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Blood circulating microparticle species in relapsing–remitting and secondary progressive multiple sclerosis. A case–control, cross sectional study with conventional MRI and advanced iron content imaging outcomes

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ABSTRACT

Background: Although multiple sclerosis (MS) is thought to represent an excessive and inappropriate immune response to several central nervous system (CNS) autoantigens, increasing evidence also suggests that MS may also be a neurovascular inflammatory disease, characterized by endothelial activation and shedding of cell membrane microdomains known as 'microparticles' into the circulation.
Objective: To investigate the relationships between these endothelial biomarkers and MS.
Methods: We examined the relative abundance of $CD31^+$/PECAM-1, $CD51^+CD61^+$ (αV–$\beta 3$) and $CD54^+$ (ICAM-1) bearing microparticles in sera of healthy individuals, patients with relapsing–remitting MS, and secondary-progressive MS. We also investigated the correlation among circulating levels of different microparticle species in MS with conventional MRI (T2- and T1-lesion volumes and brain atrophy), as well as novel MR modalities [assessment of iron content on susceptibility-weighted imaging (SWI)-filtered phase].
Results: Differences in circulating microparticle levels were found among MS groups, and several microparticle species ($CD31^+$/$CD51^+$/$CD61^+$/$CD54^+$) were found to correlate with conventional MRI and SWI features of MS.
Conclusion: These results indicate that circulating microparticles' profiles in MS may support mechanistic roles for microvascular stress and injury which is an underlying contributor not only to MS initiation and progression, but also to pro-inflammatory responses.

1. Introduction

Multiple sclerosis (MS) is a presumably immune-mediated neurovascular disease whose etiology is influenced by genetic, environmental and even hemodynamic factors [1]. Magnetic resonance imaging (MRI) in MS positively correlates with increased disability (Expanded Disability Status Score, 'EDSS') [2], and remains the most widely applied tool for diagnosing and monitoring MS. MRI also provides important mechanistic clues to the potential bases of MS. Although MRI remains the best objective tool to calculate MS burden, it is expensive and time-consuming and alternative methods are still needed to monitor disease-activity. While lymphocytes and macrophages play important roles in MS [3], additional blood biomarkers are also altered in MS which provide important clues to its pathogenesis. In particular, endothelial and platelet activation has been reported in MS [4], suggesting that microvascular activation contributes to MS pathogenesis. In RRMS platelet activation [5], blood–brain barrier (BBB) disruption correlates with contrast enhancing lesions (CEL) signifying diminished endothelial integrity which underlie CELs.

Microparticles (MPs) are small (<0.1 μm) circulating remnants of endothelial, platelet and other immune cell membranes, which present an important new class of clinical biomarkers. The MP profile may

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foreshadow BBB failure [9,6] and serve as 'surrogate' markers of vascular stress in MS. Importantly, MPs carry endothelial adhesion molecules ('ECAMs'), apoptotic, and other molecular markers from their parent cells, which may link vascular activation to disease activity in MS. MP analysis, therefore, may represent a simple method to monitor MS activity and response to treatment. When correlated with specific brain MRI parameters, MPs can provide important mechanistic insights into MS initiation pathogenesis.

Several endothelial and platelet ECAMs have now been described as MP markers in MS including CD51/CD61 (αV/β3-integrin), CD31 (platelet endothelial cell adhesion molecule-1) [7] and CD54 (intercellular adhesion molecule-1/ICAM-1) [7]. CD31 is an immunoglobulin-superfamily ECAM constitutively expressed on apical and junctional endothelial surfaces, and on platelets, myeloid cells and lymphocytes. $CD31^+$ MPs are released during MS exacerbations [4]; and appear to correlate with early changes in BBB disruption and axonal injury, often abating during remission and therapy [7]. Another MP marker, CD51 (integrin αV) forms several complexes which bind fibronectin and vitronectin and regulates vascular structure and stability [8]. Complexed with CD61, they form integrin αV/β3 which is expressed by endothelial cells, platelets but also macrophages and neutrophils. $CD51^+/61^+$ MPs increase after MS onset [4] but does not diminish during remission or therapy [7]. MP profiling has thus suggested $CD51^+/61^+$ as a vascular marker of underlying MS disease. CD54 (ICAM-1), is another MP marker constitutively expressed on brain endothelial cells which binds leukocyte LFA-1/Mac-1: CD54 is upregulated by T-helper (Th)-1 cytokines and may regulate BBB [9], and vasomotion. Consequently, the release of $CD54^+$ MP has been used as a circulating biomarker of endothelial activation.

Comparisons of these MP markers in different forms of MS suggest that MP may provide a means of evaluating and distinguishing MS subtypes, and potentially, measuring the therapeutic effectiveness of disease modifying drugs. Lastly, annexin-V binding to MP suggests that MP can reflect early stages of apoptosis and in conjunction with endothelial MPs, annexin-V^+ reveals inflammatory injury, and in this report, were studied as markers of cell stress and activation [6]. The present study correlated several circulating MP species in RRMS and SPMS (and healthy controls) with conventional and advanced MRI outcomes to evaluate this approach as a non-invasive metric for use by clinicians to assist in documentation of disease activity in MS.

