Evaluation of cerebral perfusion with transcranial Doppler and near-infrared spectroscopy in patients with multiple sclerosis

Thesis neergelegd voor het behalen van de graad van Master in de Geneeskunde

Ilse Peeters

Academiejaar 2014 - 2015

Promotor: Dr. Miguel D’haeseleer
Co-promotor: Prof. Dr. Jacques De Keyser
TABLE OF CONTENTS

ABSTRACT .................................................................................................................................................. 3
Objective ....................................................................................................................................................... 3
Methods ......................................................................................................................................................... 3
Results .......................................................................................................................................................... 3
Conclusion .................................................................................................................................................... 4

METHODS .................................................................................................................................................. 5
Subjects ......................................................................................................................................................... 5
Procedures ................................................................................................................................................... 5
Statistical analysis ....................................................................................................................................... 7

RESULTS ..................................................................................................................................................... 7

DISCUSSION ............................................................................................................................................... 9

ACKNOWLEDGMENTS .......................................................................................................................... 12

TABLE OF TABLES

Table 1: Demographics of MS patients and control subjects ................................................................. 8
Table 2: Baseline MCA TCD results in MS patients and controls* .......................................................... 8
Table 3: Effect of bosentan on TCD and NIRS parameters in MS patients* ......................................... 8
ARTICLE

Evaluation of cerebral perfusion with transcranial Doppler and near-infrared spectroscopy in patients with multiple sclerosis

Ilse Peeters¹, Jacques De Keyser¹,², Miguel D’haeseleer¹,³

¹ Department of Neurology, Universitair Ziekenhuis Brussel, Center for Neurosciences, Vrije Universiteit Brussel (VUB), Brussels, Belgium.
² Department of Neurology, Universitair Medisch Centrum Groningen, Groningen, The Netherlands.
³ Nationaal Multiple Sclerose Centrum, Melsbroek, Belgium

ABSTRACT

Objective Recent magnetic resonance imaging (MRI) studies have demonstrated that patients with multiple sclerosis (MS) have a globally decreased cerebral blood flow (CBF), which can be restored by interfering with the endothelin-1 (ET-1) system, and reduced cervical arterial blood flow velocities. The aim of this study is to evaluate cerebral perfusion with transcranial Doppler (TCD) and near-infrared spectroscopy (NIRS), which are non-invasive bedside techniques, in patients with MS.

Methods Fifteen patients with MS and 15 age and sex-matched controls underwent TCD in order to evaluate middle cerebral artery (MCA) blood flow velocity (BFV), pulsatility index (PI) and resistance index (RI). NIRS was performed simultaneously to measure absolute cerebral tissue oxygenation (SctO₂) in the frontal cortex. The full procedure was repeated in the patients with MS (not in controls) 4 hours after the intake of the ET-1 receptor antagonist bosentan.

Results Mean BFV was comparable between the MS and control group. PI (1.04 ± 0.23 versus 0.87 ± 0.10; P = 0.015) and RI (0.61 ± 0.07 versus 0.56 ± 0.03, P = 0.045) were significantly elevated in patients with MS, as compared to controls. SctO₂ was comparable between both groups. After administration of bosentan, mean BFV significantly decreased in the patients with MS, as compared to baseline (48.9 ± 16.7 versus 53.7 ± 16.5 cm/s, P = 0.015), whereas PI, RI and SctO₂ remained at the same level.
**Conclusion** The elevated PI and RI in the MCA of patients with MS suggest an increased distal vascular resistance to blood flow. These findings support the hypothesis that cerebral hypoperfusion in MS is mediated by ET-1 induced arteriolar vasoconstriction. TCD and NIRS appear to be less sensitive than MRI to detect CBF changes in MS.

**Keywords** Multiple sclerosis; Transcranial Doppler; Near-infrared spectroscopy; Bosentan; Pulsatility index; Cerebral blood flow

**INTRODUCTION**

Multiple sclerosis (MS) is a chronic inflammatory demyelinating and degenerative disorder of the central nervous system. Worldwide up to 2.5 million people are estimated to be affected, but the exact causative mechanism remains unknown. Most patients start with a relapsing-remitting pattern in which episodes of neurological dysfunction, evolving over days to weeks, interchange with periods of at least partial recuperation and stabilization. Autoimmune T-cell mediated responses play an important role in the development of hallmark focal inflammatory demyelinating lesions, which form the pathological substrate for the relapses. In contrast, patients may also experience a continuous downhill course with few or no relapses, which can occur from disease onset but more commonly installs after several years of relapsing-remitting disease. Axonal degeneration is the main determinant of the progressive phase but corresponds poorly with inflammatory disease activity.

