage correction significantly improved the accuracy of rCBV estimation in gliomas with relevant contrast extravasation.

It should also be taken into account that as rCBF inherently depends on the manually defined AIF, which strongly depends on the vascular structure. An accurate acquisition of the AIF is essential for accurate results (van Osch et al. 2003). The AIF was defined by four manually chosen voxels in order to fully control this important process. However, it is possible that the four chosen voxels do not represent the arterial input sufficiently well, resulting in a bias in CBF. Generally, the arterial input will not be the same everywhere in the brain owing to circulation and vascular state as well as local vasoactivity (Mottet et al. 1997; Conturo et al. 2005). AIF signal-to-noise-ratio was optimised in this study by choosing a distant branch of the medial cerebral artery as suggested by Ebinger et al. (2010). But using AIF-derived parameters is still afflicted with some uncertainty. Additionally, a rater bias cannot be excluded. But a rater bias does not seem particularly likely, as there was only one rater and definition of the AIFs followed strict criteria (cf. Section 2.2.3).

4.2.1 Group Comparisons

There is no significant group difference between gCIS and gMS with respect to both, rCBV and rCBF, for each respective ROI, suggesting at first glance that there is no real difference in perfusion between both groups. The literature on perfusion changes in MS is heterogeneous, with respect to methods as well as with respect to results.

Comparing subgroups with respect to cerebral blood volume (CBV)

Peruzzo et al. (2012) found a significant reduction in CBV in cortical lesions compared with NAGM in RRMS patients. Adhya et al. (2006) found reduced CBV values in NAWM in RRMS patients compared to healthy controls. Papadaki et al. (2012) found comparable CBV values in RRMS patients and healthy controls. Inglese et al. (2007) also found no difference between RRMS patients and healthy controls with respect to CBV. However, Papadaki et al. (2012) found elevated CBV values in CIS patients compared to RRMS patients. But it should be kept in mind that subtle differences between the two subject groups might simply be obscured by the inherent methodological uncertainties. Overall, the results presented here most likely support the findings of Papadaki et al. (2012) and Inglese et al. (2007).

Comparing subgroups with respect to CBF

Findings on CBF are more homogeneous, as most studies found a reduction in CBF when comparing patients to healthy controls. Two studies found a significant reduction in CBF for the nucleus caudatus and the thalamus in CIS patients compared to healthy controls (Papadaki et al. 2012; Papadaki et al. 2014b). However, there was no difference between CIS patients and healthy controls with respect to CBF values in the putamen and the NAWM (Papadaki et al. 2012). Using arterial spin labelling (ASL), Rashid et al. (2004) found reduced perfusion in CIS patients compared to healthy controls in both cortical and deep GM. Hojjat et al. (2016) found a significant reduction in CBF values in cognitively impaired RRMS patients compared to healthy controls in several cortical regions. Hojjat et al. (2016) also found a significant reduction in several deep grey matter regions including the thalamus and the putamen. Debernard et al. (2014) found reduced CBF values in RRMS patients compared to healthy controls in several cortical areas and deep grey matter as well. Papadaki et al. (2012) found significantly decreased CBF

values in all deep GM in RRMS patients compared to healthy controls. Inglese et al. (2007) also found CBF values in the thalamus, the putamen and the caudate nuclei to be reduced in RRMS patients compared with healthy controls. Conversely, Varga et al. (2009) only found a significant reduction in CBF in the putamen of RRMS patients in comparison to healthy controls, but not for the rest of the deep GM. Using ASL, Rashid et al. (2004) found reduced perfusion in RRMS patients compared to healthy controls in both cortical and deep GM. Ge et al. (2005b) also found reduced CBF in lesions and NAWM in RRMS patients compared with healthy controls. Papadaki et al. (2012) found significantly decreased CBF values in NAWM in RRMS compared to healthy controls. Adhya et al. (2006) and Law et al. (2004) also found reduced CBF in NAWM in RRMS patients compared with healthy controls. But conversely, Rashid et al. (2004) also found elevated perfusion in cerebral white matter (WM) in RRMS patients compared to healthy controls using ASL. Varga et al. (2009) found RRMS patients to show significantly reduced CBF values in the putamen in comparison with CIS patients. Similarly, Papadaki et al. (2012) found RRMS patients to show significantly reduced CBF values in NAWM and deep GM compared to CIS patients, with the exception of the caudate nuclei. Papadaki et al. (2012) even found that there is no overlap in the distribution of CBV between CIS and RRMS patients, even after correcting for disease duration, EDSS score, T1-weighted lesion volume and T2w lesion volume.