2. Methods

2.1. Subject identification and MS diagnosis

RRMS and SPMS patients and age- and sex-matched healthy controls (HC) were enrolled at the Department of Neurology, University of Buffalo, Buffalo, NY. Inclusion criteria were MS diagnosis according to McDonald criteria [10], RR or SP disease [11], having an MRI exam performed ≤30 days of clinical examination with the standardized study protocol, age 18–80 years and EDSS = 0–8.5. Exclusion criteria included relapse/exacerbation or steroid treatment ≤30 days of study, pre-existing conditions associated with non-MS brain pathology or pregnancy. HC subjects were recruited from hospital personnel and respondents to local advertisements. HC underwent physical examination, and were assessed for demographic characteristics, autoimmune and other concomitant diseases, vascular and environmental risks and personal habits. Serum samples were stored −80 °C. Samples of serum were analyzed at Louisiana State University Health Science Center-Shreveport (LSUHSC-S). The study was approved by Institutional Review Board at the University at Buffalo.

2.2. MP isolation, labeling, and flow cytometry analysis

Blood was drawn into serum collection tubes and sera removed and aliquots frozen at −80 °C. Serum samples were first centrifuged for 10 min (160 ×*g*) and then centrifuged for 6 min at 500 ×*g*. Cleared supernatants from these spins were then centrifuged at 140,000 ×*g* for 1 h and the MP pellets resuspended in PBS. 50 μl of each sample was incubated with 4 μl of anti-CD31-PE (Abcam), anti-CD51/61-FITC (Pharmingen), anti-CD54 PE-Cy5 (ICAM-1, BD) and anti-annexin V-APC-Cy7 for 20 min with orbital shaking. 1 mL of PBS was added to each sample prior to flow cytometry. MPs were assayed by flow cytometry using FACSVantage SE counter (Beckman Coulter) at medium flow rate setting and 30-second stop time, with log gain on light scatter and fluorescence. Detection was set to trigger by fluorescence signal > noise. Fluorescent microparticles were separated on another histogram based on size (forward light scatter). Flow cytometry analysis was performed using CellQuest for data acquisition and data analysis. Species of MP defined by flow profiling are shown in Table 1.

3. MRI analysis

All scans were acquired on a 3 T GE Signa Excite HD 12.0 TwinSpeed 8-channel scanner (General Electric 'GE', Milwaukee, WI, USA), with a maximum slew rate of 150 T/m/s and maximum gradient amplitude in each orthogonal plane of 50 mT/m (zoom mode). A multi-channel head and neck (HDNV) coil (GE) was used to acquire the following sequences: 2D multiplanar dual fast spin-echo (FSE), proton density (PD) and T2-weighted image (WI); fluid-attenuated inversion-recovery (FLAIR); 3D high resolution (HIRES) T1-WI using a fast-spoiled gradient echo (FSPGR) with magnetization-prepared inversion recovery (IR) pulse; susceptibility-weighted imaging (SWI); and SE T1-WI both with and without a single dose intravenous bolus of 0.1 mM/kg gadolinium (Gd)-DTPA (Gd-DTPA given only to MS subjects). All scans were prescribed in an axial-oblique orientation, parallel to the subcallosal line. One average was used for all pulse sequences.

With the exception of SWI, all sequences were acquired with a 256 × 192 matrix (freq. × phase), field-of-view (FOV) of 25.6 cm × 19.2 cm (256 × 256 matrix with phase FOV = 0.75), for an in-plane resolution of 1 × 1 mm. For all 2D scans (PD/T2, FLAIR and SE T1), we collected 48 slices (3 mm thickness, no gaps between slices.) For the 3D HIRES IR-FSPGR, we acquired 184 locations (1 mm thick, providing for isotropic resolution).

Other relevant parameters were as follows: for dual FSE PD/T2, echo and repetition times (TE and TR) TE1/TE2/TR = 9/98/5300 ms, flip angle (FA) = 90°, echo train length ETL = 14; for FLAIR, TE/TI/TR = 120/2100/8500 ms (inversion time, IT), FA = 90°, ETL = 24; for SE T1-WI, TE/TR = 16/600 ms, FA = 90; for 3D HIRES T1-WI, TE/TI/TR = 2.8/900/5.9 ms, FA = 10°.

All analyses were performed by operators blinded to participants' disease status. SWI was acquired using a 3D flow-compensated gradient echo (GRE) sequence with 64 partitions, 2 mm thickness, a 512 × 192 matrix, FOV = 25.6 cm × 19.2 cm (512 × 256 matrix with Phase FOV = 0.75), for an in-plane resolution of 0.5 × 1 mm, flip angle FA = 12, TE/TR = 22/40 ms.

3.1. Global atrophy and lesion analyses

The SIENAX cross-sectional software tool (version 2.6) was used, with correction for T1-hypointensity misclassification, for brain extraction and tissue segmentation on 3D-T1-WI [12]. We acquired normalized measures of whole brain volumes (NBV), gray matter volumes (NGMV) and white matter volumes (NWMV). T2- and T1-lesion volumes (LV) were measured on FLAIR and SE T1-WI, respectively, using a semi-automated edge detection contouring/thresholding technique (previously described [13]). Normalized volumes were obtained for all subcortical deep gray matter (SDGM) structures with FMRIB's integrated registration and segmentation tool (FIRST) on 3D-T1-WI [14].