Recent dynamic susceptibility-weighted (DSC) and arterial spin labelling (ASL) magnetic resonance imaging (MRI) studies have demonstrated that there is a decreased cerebral blood flow (CBF), as compared to control subjects, throughout the normal appearing white matter (NAWM), deep and cortical grey matter, and cerebellar hemispheres of patients with relapsing-remitting and progressive MS. A similar CBF reduction was found in the NAWM and deep grey matter of individuals with clinically isolated syndromes suggestive for MS. In addition, total cervical arterial blood flow velocities appear to be reduced in patients with MS when evaluated with phase-contrast MRI. These data indicate that widespread cerebral hypoperfusion is an integral part of MS pathology, but its precise role remains to be determined.

Magnetic resonance spectroscopy and combined diffuse tensor and perfusion MRI findings argue against the hypothesis that reduced CBF in MS is just a secondary phenomenon to impaired axonal integrity with decreased metabolic demands. In contrast, enhanced
expression of the transcription factor hypoxia-inducible factor-1α and its target genes, a pathway involved in the homeostatic response to tissue hypoxia, suggest that there might be a relevant ischemia in the NAWM of patient with MS. It has recently been demonstrated that elevated levels of the potent vasoconstrictive compound endothelin-1 (ET-1) are released in the cerebral circulation of individuals with MS, likely by reactive astrocytes, and moreover, that the reduced CBF can be restored by administering the non-selective ET-1 receptor antagonist bosentan.(10)

The aim of this study was to evaluate the arterial intracranial circulation in patients with MS by means of transcranial Doppler sonography (TCD), which is an easy applicable and non-invasive bedside technique. Near-infrared spectroscopy (NIRS) was performed to measure brain tissue oxygen saturation.

METHODS

Subjects
Fifteen patients with MS (six with relapsing-remitting, seven with secondary progressive and two with primary progressive disease), according to the 2010 revised McDonald criteria,(1) and 15 healthy controls, matched for age and gender, were included in this study. All MS patients were clinically stable without evidence of an exacerbation within the three months prior to inclusion. MS patients with a known contraindication for bosentan (liver dysfunction, use of cyclosporin A, allergy to bosentan) were excluded. The ethics committee of Universitair Ziekenhuis Brussel (Belgium) approved the study and a written informed consent was obtained from all subjects.

Procedures
TCD and NIRS examinations were performed simultaneously on each subject (MS patients and controls) in supine position. They were allowed to rest in a dimmed and quiet room, laying comfortably and breathing room air. All participants were asked to abstain from caffeine-containing products, food intake and cigarette smoking for at least two hours before the examination, since this could temporary influence cerebral perfusion.(11,12)

The M1 segment of the middle cerebral arteries (MCA) was bilaterally insonated through the temporal bone window. A TCD device (SONARA TCD System, Natus Medical Incorporated, Pleasanton, USA) was used with 2 MHz probes mounted on a head frame to ensure a constant
angle of insonation during the procedure. MCA waveforms were identified at a depth range of 40–60 mm. A stable forward unidirectional waveform with good intensity was selected. Mean, systolic and diastolic blood flow velocities (BFV) were bilaterally recorded six times with an interval of 1 minute between each measurement. Pulsatility index (PI) was calculated according to the formula of Gosling as follows: PI = (systolic BFV - diastolic BFV) / mean BFV.(13) Cerebrovascular resistance index (RI) was calculated according to Tranquart and colleagues: RI = (systolic BFV – diastolic BFV) / systolic BFV.(14)

Heart rate (HR), systolic blood pressure (BP), diastolic BP and mean arterial BP (MABP) were continuously assessed by a non-invasive, beat-to-beat finger plethysmograph (Nexfin, BMEYE, Amsterdam, The Netherlands). The finger cuff was preferably applied to the middle finger or index of the left hand and was calibrated with the hand placed at the heart level on the chest. Once the system was calibrated, patients could choose their hand position.

