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Investigation of cerebral microbleeds in multiple sclerosis as a potential marker of blood-brain barrier dysfunction

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ABSTRACT

Objective: In multiple sclerosis (MS) lesions blood-brain-barrier (BBB) breakdown is a common phenomenon delineating the phase of focal inflammation in developing MS lesions. In other pathologies like cerebral amyloid angiopathy or arteriosclerotic cerebral small vessel disease permanent cerebral microbleeds (CMB) have been shown to be sensitive markers indicating BBB dysfunction. We were interested in the potential role of T^{2}*-weighted MRI and CMBs as BBB integrity markers in MS.
Methods: A large cohort of 189 MS patients (179 relapsing remitting MS and 10 secondary progressive MS) was investigated on a 3 T MRI system with conventional and T^{2}*-weighted gradient echo MRI (T^{2}*w) sequences. T^{2}*w images were analysed for CMBs by experienced raters.
Results: None of the MS patients showed a CMB.
Conclusion: On T^{2}*w MRI the prevalence of CMBs is not higher in MS patients than what is to be expected in young healthy people. In contrast to pathologies with structural vascular changes like small vessel disease or cerebral amyloid angiopathy, CMBs are not seen in MS where the immune reaction is causing a functional change in the BBB.

1. Introduction

Multiple Sclerosis (MS) is traditionally considered to be an autoimmune inflammatory demyelinating disease of the central nervous system (Compston and Coles, 2008; Weiner, 2009). An increase of the permeability of the blood-brain-barrier (BBB) represents a crucial step in the development of new acute contrast-enhancing lesions that can be demonstrated by magnetic resonance imaging (MRI). T^{2}*-weighted gradient echo MRI (T^{2}*w) and susceptibility-weighted imaging (SWI) have the potential to detect small hemorrhagic residuals, cerebral microbleeds (CMBs) containing deoxyhemoglobin, ferritin, and hemosiderin as hypointense areas in focal lesions and the normal-appearing brain tissue with high sensitivity (Ge et al., 2008l; Tallantyre et al., 2011). In the normal population the prevalence of CMBs is age dependent (Vernooij et al., 2008) while in other pathologies with angiopathies and blood-brain-barrier disturbances, such as cerebral amyloid angiopathy, the prevalence of CMBs is higher due to increased vascular permeability. We investigated the prevalence of CMBs in MS, a disease with a high prevalence of a blood-brain-barrier disturbance.

2. Methods

2.1. Subjects

We performed a prospective cohort analysis of MS patients admitted to our institution with relapsing-remitting or secondary progressive MS (Polman et al., 2011). None of the patients was treated with steroids for at least three months prior to MRI and none had evidence of cerebrovascular disease, cardiovascular disease or a neurological disease other than MS.

2.2. MRI studies

All MRI studies were performed on a 3.0 T MR system (MAGNETOM Skyra, Siemens, Erlangen, Germany, 20-channel head coil,

Abbreviations: BBB, blood-brain-barrier; CMB, cerebral microbleeds; EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; MS, multiple sclerosis; SVD, small vessel disease; T^{2}*w, T^{2}*-weighted MRI; WML, white matter lesions

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50 cm field of view). A standardized protocol was used in all patients: (1) axial, coronal and sagittal localizing sequences followed by axial oblique contiguous 3 mm slices aligned with the inferior borders of the corpus callosum; (2) DW echo planar [EP] images DW EP-SE (TR 5300 ms/TE 68 ms/b=0/500/1000 s/mm^2, FOV 220 mm, slice thickness 3 mm); (3) T^2-w images (TR 4000 ms/TE 78 ms, FOV 220 mm, slice thickness 3 mm, voxel size $0.4 \times 0.4 \times 3$ mm); (4) fluid-attenuated inversion recovery (FLAIR)-images (TI 2500 ms/TR 8500 ms/TE 136 ms, FOV 220 mm, slice thickness 3 mm, voxel size $0.4 \times 0.4 \times 3$ mm) (5) T^1-w images (TR 225 ms/TE 2.5 ms, FOV 220 mm, slice thickness 3 mm, voxel size $0.7 \times 0.7 \times 3$ mm); (6) T^2*w images (TR 602 ms/TE 20 ms, FOV 220 mm, slice thickness 3 mm, voxel size $0.7 \times 0.7 \times 3$ mm); followed by (7) T^1-w images ten minutes after manual injection of single dose contrast agent (Dotarem, Guerbet) identical to (5).

2.3. Data processing and analysis

All MR images were examined jointly by two experienced readers (P.E., A.G.). Readers were unaware of clinical data and patient identification information. Image interpretation was performed on a standard picture archiving and communication system workstation. A structured reporting scheme was used. FLAIR images were used for the identification of white matter lesions (WML). T^2*w images were examined for the occurrence of CMBs. Identification and interpretation of CMBs was performed in a standardized protocol following recommended criteria (Greenberg et al., 2009).

2.4. Standard protocol approvals

This study was approved by the local Institutional Review Board and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained in written form from all patients.