In summary, all existing studies found reduced CBF values in MS patients, regardless of the subtype. The findings of this study neither argue for such a difference in CBF between patients and healthy controls, nor against it. If CBF was unaffected in both subgroups of subjects, there would be no difference. But equally, there would be no difference between both patient subgroups, if CBF was affected to a similar degree in both subgroups. Since additional to all the studies, which found a decrease in CBF in MS patients compared to healthy controls, there is considerable evidence that CIS patients already show signs of demyelination and diffuse axonal damage as well (Iannucci et al. 2000; Brex et al. 2001; Miller et al. 2005a), the most likely interpretation of the presented data is that the deficit in rCBF is similar in both subgroups. But in direct comparison several studies found a difference between CIS and RRMS patients, suggesting that CBF is more severely affected in RRMS. This is not reflected in the findings of this study. But it should be kept in mind that subtle differences between the two subject groups might simply be obscured by the inherent methodological uncertainties. Moreover, it

should be noted that most of the results detailed above found differences in CBF but not in CBV. While this might simply reflect a real effect, it could equally well reflect the dependence of CBF on a somewhat arbitrarily defined AIF.

Comparing ROIs within subject subgroups

Even though there is no difference between the two subgroups for any ROI, there are differences between several pairs of ROIs within each group. Within both groups, there is a significant difference for each pair of a grey matter ROI (GMCort, THAL, PUT) and WMROI, respectively. This is expected considering the different structure of grey matter and white matter. There is a significant difference between PUT and THAL for rCBV in gCIS and gMS and for rCBF only in gCIS. That the difference in rCBF between THAL and PUT only exists for gCIS could suggest that rCBF is affected to the same degree in THAL and PUT in gMS, but not in gCIS. This finding is explicitly consistent with the results and the idea of Varga et al. (2009) that perfusion change is an ongoing process in MS, which reaches the putamen before it reaches the thalamus. Such a spreading perfusion change would result in exactly the pattern of differences in rCBF between THAL and PUT reported above. But Papadaki et al. (2012) found that both, the putamen and the thalamus displayed reduced CBF values in RRMS patients compared to CIS patients, which seems to be incompatible with the findings discussed above. However, it should be noted that these results could also be consistent with the presented findings, as a difference between THAL and PUT does not reflect whether ROI are affected, but whether they are affected to a different degree. The idea that rCBF is the first parameter to be affected, is in line with the findings of Law et al. (2004) and Varga et al. (2009), who found a difference in CBF between different groups of subjects, but not in CBV. Yet, since this study comprises only cross-sectional data and the gMS patients are not the ones who were in gCIS before, the evidence in support of a dynamically spreading change in perfusion remains circumstantial. And it should also be kept in mind that the results presented here are equally open to the interpretation that there is no difference between THAL and PUT in gMS, because both regions are unaffected in gMS, while one of them is affected in gCIS.