Absolute cerebral tissue oxygen saturation (SctO₂) in the frontal cortex was continuously monitored by the FORE-SIGHT cerebral oximeter (CAS Medical Systems Inc, Branford CT, USA), a technology based on NIRS. NIRS is a safe, non-invasive bedside technique based on the principle that blood contains haemoglobin in two forms, oxygenated haemoglobin (HbO₂) and deoxygenated haemoglobin (Hb), which absorb infrared light in different ways according to a modified Beer-Lambert law. Details about the technical background of NIRS are described by Scheeren et al.(15) SctO₂ is defined as the ratio of HbO₂ to total haemoglobin (tHb) concentrations in the cerebral tissue. It can be represented as follows: SctO₂ = HbO₂ / (Hb + HbO₂). The used device is a continuous wave spatially resolved near infrared spectrometer that measures absolute SctO₂ without the need for calibration. Two large disposable sensors were placed concurrently on the right and left forehead, after cleaning the skin with an alcohol-based swap and avoiding positioning on hair or air frontal sinus. Each sensor contains a 4-wavelength (690, 779, 808, and 850 nm) laser source and two photodiode detectors, i.e., a scalp and a brain detector. All SctO₂ measurements were obtained from right and left sensors continuously over 60 seconds, with a device refresh rate every 2 seconds.

In the MS patients the full TCD/NIRS procedure was repeated on the same day and under the same conditions as described above, 4 hours after the oral intake of 62.5 mg bosentan (when peak plasma concentrations are expected).(16)
**Statistical analysis**

All statistical analyses were performed with SPSS software (Version 17.0; SPSS, Inc., Chicago, IL, USA). Mann Whitney U of Wilcoxon signed rank tests were used to compare group differences where appropriate. All reported P values are two-tailed and considered statistically significant at the 0.05 level.

**RESULTS**

Demographics of all participants are summarized in Table 1. Baseline BFV values were comparable between the MS and control group. PI and RI were significantly increased in the patients with MS, as compared to the controls (Table 2). There were no significant differences between both groups (controls versus MS) for HR (62.5 ± 5.7 versus 67.5 ± 10.2 beats per minute; P = 0.250), systolic BP (134.4 ± 13.3 versus 136.3 ± 19.6 mmHg; P = 0.713), diastolic BP (76.5 ± 8.5 versus 74.1 ± 9.4 mmHg, P = 0.486), MABP (99.0 ± 10.7 versus 98.0 ± 12.7 mmHg, P = 0.838). Frontal cortex SctO₂ was comparable between both groups (71.0 ± 2.4 versus 73.3 ± 4.3 %; P = 0.230).

After administration of bosentan, mean BFV in the MCA of patients with MS significantly decreased, as compared to baseline. PI and RI did not change significantly; neither did SctO₂ (Table 3). No significant difference in systolic BP (80.6 ± 27.0 versus 90.1 ± 28.9; P = 0.112), diastolic BP (32.1 ± 12.63 versus 34.64 ± 12.21; P = 0.061) and MABP (97.6 ± 14.5 versus 98.0 ± 12.7 mmHg, P = 0.933) was seen, whereas HR was significantly increased (76.7 ± 12.8 versus 67.5 ± 10.2 beats per minute; P = 0.001) compared to baseline.

TCD was repeated in five healthy controls under the same conditions as the first examination, without the administration of bosentan, to control intra-observer variability. There were no significant differences between both TCD examinations for mean BFV (49.0 ± 4.2 versus 47.4 ± 5.1 cm/s, P = 0.424), systolic BFV (77.2 ± 7.7 versus 73.9 ± 6.8 cm/s, P = 0.387), diastolic BFV (31.9 ± 3.48 versus 31.1 ± 4.5 cm/s, P = 0.616), RI (0.58 ± 0.04 versus 0.58 ± 0.05, P = 0.885) and PI (0.92 ± 0.13 versus 0.91 ± 0.14, P = 0.896).
**Table 1: Demographics of MS patients and control subjects**

<table>
<thead>
<tr>
<th></th>
<th>MS (n=15)</th>
<th>Controls (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex; number</td>
<td>9</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Mean age (SD); years</td>
<td>52 (9)</td>
<td>52 (9)</td>
<td>-</td>
</tr>
<tr>
<td>Median EDSS score (IR)</td>
<td>3.5 (4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Median disease duration (IR); years</td>
<td>11 (16)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

MS = multiple sclerosis, EDSS = Expanded Disability Status Scale, IR = interquartile range.