3. Results

189 MS patients (179 relapsing remitting MS and 10 secondary progressive MS; 142 women, 47 men; mean age 36 years; range 18–69 years) were included in the study. Patient characteristics are provided in Table 1. The median Expanded Disability Status Scale (EDSS) was 2 (range 0–7). 145 patients were on best individually selected disease modifying treatment either with Interferon-beta, glatiramer acetate, dimethyl fumarate, natalizumab or fingolimod. 66 patients (35%) had an acute relapse at the time of MRI. None of the MS patients demonstrated CMBs on T^2*w images. Fig. 1 shows an exemplary MRI of a MS patient.

Table 1
Characteristics of MS patients.

	Overall (n=189)	RRMS (n=179)	SPMS (n=10)
Age in years, mean (range)	36 (18–69)	35 (18–59)	48 (29–69)
Gender female, %	75%	75%	40%
EDSS (median, range)	2 (0–7)	2 (0–6)	6 (5–7)
Disease-modifying therapy, N (%)	145 (77%)	141 (79%)	4 (40%)
Interferon-Beta, N (%)	54 (29%)	53 (30%)	1 (10%)
Glatirameracetate, N (%)	11 (6%)	11 (6%)	0 (0%)
Dimethyl fumarate, N (%)	3 (2%)	3 (2%)	0 (0%)
Natalizumab, N (%)	68 (36%)	68 (38%)	0 (0%)
Fingolimod, N (%)	9 (5%)	6 (3%)	3 (30%)

4. Discussion

In this study we found no higher prevalence of CMBs than what is to be expected in healthy young people (Vernooij et al., 2008). This is an interesting finding, as one might have assumed, that in a disorder that is characterized by repeated focal brain tissue inflammation and clearly demonstrable BBB opening also hemorrhagic residuals due to leakage might be detectable. But despite using a 3 T MRI system and a sensitive T^2*w sequence we did not detect CMBs in this rather large cohort of MS patients.

Our results also contrast to the situation in patients with BBB disturbances due to primary vascular pathologies. In patients with cerebral small vessel disease (SVD) (type 1 (arteriosclerosis) and type 2 (sporadic and hereditary cerebral amyloid angiopathy)) WML are commonly identified on MRI (Pantoni, 2010; Schmidt et al., 2006). CMBs in both disease types are assumed as a consequence of a leakage from vessels with disrupted vascular architecture. In sporadic cerebral amyloid angiopathy, the deposition of ß-amyloid in the basement membrane of small arteries and arterioles, but also small veins and capillaries, leads to a loss of smooth muscle cells, fibrinoid necrosis and focal aneurysm formation (Weller et al., 2008). In SVD type 1, fibrinoid necrosis occurs in the context of (hypertension-induced) lipohyalinosis, possibly attenuated by endothelial dysfunction (Hassan et al., 2003) and an inflammatory component (Rouhl et al., 2012).

However, recently another mechanism of CMB development than direct cell extravasation has been postulated in the form of ischemia-mediated iron store release by oligodendrocytes and phagocytosis of red cell microemboli into the perivascular spaces (a mechanism termed “angiophagy”) (Grutzendler et al., 2014). Indeed, a current study investigating combined radiological and histological post mortem tissue found that areas of hemorrhage were frequently associated with microinfarcts, suggesting a secondary mechanism of CMB development (Janaway et al., 2014).

In MS, the situation appears to be different to those disorders with a primarily vascular pathology. Firstly histopathological studies demonstrate that in MS subcortical white matter lesions develop as a consequence of inflammation around small veins that drain into deep and superficial venous pathways (Adams, 1975; Fog, 1964), while the arterial vasculature does not seem to be affected. Secondly, structural changes of the vasculature have not been documented in MS patients. Whereas massive inflammation with invasion of primarily CD4+T-cells leads to destructive changes of the white matter with demyelination and axonal loss, the inflammatory impact on the BBB seems to be of a transient and dynamic nature. Increased levels of inflammatory cytokines like TNF-alpha or IFN-gamma during active periods of the disease can modulate the molecular composition of tight and adhaerens junctions of the BBB, resulting in BBB dysfunction (Minagar and Alexander, 2003). In turn, the effect of IFN-gamma on tight junctions can be blocked by interferon-beta, indicating the reversibility of BBB changes in MS (Minagar et al., 2003). Of note, also in SVD systemic evidence of inflammation from serum biomarkers has been found. It has been suggested, that this might be one of the reasons for the endothelial dysfunction, vasculopathy and formation of CMBs in SVD (Fornage et al., 2008, Wardlaw et al., 2013) and a recent study suggested an association between levels of TNF-alpha and CMBs (Shoamanesh et al., 2015). As increased TNF-alpha serum levels appear to be common in MS patients (Polachini et al., 2014), it is unlikely that an inflammatory process in isolation is the reason for MB development but that further or other factors are responsible for the formation of CMBs also in SVD.

The absence of CMBs in MS is also interesting when considering recent studies performed with SWI. Several MS studies have reported hypointense rims around developing MS lesions on SWI (Absinta et al., 2013; Haacke et al., 2009; Hagemeier et al., 2012)