Myeloid Microvesicles and Risk of Multiple Sclerosis In Patients with Clinically Isolated Syndromes

Dalla Costa G, Croese T, Finardi A, Moiola L, Colombo B, Comi G, Martinelli V, Furlan R

San Raffaele Hospital, Milan, Italy

Introduction and purpose

- Microglial cells are phagocytic cells of myeloid origin, that are constantly patrolling cerebral microenvironment. As resident innate immune cells of CNS, they respond quickly to pathogens and injury, rapidly changing their morphology and promoting expression of a wide variety of pro-inflammatory molecules (microglia activation). Morphologically activated microglial cells display diverse phenotypes that critically depend on the sequence and duration of their exposure to various stimuli, on the disease type, disease stage and patient's individual characteristics. They also tend to accumulate in regions of neurodegeneration [1,2].
- Microvesicles (MVs) are small (0.05-1.0μm), heterogeneous, plasma membrane-derived vesicles. Shedding of membrane microparticles is a physiological process during cell growth and activation, that is enhanced by cytokines, reactive oxygen species, activation of apoptotic pathways, or intracellular calcium increase. In stress conditions the number of released MVs is increased [3,4].
- The aim of the current study was to investigate CSF MVs levels in patients with clinically isolated syndromes and assessed their ability to predict conversion to clinically definite MS (CDMS) and their relation to other markers of disease activity.

Methods

- 50 patients with the shortest time to conversion to McDonald 2010 MS [5] (< 1 year, fast converters (FC)) and the 50 with the longest follow-up time in the absence of conversion (non-converters (NC). Expanded disability status scale (EDSS), the number of T2 hyperintense and gadolinium enhancing (Gd+) lesions on cranial MRI and the presence of IgG oligoclonal bands (OCB) in the CSF at the time of CIS were assessed. 50 patients with other non-inflammatory neurological disorders (normal pressure hydrocephalus, mild cognitive impairment and Alzheimer disease) were also enrolled as controls(CG)
- PBMCs from anticoagulated whole blood were separated by density centrifugation using Ficoll-PaqueTM Plus (GE Healthcare Bio-Sciences). CD14+ Monocytes were positive selected from PBMCs with CD14 immunomagnetic MicroBeads (MACS® Miltenyi Biotec) and then incubated overnight at 37°C and 5% CO2. 300 µl of supernatant cells were analysed by flow cytometry, while the remaining were used for gene expression analysis
- Normalized (log10) MVs levels were used for all analyses. We applied logistic regression models to assess the ability of MVs to predict disease status (FC vs HC, NC vs HC and FC vs NC). We used linear regression to test the ability of other markers of disease activity (T2 lesions, Gd+ lesions, OCB and EDSS) to predict CSF MVs levels.

Results

Table 1. General characteristics of CIS patients and controls included in the study.

Variable	Category	FC (n=50)	NC (n=51)	CG (n=50)
Age	-	32.0 (24.3-35.4)	37.5 (27.6-44.7)	56.6 (48.6-64.6)
Sex	F	27 (54.0%)	29 (56.9%)	29 (56.9%)
OCB	Positives	41 (82.0%)	34 (66.7%)	-
T2 lesions	0 to 1	3 (6.0%)	14 (27.5%)	-
	2 to 9	17 (34.0%)	13 (25.5%)	-
	> 9	30 (60.0%)	24 (47.1%)	-
Gd+ lesions	Positives	21 (42.0%)	12 (23.5%)	-
EDSS	-	1.5 (1.0-2.0)	1.5 (1.0-2.0)	-
MMVs per ml	-	855.0 (415.0-2407.5)	423.7 (235.0-1010.0)	320.0 (215.0-542.5)

FC=fast converters to MS; NC=non-converters to MS; CG=control group; OCB=oligoclonal bands in CSF; T2=T2 hyperintense lesions; Gd+=gadolinium enhancing lesions; EDSS=Expanded Disability Status Scale; MMVs=myeloid microvesicles in the CSF.

Results

Figure 1. CSF microvesicles (MMVs) concentrations across the three investigated groups (FC, fast converters to McDonald 2010 multiple sclerosis (MS); NC, non-converters to MS; CG, control group). Each dot represents CSF MMVs in a single individual. Box plots indicate median and IQR.