**Table 2: Baseline MCA TCD results in MS patients and controls***

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BFV (cm/s)*</td>
<td>53.7 ± 16.5</td>
<td>49.9 ± 7.1</td>
<td>0.512</td>
</tr>
<tr>
<td>Systolic BFV (cm/s)*</td>
<td>90.1 ± 28.8</td>
<td>76.8 ± 11.1</td>
<td>0.250</td>
</tr>
<tr>
<td>Diastolic BFV (cm/s)*</td>
<td>34.6 ± 12.2</td>
<td>33.4 ± 5.4</td>
<td>1.000</td>
</tr>
<tr>
<td>PI°</td>
<td>1.04 ± 0.23</td>
<td>0.87 ± 0.10</td>
<td>0.015</td>
</tr>
<tr>
<td>RI°</td>
<td>0.61 ± 0.07</td>
<td>0.56 ± 0.03</td>
<td><strong>0.045</strong></td>
</tr>
</tbody>
</table>

MCA = middle cerebral artery, TCD = transcranial Doppler, MS = multiple sclerosis, BFV = blood flow velocity, PI = pulsatility index, RI = resistance index, SctO₂ = absolute cerebral tissue oxygen saturation.

* Data are expressed as mean ± SD
○ Recorded at the M1 level of the middle cerebral artery

**Table 3: Effect of bosentan on TCD and NIRS parameters in MS patients***

<table>
<thead>
<tr>
<th></th>
<th>Before bosentan</th>
<th>After bosentan</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BFV (cm/s)*</td>
<td>53.7 ± 16.5</td>
<td>48.9 ± 16.7</td>
<td><strong>0.015</strong></td>
</tr>
<tr>
<td>Systolic BFV (cm/s)*</td>
<td>90.1 ± 28.8</td>
<td>80.6 ± 27.0</td>
<td>0.112</td>
</tr>
<tr>
<td>Diastolic BFV (cm/s)*</td>
<td>34.6 ± 12.2</td>
<td>32.1 ± 12.6</td>
<td>0.061</td>
</tr>
<tr>
<td>PI°</td>
<td>1.04 ± 0.23</td>
<td>1.02 ± 0.16</td>
<td>0.776</td>
</tr>
<tr>
<td>RI°</td>
<td>0.61 ± 0.07</td>
<td>0.61 ± 0.06</td>
<td>0.972</td>
</tr>
<tr>
<td>Frontal cortex SctO₂ (%)</td>
<td>73.2 ± 4.3</td>
<td>72.9 ± 3.3</td>
<td>0.345</td>
</tr>
</tbody>
</table>

TCD = transcranial Doppler, NIRS = near-infrared spectroscopy, MS = multiple sclerosis, BFV = blood flow velocity, PI = pulsatility index, RI = resistance index, SctO₂ = absolute cerebral tissue oxygen saturation.

* Data are expressed as mean ± SD
○ Recorded at the M1 level of the middle cerebral artery
DISCUSSION

Recent DSC and ASL MRI data have associated MS with a globally reduced CBF, regardless of the clinical subtype (relapsing and progressive disease) and which is already present at the time of the first clinical manifestations.\(^4\to7,10\) This is the first study investigating cerebral perfusion in patients with MS by means of combined TCD and NIRS. The most important finding is that MCA PI and RI were significantly elevated in patients with MS as compared to healthy people, suggesting an increased distal resistance to blood flow.\(^17,18\) PI and RI both represent the pulsatility of Doppler velocity waveforms with, as can be seen above, the denominator as the only difference in the respective formulas.\(^19\) Whereas RI is easier calculated and therefore preferable when waveforms are noisy,\(^19\) we expect PI to have a more linear relation to vascular resistance than RI.\(^20\to22\) Our results are in accordance with the hypothesis that the increased levels of ET-1 in the cerebral circulation of individuals with MS induce long-lasting vasoconstriction of the precapillary arterioles.\(^10\) Furthermore, it has been shown that these arterioles have an impaired dilatatory capacity in response to hypercapnic vasomotor stimulation.\(^23\)