4.2.2 Correlations

A multiple regression model comprising all ROIs with respect to either rCBV or rCBF, as well as age, gender and disease duration was used to try to explain the variation in NBV and EDSS, respectively. NBV was chosen as the target variable because brain volume loss is considered to be a sensitive measure of neurodegeneration (Zivadinov and Bakshi 2004; Zivadinov et al. 2008; Barkhof et al. 2009). The extent of (early) brain volume loss has also been shown to be a predictor of more severe progression in terms of cognitive impairment and disability in MS patients (Bermel and Bakshi 2006; Simon 2006; Amato et al. 2007; Minneboo et al. 2008; Fisniku et al. 2009; Filippi and Rocca 2011; Zivadinov et al. 2013b; De Stefano et al. 2014; Jacobsen et al. 2014). EDSS was chosen as a comprehensive marker of clinical affection. The resulting adjusted R^2 are comparatively bad, suggesting the model does not capture most of the systematic variation in NBV and EDSS, respectively. However, this is only mildly relevant, as the purpose is to simultaneously analyse correlations – and not to find the best predictors for NBV and EDSS, respectively.

On a note of caution, it should be noted that brain volume loss has been found in various studies to proceed at a rate of approximately 0.5 – 1.05% per year in MS patients and at a rate of approximately 0.1 – 0.3% per year in healthy individuals (Simon 2006; Fotenos et al. 2008; Fisher et al. 2008; Barkhof et al. 2009; De Stefano et al. 2010; De Stefano et al. 2014; Vollmer et al. 2015). However, SIENAX can reliably (Smith 2002; Sormani et al. 2004; Anderson et al. 2006; Smith et al. 2007) estimate brain volume with an accuracy of 0.5 – 1% (Smith et al. 2001; Smith 2002; De Stefano et al. 2007). As the enrolled CIS patients and most of the enrolled MS patients find themselves at the beginning of the course of the disease, the brain volume loss attributable to MS will probably be small. Therefore the brain volume loss due to the disease is expected to be only in the range of the accuracy of the method. Also, considering that likely only a fraction of the change in NBV which are attributable to the disease is caused by perfusion changes, the overall goodness of fit (as represented by adjusted R^2) is expected to be comparatively small even in ideal situations. This does not invalidate the approach as a means of simultaneously examining correlations.

Based on the group comparisons there is no a priori justification for excluding specific ROIs from the regression models. Therefore all ROIs were included. No model selection

algorithms were applied and no further effort was made to optimise the amount of variability in NBV and EDSS explained by the respective model. Adhering to this argument, it would make most sense to include all perfusion parameters in the same model. However, this would likely render all perfusion-related predictors artificially insignificant, as rCBV and rCBF are inherently linearly correlated. Therefore rCBV and rCBF were investigated separately. Generally, it should be kept in mind that existing correlations between any two or more predictors would render all of the respective t-tests insignificant. Also, an existing weak correlation could manifest itself only in rCBF and not in rCBV, if it was amplified by AIF. Conversely, the AIF could blur an existing correlation in the model with respect to rCBV, so that it did not have a visible statistical effect in the model with respect to rCBF anymore.

Predicting Normalized Brain Volume

Neither the model containing rCBV nor the model containing rCBF contributes significantly to explaining the systematic variability in NBV for gMS. For gCIS both models, that containing rCBV as well as that containing rCBF, contribute significantly to explaining the variation in NBV, but with somewhat different predictors. This argues strongly in favour of a systematic difference between gCIS and gMS with respect to the perfusion metrics, even though this difference is not reflected in the simple group comparisons.

In more detail, mean rCBV of GMCort is a significant predictor for NBV in gCIS, but mean rCBF of GMCort is not a predictor for NBV. Conversely, both, mean rCBV of WMROI and mean rCBF of WMROI are significant predictors for NBV in gCIS. Firstly, this indicates that there is no correlation between either rCBV in GMCort and WMROI or rCBF in GMCort and WMROI, as such a correlation would render both predictors insignificant. This is consistent with the study of Varga et al. (2009), who did not find a correlation between NAWM and NAGM in terms of perfusion metrics in CIS patients. Secondly, this indicates that ratio of rCBV and rCBF is not constant over all ROI in gCIS. Assuming this change in ratios is at least partly driven by a change in perfusion, these findings are generally consistent with the studies of Law et al. (2004) and Varga et al. (2009), who both found perfusion changes in CBF but not in CBV. However, it is worth noting that while the findings of this study support the idea of a different development in rCBF and rCBV, there is no information on which of the perfusion

