FUNCTIONAL AND STRUCTURAL MRI PREDICTORS OF DISABILITY WORSENING IN MULTIPLE SCLEROSIS: A 4-YEAR FOLLOW-UP STUDY

[1]F. Pirro, [1]P. Valsasina, [1]E. Pagani, [1]A. Meani, [2]M. Copetti, [3]F. Martinelli-Boneschi, [3]V. Martinelli, [3]G. Comi, [4]A. Falini, [1,3]M. Filippi, [1,3]M.A. Rocca.

[1]Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, [3]Dept. of Neurology, [4]Dept. of Neuroradiology, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy; [2]Biostatistics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy.

INTRODUCTION AND AIMS

In multiple sclerosis (MS), the accumulation of severe disability and the development of cognitive impairment have a dramatic effect on patients' daily life activity. As a consequence, the identification of biomarkers able to predict disease worsening is of paramount importance to optimize patients' treatments. Conventional MRI (cMRI) is sensitive in revealing focal white matter (WM) lesions and disease activity. However, its prognostic value has been proven only in patients at presentation with a clinically isolated syndrome (CIS) suggestive of MS, while the prognostic role of cMRI in patients with definite MS is still debated. Recent evidences have suggested that grey matter (GM) atrophy is better associated with long-term disability and cognitive impairment than focal WM lesion volumes [1].

Advanced MRI techniques, including diffusion tensor (DT) MRI and resting state (RS) functional MRI (fMRI) [2], might contribute to improve the understanding of the mechanisms responsible for the accumulation of irreversible clinical and cognitive deficits in MS patients.

Aims of the study were to identify the MRI predictors of medium-term disability and cognitive impairment accrual in patients with the main MS clinical phenotypes. To do this, we assessed:

- the value of cMRI, DT MRI and RS fMRI in predicting clinical improvement and worsening (including evolution towards a more severe clinical phenotype) after 4 years of follow-up (FU) in MS patients;

- the value of the previous MRI quantities in predicting cognitive deterioration after 4 years.

METHODS

- **Subjects:** 248 right-handed MS patients and 98 age- and sex-matched healthy controls (HCs) were enrolled.
- Clinical and neuropsychological assessments were performed at baseline and after a mean period of 4.0 years (range 2.3-7.9) of FU. At baseline, a MRI evaluation was also obtained.
- **Neurological evaluation:** definition of disease clinical phenotype and quantification of EDSS score [3].
  - At FU, a patient was defined clinically worsened if EDSS score increased ≥1.0, when baseline EDSS was <6.0, or if EDSS score increased ≥0.5, when baseline EDSS was ≥ 6.0.
  - Clinical improvement was defined if EDSS score decreased ≥1.0.
- **Neuropsychological assessment (Rao's BRB of Neuropsychological Tests)** [4]**:**
  - Cognitive impairment was defined if a patient had at least 2 abnormal tests (a score below 2 SD of the normative value);
  - At FU, a patient was defined as cognitively worsened if the number of tests failed at FU was greater than at baseline.
- **Brain MRI acquisition** (3.0 Tesla Philips Intera scanner) (baseline):
  - Axial dual-echo turbo spin echo for lesion assessment;
  - Axial high-resolution 3D T1-weighted fast field echo (FFE) for atrophy assessment;
  - Pulsed-gradient SE echo-planar imaging (EPI) for DT MRI and assessment of WM tracts;
  - T2*-weighted single-shot EPI for assessment of RS functional connectivity (FC).
- **Structural MRI analysis:**
  - Quantification of T2 hyperintense and T1-hypointense lesion volumes (LV) (Jim 6.0 software);
  - Estimation of baseline normalized brain (NBV), GMV and WMV volumes (SIENAx software), after T1-hypointense lesion refilling;
  - Calculation of total volume of deep GM nuclei (including the thalamus, putamen, pallidum, caudate, amygdala and accumbens) (FSL FIRST software).
- **Diffusion MRI analysis:**
  - Eddy current correction and DT estimation;
  - Calculation of average fractional anisotropy (FA) and mean diffusivity (MD) values within lesions, normal appearing (NA) WM and MD in the GM;
  - Derivation of average FA and MD values from the 48 WM regions of the ICBM-DTI-81 white-matter label atlas (**Figure 1**) [5].
- **RS FC analysis:**
  - Pre-processing (SPM12 and REST: realignment, normalization to MNI space, linear detrend, band-pass filtering, 6 mm^3 Gaussian smoothing);
  - Assessment of RS FC using independent component analysis (ICA) with the GIFT software;
  - Extraction of 11 RS networks of interest, as shown in **Figure 2**;
  - Single network mean RS FC was computed by averaging the Z-scores of above-thresholded voxels for each study subject.