An increased precapillary vascular resistance, evidenced by elevated PI and RI in the MCA, would be expected to decrease MCA mean BFV.\(^19,24\) However, mean BFV were comparable between MS patients and healthy controls in this study. TCD is unable to exactly quantify absolute blood flow volume per time unit due to an unknown vessel diameter, but a velocity change can be proportionate to the change in CBF.\(^25,26\) The CBF reduction observed in MS patients with perfusion-weighted MRI, as compared to control subjects is approximately 20\%,\(^10\) which could be too small to alter TCD measured mean BFV. The same discrepancy between PI and mean BFV was observed in patients with amnestic mild cognitive impairment in a study by Viola et al.\(^27\) Actually, several studies show the exact relationship between velocity and pulsatility changes is complex.\(^22,28\)

Cerebral hypoperfusion in MS would be expected to lead to decrease in HbO\(_2\) delivery and subsequent decrease in NIRS-obtained SctO\(_2\). In contrast, in this study no significant difference in SctO\(_2\) between MS patients and controls was found. The main reason for this discrepancy might be that SctO\(_2\) represents the ratio of HbO\(_2\) to tHb, which is in his turn the sum of Hb and HbO\(_2\). NIRS takes a mixture of the entire microvascular system (i.e., arterioles, capillaries and venules) into account, \(^29\) in which an arterial/venous blood ratio of 3/7 is estimated based on previous positron emission tomography studies of the human cerebral circulation.\(^30,31\) In
Other words, the cerebral oxygenation measurement represents a balance between cerebral oxygen delivery and consumption. (32) Two studies with high-field susceptibility-weighted magnetic resonance venography, a technique in which cerebral veins are directly imaged by exploiting the magnetic susceptibility effect of paramagnetic deoxygenated haemoglobin, showed a decreased venous vascular visibility in patients with MS. (33, 34) Decreased levels of venous deoxygenated haemoglobin probably result from a widespread decrease in cerebral white matter and cortical glucose metabolism and oxygen utilization. (35) When both HbO$_2$ and dHbO$_2$ are decreased, the measured SctO$_2$ might thus remain unchanged in MS. Additionally, it cannot be ruled out that there might be poor correlation between TCD and NIRS estimates of CBF, as NIRS assesses frontal cortex oxygenation, which is partially supplied by the anterior cerebral artery. (36)

Another interesting finding in this study is that MCA mean BFV significantly decreased after the administration of bosentan in the patients with MS, whereas no effect on PI and RI was seen. As stated before, in MS is endothelin-1 probably released by reactive astrocytes in the brain’s microcirculation (10) However, ET-1 is mostly produced by endothelial cells, which are found throughout whole the human body, including the brain and its great intracranial arteries. (37) Bosentan probably acts on MCA and distal arterioles, since ET-1 receptors are present in both great and small intracranial blood vessels. (38, 39) A decrease in tension of smooth muscle in small arteries and arterioles will decrease distal vascular resistance and consequent decrease pulsatility, but in MCA is it unlikely to influence vascular resistance because basal arteries are too wide. Instead, bosentan can increase MCA compliance which may result in an increase in pulsatility. This may mask the effect of distal vasodilation and therefore PI and RI may remain unchanged. (40) The obtained MCA mean BFV, meanwhile, will decrease because arterial compliance of the MCA is probably increased at the insonation site. Additionally, we cannot exclude that the same effect could be seen in patients without MS since we did not administer bosentan in healthy controls. In fact, the most common side effect of bosentan in clinical practice (for treating pulmonary hypertension) is headache, probably induced by cerebral vasodilatation. (41, 42) Bosentan did not induce changes in SctO$_2$ obtained by NIRS in MS patients. It is possible that the sensitivity of NIRS is too low to detect a small effect of bosentan on the frontal cortex microcirculation. Currently, the technique is principally used to monitor cerebral perfusion during chirurgical interventions at risk for cerebral hypoxia, such as cardiac surgery (43) and carotisendarterectomy (44). In these situations, in contrast to our study, abrupt and great changes in cerebral oxygenation are tracked.
There is evolving evidence that cerebral hypoperfusion might play an important role in the pathophysiology of MS. First, several results suggest that reduced CBF may contribute to focal lesion formation. Luchinetti and co-workers have distinguished four histologically different demyelination patterns of which type III lesions show remarkable similarity with classic vascular white matter injury. Some MS patients develop new plaques with reduced MRI mean diffusivity, similar to what is observed in acute ischemic stroke. Also with MRI, it has been shown that hyperintense T2-weighted MRI lesions in patients with MS are situated preferentially in lower perfused white matter areas, and that T1-hypointense lesions occurred almost exclusively in brain regions with lower CBF, of which the latter generally are considered as irreversible tissue damage with poor clinical prognosis. Second, axonal degeneration seems to be associated with mitochondrial energetic failure and oxidative stress. Chronic cerebral hypoperfusion appears to induce mitochondrial dysfunction leading to neuronal damage in animal models. In human MS cortex, gene products specific for the mitochondrial electron transport chain seem to be reduced in the human MS cortex and cortical mitochondria seem to have a lower capacity to exchange electrons in certain respiratory chain complexes. In experimental models of central nervous system white matter hypoxia and ischemia, the mitochondrial electron transport chain ionic cascade has been clearly reported as the cause of myelinated axonal degeneration. Third, reduced CBF in patients with MS has been associated with clinical repercussions of MS. Correlations between reduced white and grey matter CBF and cognitive manifestations in MS have been found. One study found that reduced deep grey matter perfusion in MS negatively correlated with fatigue, which is a common cause of impaired activities of daily living in patients with MS. These factors all contribute to the growing evidence of an association between cerebral hypoperfusion and MS.