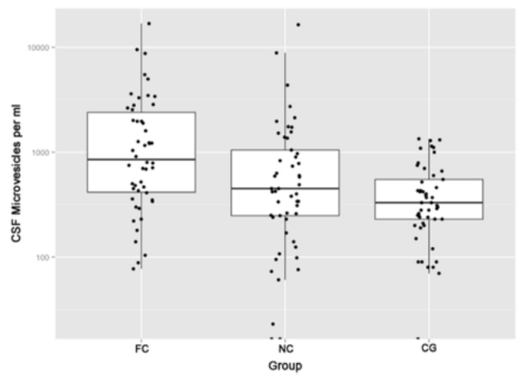
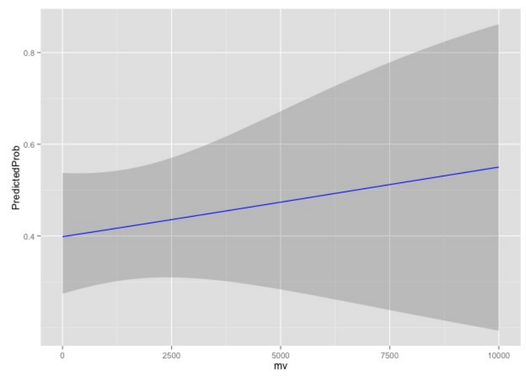


Figure 2. Predicted probability of conversion to McDonald 2010 multiple sclerosis according to CSF microvesicles (MMVs) in patients with the absence or presence of Gadolinium enhancing lesions (panel a and b, respectively)



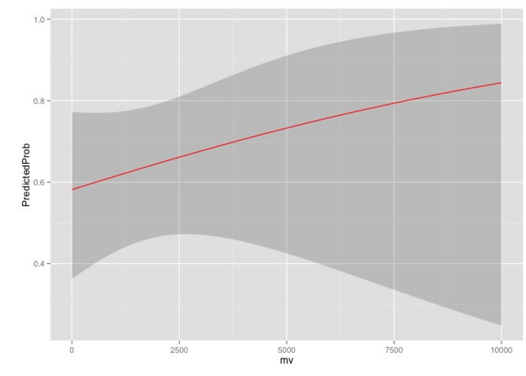


Table 2: Results of logistic regression models investigating CSF MMVs, OCB, T2, Gd+ and EDSS as predictors of disease status (adjusted by age and sex) and results of logistic and ordinal regression models investigating MMVs concentration as predicts of OCB, EDSS, T2 and Gd+ lesions (adjusted by age and sex).

	Predictor	OR	95%CI	p
Upper panel				
Comparison				
NC vs CG	MMVs	0.88	0.38 - 1.99	0.761
FC vs CG	MMVs	6.41	1.38 - 4.15	0.030
FC vs NC	MMVs	2.38	1.19 - 5.32	0.023
FC vs NC	MMVs	2.84	1.27 - 7.50	0.025
	OCB	1.98	0.87 - 4.08	0.094
	T2 (2-9 lesions)	6.23	1.35 - 37.40	0.028
	T2 (>9 lesions)	6.92	1.67 - 38.61	0.014
	Gd+	1.94	0.91 - 4.83	0.123
	EDSS (1.5 - 2.0)	0.86	0.29 - 2.45	0.771
	EDSS (> 2.0)	1.49	0.42 - 5.43	0.536
Lower panel				
Predicted variable				
OCB	MMVs	1.13	0.56 - 2.23	0.718
T2	MMVs	0.69	0.38 - 1.17	0.183
Gd+	MMVs	2.07	1.02 - 4.63	0.059
EDSS	MMVs	0.68	0.39 - 1.16	0.161

OR=odds ratio; 95%CI=95% confidence interval; SE=standard error; NC= non-converters to MS; FC= fast converters to MS; CG=control group; MMVs= myeloid microvesicles in CSF; OCB=oligoclonal bands in CSF; T2=T2 hyperintense lesions; Gd+=gadolinium enhancing lesions; EDSS= Expanded disability status scale.

Conclusions

- CSF MVs level is significantly higher in FC than in NC and this confirms the active role of microglial cells in MS pathogenesis;
- MVs level was significantly associated with fast conversion to MS independently from other known prognostic factors (T2 and gadolinium enhancing lesions as baseline MRI, presence of oligoclonal bands, baseline disability);
- MVs level was significantly higher in patients with Gd enhancing lesions, and it better predicted conversion to MS in these patients than in patients without active lesions;
- Larger prospective studies are needed to validate these results and to assess through genes expression analysis whether different MVs exist, reflecting different role of microglial cells in MS pathogenesis according to their polarization state.

References

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