parameters is changing. This study can be interpreted both ways, depending on the sensitivity of NBV to reflect the degree of damage due to the disease. Coming back to the string of arguments presented in Section 4.1, it seems not unlikely that NBV does not (yet) capture the diffuse damage induced by the disease. In this case, it would make perfect sense for the correlation of rCBF and NBV to break down, if rCBF is being affected while NBV is not. This notion is supported by the fact that all correlations break down in gMS, suggesting that the development is towards less association in more severely affected patients. It is also in line with the results of previous studies, which found that rCBF was affected in CIS and RRMS patients, while rCBV remained unaffected (Law et al. 2004; Varga et al. 2009).

For deep GM, only mean rCBV of THAL as well as mean rCBF of THAL for gCIS are significant predictors for NBV. Mean rCBV as well as mean rCBF of PUT are not significant predictors for NBV in gCIS. This argues in favour of a general difference between the perfusion in THAL and PUT. Coming back to the argument that a breakdown of the correlation with NBV represents a further advanced change, the results are in line with the idea of Varga et al. (2009) that the putamen is affected by changes in perfusion before the thalamus. However, the results presented here run counter to those of Papadaki et al. (2014b), who found all CIS patients included in their study to display elevated CBV and reduced CBF in the thalamus compared to healthy controls, suggesting that thalamus perfusion is also already affected in CIS patients. Furthermore, the existing correlation between mean rCBV and rCBF of THAL, respectively, and NBV argues against the idea that there is a significant change in one perfusion metric but not in the other.

Gender is a significant predictor for NBV in gCIS in the model with respect to rCBV, but not in the model with respect to rCBF. Gender is known to have an effect on NBV (Luders et al. 2005), so it would be expected to be a significant predictor. But, more importantly, since both models are based on the same dataset where gender and NBV are concerned, there is no conceivable reason why gender would be correlated to NBV in one case but not in the other. The most likely reason for this behaviour is an existing correlation between gender and another predictor in the model with respect to rCBF. And since all non-perfusion-related predictors are identical in both models, this would have to be a correlation between gender and mean rCBF for at least one of the ROIs, GMCort or PUT, which do not show up as significant predictors. Assuming an

underlying correlation to be responsible for this behaviour, GMCort is the most likely candidate.

DisDur is not a significant predictor of NBV, regardless of the perfusion metric. This is consistent behaviour, since both models represent exactly the same dataset with respect to DisDur and NBV. However, brain volume loss has been shown to proceed at a significantly higher rate per year in RRMS patients than in healthy controls (Simon 2006; Fotenos et al. 2008; Fisher et al. 2008; Barkhof et al. 2009; De Stefano et al. 2010; De Stefano et al. 2014; Vollmer et al. 2015), so a correlation between NBV and DisDur is an expected finding. There are two reasons for the absence of this correlation. Firstly, there could be a correlation between DisDur and mean rCBV or mean rCBF, respectively, for at least one of the ROI, which are not significant predictors. This is possible, but not likely, compare Figure 3.3. Also, Varga et al. (2009) found no correlation between DisDur and either perfusion metrics. Secondly, the reason could simply be that NBV does not (yet) reflect systematic disease-induced brain volume loss in the data underpinning this study, as explained above. This the most likely reason for the absence of a correlation between DisDur and NBV.