There are several limitations to our study. First, our sample size was relatively small and patients of variable clinical subtypes and disease severity were included in the MS group. Second, individuals with atherosclerotic cerebral small vessels disease and its risk factors (e.g. arterial hypertension, diabetes mellitus, smoking, dyslipidaemia) were not excluded from the analysis. Elevations in MCA PI has also been reported in individuals with small vessel disease, and could therefore confound TCD assessment. Increased risk for ischaemic stroke and endothelial dysfunction, the first step towards overt atherosclerosis, has been associated with MS.
Third, there is no standardised method to determine the accuracy of NIRS devices.(27) but the device used in this study is equipped with a 4-wavelength laser source and a scalp detector which both improve measurement accuracy of HbO₂ and Hb.(30)

Fourth, inter- and intra-observer variability may complicate TCD assessments.(12) In an attempt to solve this problem, all TCD examinations in our study were performed by the same researcher. Five control subjects underwent second evaluation, which did not show significant changes compared to the first examination. Furthermore, PI and RI seem to not depend on the insonation angle applied by the performer.(22,62). Nevertheless, both indices are only approximate indicators of distal vascular resistance because they seem to be related to cerebral perfusion pressure, pulse amplitude of arterial pressure, compliance of the cerebral arterial bed and heart rate as well.(24,63)

In conclusion, the underlying mechanism of MS pathology remains incompletely understood but recent perfusion-weighted MRI studies suggest that a globally reduced CBF might be involved. In this study, TCD and NIRS were simultaneously used to evaluate cerebral perfusion in MS and revealed, most importantly, elevated PI and RI in the MCA of patients with MS. These findings suggest an increased distal vascular resistance to blood flow and support the hypothesis that cerebral hypoperfusion in MS is mediated by ET-1 induced arteriolar vasoconstriction. TCD and NIRS appear to be less sensitive than MRI to detect CBF changes in MS. Future studies with a larger, homogenous MS population, excluded for possible confounding factors (e.g., atherosclerosis) will be required to determine whether PI is a useful measure of cerebral hypoperfusion in MS. It would also be of interest to investigate the effect of bosentan on intracranial arterial vessels in healthy controls and to further investigate the underlying mechanism of ET-1 elevation in MS. This could evolve as a new potential therapeutic target.

ACKNOWLEDGMENTS
I thank Miguel D’haeseleer, promotor of this masterthesis, and Jacques De Keyser, co-promotor, for supporting me during the whole study and for commenting on the manuscript. I’m thankful to patients who participated in this study and to Karolien Flamee and Annick Van Merhaeghen-Wieleman for recruiting them. I would also like to thank Saskia De Vos and Ellen Boels for teaching me transcranial Doppler examinations. This study has been made possible by the great flexibility of the staff working at the Department of Neurology at UZ Brussel, for which I thank everyone involved.
REFERENCES


2 Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372(9648):1502-17


43 Grocott HP. Monitoring the brain in cardiac surgery—an evolving area for research. Anaesthesia 2012; 67(3):216-9