Age is a significant predictor in case of rCBF, but not in case of rCBV. This is counterintuitive at first glance, as age is known to be correlated to brain volume loss (e.g. Simon 2006; Fotenos et al. 2008; Fisher et al. 2008; Barkhof et al. 2009; De Stefano et al. 2010; De Stefano et al. 2014; Vollmer et al. 2015). Also, both models are based on exactly the same dataset where age and NBV are concerned, so either age is correlated NBV or it is not. However, it could simply be a statistical effect induced by a weak correlation between age and either of the non-significant predictors in the model with respect to rCBV. If such an existing correlation was sufficiently distorted by the AIFs, age could become a significant predictor in the model with respect to rCBF in turn.

When comparing the respective models for the two subject subgroups, gCIS and gMS, the results convincingly indicate that there is a difference in perfusion between gCIS and gMS. This seems to contradict the results from the simple group comparisons. But it probably only reflects that the differences in perfusion are too subtle and possibly also too local to be captured by simply comparing group medians. However, it is not always straightforward to translate the regression results to differences between the two subject subgroups for specific ROI. In case of PUT, neither mean rCBV nor mean rCBF are correlated with NBV. Superficially this differs from the findings of Varga

et al. (2009) and Papadaki et al. (2012), who found CBF in the putamen to be reduced in RRMS patients compared to CIS patients. But keeping in mind that NBV is not likely to capture the disease-induced change (yet), the absence of a correlation in both models only implies that perfusion in the putamen is affected in both groups. However, it does not imply that putamen perfusion in the respective group, gCIS and gMS, is affected to the same degree. So the results of the presented do not contradict previous findings. In case of THAL, both perfusion metrics are correlated to NBV in gCIS, while none of the perfusion metrics is correlated to NBV in gMS. This could be due to correlations between the perfusion metrics of different ROIs, which only exist in gMS, but it more likely presents a systematic difference with respect to thalamus perfusion between gCIS and gMS. As discussed above, the existing literature is inconsistent a possible difference between CIS patients and RRMS patients in thalamus perfusion, so the results presented here fit in reasonably well. In view of the idea of a spreading change in perfusion (Varga et al. 2009), these findings are consistent with an ongoing process in the thalamus. However, it is impossible to derive only from these results, whether the effect in our models is caused by more severe change in gMS. But accepting the string of arguments presented above that NBV does not (yet) sufficiently reflect the changes induced by the disease, the model results indicate that thalamus perfusion is only affected as the disease progresses as Varga et al. (2009) suggested. The same arguments hold true for the perfusion of WMROI, which exhibits the same behaviour as the perfusion of THAL with respect to our models. There is evidence that perfusion in NAWM is more severely affected in RRMS patients than in CIS patients (Papadaki et al. 2012), which is consistent with the presented findings. However, there is also evidence that WM perfusion is already affected in CIS patients (Papadaki et al. 2012). But if NBV is not capturing the disease-induced damage, an already disturbed WM perfusion should also destroy a correlation between WMROI perfusion and NBV in gCIS. Therefore there is not much support in the results of the regression models for the idea of Varga et al. (2009) that NAWM is the first region to show relevant perfusion changes. But to put that into perspective, most studies found changes (only) in specific subregions of NAWM. Since WMROI also comprises the comparatively large part of still only lightly affected tissue, the early change in WM perfusion might be concealed in the regression models used in this study.

Interestingly, pooling all data results in a model which comprises more significant in-

dividual predictors for both perfusion metrics, rCBV and rCBF, than a simple union of both sets of significant predictors from the subgroup models would contain. This suggests that pooling the data either uncovers more correlations between the respective predictors and NBV, or that pooling distorts existing correlations between predictors enough to make these predictors assume statistical significance. Superficially, this also seems similar to the results of Varga et al. (2009), who did not find a correlation between NAWM and NAGM perfusion metrics for either CIS patients or MS patients, but found such a correlation in the pooled data of all patients. However, the findings presented here run counter to the findings of Varga et al. (2009) on closer inspection, as more pronounced correlations between the predictors would lead to a smaller number of significant predictors in the regression model for all pooled data.

Generally, the results presented here argue in favour of a difference in perfusion metrics and/or internal correlations between gCIS and gMS, which is in line with the discussed earlier studies, which is in line with the results of studies, which directly compared CIS patients and RRMS patients (Varga et al. 2009; Papadaki et al. 2012). But it should be kept in mind that those differences were derived from group comparisons, not from the existence of correlations. In terms of correlations, several studies found an inverse correlation between verbal memory and executive motor task function and elevated CBV values in NAWM and deep grey matter (Papadaki et al. 2012; Papadaki et al. 2014a; Papadaki et al. 2014b). Additionally, Inglese et al. (2007) found that both, CBV and CBF, correlated with the fatigue score of RRMS patients. None of the existing perfusions studies using DSC imaging found relevant correlations between perfusion metrics and any volumetric parameters such as NBV. A possible reason for this is the use of different statistical methods to investigate possible correlations. Interestingly, Marshall et al. (2014) not only found an inverse correlation between GM cerebrovascular reactivity and lesion volume, but also a correlation between GM cerebrovascular reactivity and GM atrophy using ASL imaging. So there might be other methodically issues apart from statistics, which should be investigated in future studies.

Predicting Expanded Disability Status Scale

Neither the model with respect to rCBV nor the model with respect to rCBF contributes significantly to explaining the systematic variability in EDSS for either gCIS or gMS, respectively. Pooling all data does not improve the models in terms of significant pre-

dictors. This is in line with the results of Varga et al. (2009) and Papadaki et al. (2012), who did not find any correlations between perfusion metrics EDSS scores for either CIS or RRMS patients. However, the results presented here do not necessarily mean that there is no correlation between changes in perfusion metrics and progressing disability. Although EDSS is a widely used measure for clinical disability, it is comparatively unsuitable as a measure of clinical disease progression in statistical studies as it is not continuous. With regard to content, using the EDSS it is not possible to discriminate disability on any finely grained scale, which also makes its suitability in the context of this and other comparable studies questionable. Furthermore, using a (single) number to quantify the degree to which a patient is clinically affected might seem absolutely objective, but it is not. For one thing, the EDSS does not at all reflect, which part of the nervous system is affected. Therefore it is only marginally useful in trying to attribute effects to a specific region and/or system. Furthermore, the EDSS attributes disproportionately high weight to the ability to walk. This makes perfect sense in a clinical context when judging disability in its relevance to daily life. But for generally analysing the progress of disability in all different functional systems and the potentially regional influences on this change, it would make more sense to weigh every all functional systems evenly - or alternatively use measures, which are less compressed than EDSS. Additionally, two patients can score the same EDSS while one is moderately to severely affected in one functional system and the other is only slightly affected in several functional system. Again, this makes sense in a clinical context with respect to functional disability. When analysing the different functional systems, both, severity of the disability and the affected functional system is most relevant information, which the EDSS cannot always provide. For all these reasons, the EDSS is rather unlikely to sufficiently represent the effect which should be investigated. Therefore the lack of any significant correlation in all models with respect to EDSS does not indicate there is no correlation between the progress of disability and a change in perfusion metrics in defined regions of the brain.

4.2.3 Responsible mechanisms

The mechanisms responsible for the change in perfusion in CIS and MS patients are not yet fully understood. Ge et al. (2005b) and Wuerfel et al. (2007) suggested vasodi-

lation and/or angiogenesis induced by inflammation as the mechanism responsible for elevated CBV in lesions of CIS patients. Peruzzo et al. (2012) found some instances of inflammation-induced hyperperfusion in cortical lesions, but mostly they found cortical lesions to display a decrease in perfusion. They suggested a reduction of metabolism because of the loss of cortical neurons as a main reason for this reduction in perfusion (Peruzzo et al. 2012). Contrary to that, Saindane et al. (2007) suggested that the change in perfusion is driven by primary ischemia rather than decreased perfusion induced by axonal damage. They based their suggestions on the finding that decreased CBF positively correlated with mean diffusivity but not with fractional anisotropy (Saindane et al. 2007). Moreover, Debernard et al. (2014) found that RRMS patients who did not yet show any volume loss indicative of axonal loss already displayed reduced cortical and deep gray matter CBF. This does not discount neuronal metabolic dysfunction as a reason for changes in perfusion, but it argues against the idea of axonal loss being the reason for this change in metabolism.

However, changes in perfusion are not limited to lesions, but are a widespread finding in MS. There is no evidence that structural abnormalities of small blood vessels such as microthrombosis are a common occurrence in MS lesions (De Keyser et al. 2008), more or less discounting this as the mechanism responsible for decreased perfusion in cortical lesions. Varga et al. (2009) suggested that a continuum of tissue perfusion decrease due to inflammatory processes is responsible for the differences in perfusion between CIS and MS patients, spreads from white to grey matter as the disease progresses. Both assumptions were in some fashion corroborated by Lucchinetti et al. (2011), who found widespread cortical and even meningeal inflammation in cerebral biopsies of early MS patients. Data from two further studies (Law et al. 2004; Papadaki et al. 2014a; Papadaki et al. 2014b) supported the view that hemodynamic change is induced by diffuse and widespread inflammatory processes in the respective brain regions. Papadaki et al. (2012) suggested that vasodilatation and/or angiogenesis induced by inflammation are likely also responsible for elevated CBV in several normal appearing regions of the brains of CIS patients. However, the results of Marshall et al. (2014) showed a reduced dilatatory capacity of cerebral arterioles in response to vasomotor stimulation MS patients compared to healthy controls using ASL imaging. This indicates that vasodilatation might not be a key player in perfusion change in MS patients, but at the same time it emphasises that cerebrovascular reactivity is affected in MS. Vessel pathology has

received increasing attention as a potential reason for diffuse pathology in the last couple of years. Several studies found enhanced levels of the potent vasoconstrictive peptide ET-1 in the blood and some also in the cerebrospinal fluid in MS patients (Speciale et al. 2000; Haufschild et al. 2001; Pache et al. 2003). Moreover, D’haeseleer et al. (2013) found a 20% reduction of CBF in MS patients compared to healthy controls to be reversible after administration of a ET-1 receptor antagonist, which strongly indicates a causal relationship. D’haeseleer et al. (2015) also deduced that the release of ET-1 in the cerebral circulation of MS patients is likely responsible for inducing arteriolar vasoconstriction.

Generally, there is nothing in the data underpinning the study presented here to indicate any of these assumptions could be unfounded. In view of previous findings, inflammation and also ET-1 release still seem the most likely reasons for hemodynamic changes in CIS and MS patients. There is some evidence in our regression models that the change in cerebral perfusion in MS is an ongoing process, which affects almost all of the ROIs. This supports the theory of Varga et al. (2009), but not all of the findings presented here are consistent with that theory.

4.3 Conclusions and Outlook

Among the limitations of this study are its cross-sectional nature and the use of the comparatively coarse measures of disease severity and brain damage, EDSS and NBV. The use of DSC imaging also comes based on some assumptions and models. And while manual definition of the AIF is superior to automatic determination, basing the definition of the AIF on only four voxels is also a potential source of uncertainty.

In conclusion, there were no simple group differences in perfusion metrics between CIS and MS patients for the various white matter and grey matter ROIs. However, there is some evidence for existing differences in perfusion not only between CIS and MS patients, but also between several different (grey) matter ROIs within each group. Especially, the putamen displays disparate behaviour. The results of this study indicate that there are relevant hemodynamic changes early on in the course of the disease, as CIS patients show a different behaviour from the cohort of RRMS patients, even though the RRMS cohort mostly features comparatively short individual disease duration. Generally, the findings of this study suggest that the underlying mechanism responsible for

hemodynamic changes is diffuse rather than focal and seems to be subject to some sort of (temporal) evolution, as changes do not seem to occur simultaneously in all regions in the brain. But because of the limited accuracy of calculating NBV, the results are open to several interpretations with respect to the sequence in which the different regions are affected. The results further suggest that hemodynamic changes might be a much more sensitive marker than previously thought. Moreover, this study indicates that there is likely little merit in simply comparing averaged perfusion metrics per ROI as this method lacks the necessary sensitivity. The crucial information is most likely to be had from simultaneous correlation analyses.

Ideally, longitudinal studies with higher resolution ROIs are needed to further investigate the role of hemodynamic change at different stages of the disease and to evaluate the changing patterns of correlations. Known correlations between hemodynamic change and higher brain functions and neuropsychological functions, such as parts of the memory and fatigue, should be more thoroughly investigated with respect to a potential causal relationship. Considering that possibly a continuous process is driving the change in perfusion, these correlations assume a new significance not only with respect to reliable markers for disease progression but also with respect to possible predictors of disease progression. But to fully utilise these presumed relationships, it is necessary to further scientific understanding of the mechanisms responsible for hemodynamic change, particularly on a microscopic scale.

Finally, perfusion metrics deserve some attention with respect to a pharmacological point of view. Not least, changes in perfusion metric show promising potential as a very sensitive marker for drug efficacy in MS treatment. But even more importantly, the potential response to treatment merits scientific efforts. If hemodynamic change is a slowly evolving process which starts early on in the course of the disease, it might present a suitable drug target. This is especially important, as the axonal damage which can occur in the wake of hypoperfusion even makes it a potential drug target in the so far elusive progressive forms of MS.

5. Summary

A total of 106 untreated patients aged between 18 and 65, who had been diagnosed with clinically isolated syndrome (CIS) or relapsing-remitting multiple sclerosis (RRMS) were prospectively recruited, of which 95 patients were finally included in the study presented here. All patients were evaluated according to the Expanded Disability Status Scale (EDSS) and underwent MRI including perfusion imaging. Normalized brain volume (NBV), average relative cerebral blood volume (rCBV) and average relative cerebral blood flow (rCBF) were obtained for non-lesional tissue of the thalamus, the putamen, the cortical grey matter and the white matter. Subjects were dichotomised according to their diagnosis.

The two groups are similar with respect to demographic and disease characteristics except for a small difference in disease duration. There is no significant difference between both subject subgroups with respect to regional volumes or perfusion parameters, respectively, in any of the regions of interest (ROIs). The relations between pairs of ROIs within each subject subgroup are heterogeneous. Perfusion parameters are not correlated with EDSS in any of the two subgroups. Perfusion parameters are not correlated with NBV in the subgroup of RRMS patients. Mean rCBV of the GM, of the thalamus and of the WM is correlated with NBV for the subgroup of CIS patients, but only rCBF of the thalamus and of the WM is correlated to NBV.

The results from the regression models indicate that there is a difference in local perfusion between CIS and RRMS patients, even though the RRMS cohort mostly features comparatively short individual disease duration, which is largely consistent with the literature. The fact that these differences are not reflected in the results of the simple group comparisons is likely due to its comparatively small magnitude. Especially, this study indicates that there is likely little merit in simply comparing averaged perfusion metrics per ROI as this methods lacks the necessary sensitivity. The crucial information

is most likely to be had from simultaneous correlation analyses. Beside the comparatively coarse measures of disease severity and brain damage, EDSS and NBV, this study is limited by its cross-sectional nature.

In summary, the results of this study suggest that the underlying mechanism responsible for hemodynamic changes is diffuse rather than focal and is subject to some sort of (temporal) evolution. But because of the limited accuracy of calculating NBV, the results are open to several interpretations with respect to the sequence in which the different regions are affected. Ideally, longitudinal studies with higher resolution ROIs are needed to further investigate the role of hemodynamic change at different stages of the disease and to evaluate the changing patterns of correlations.

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