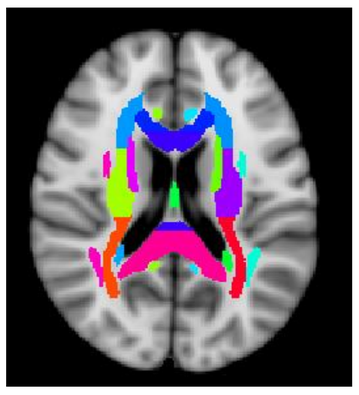
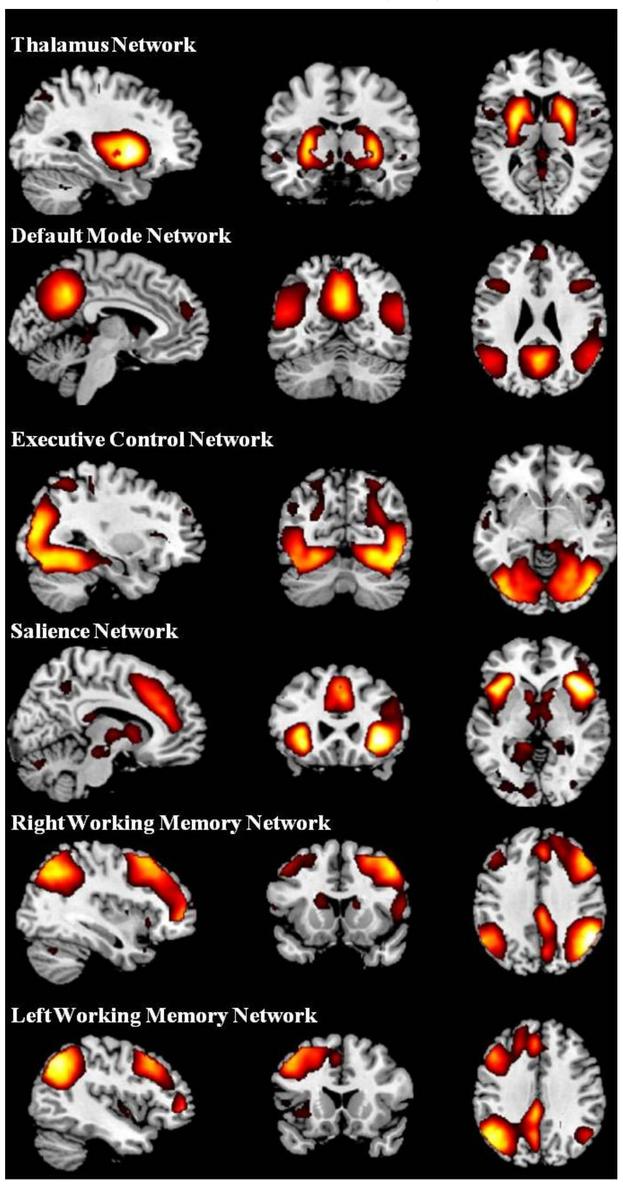


Figure 1. ICBM-DTI-81 white-matter label atlas [5].



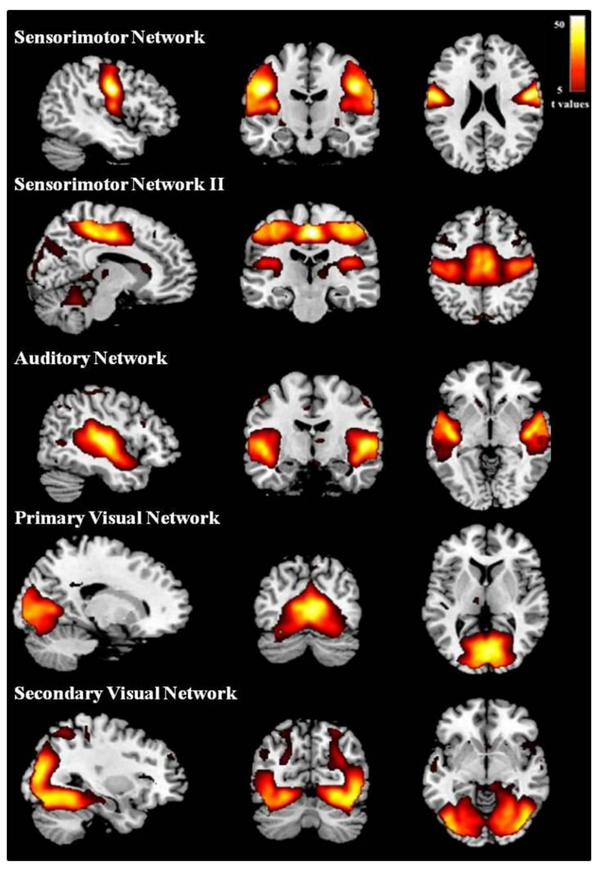


Figure 2. ICA-determined RS FC maps (p<0.05, FWE corrected) of thalamic network (ThN), default mode network (DMN), executive control network (ECN), salience network (SN), right (R) and left (L) working memory networks (WMN), sensorimotor networks, auditory network (AuN), primary and secondary visual networks. All images are in neurological convention (left side of the image corresponds to the left side of the brain).

- **Statistical analysis:**
  - Comparison of demographic, clinical and structural MRI measures using ANOVA or non-parametric tests, as appropriate;
  - Calculation of global and regional RS FC differences between MS patients and HCs (SPSS and SPM12, respectively). For each significantly different SPM cluster between MS patients and HCs, average Z-scores of RS FC were extracted using the MarsBaR toolbox;
  - Univariate logistic regression models, adjusted for FU duration, using clinical, structural and fMRI variables as predictors of clinical improvement/worsening, evolution to a more severe phenotype and cognitive deterioration;
  - Random forest (RF) approach and multivariable analysis were performed for each outcome.

RESULTS

Table 1. Demographic, clinical, lesional and atrophy measures at baseline of MS patients who were clinically stable *vs* those who had worsened or improved clinically at FU and patients who were cognitively stable *vs* those who were cognitively worsened at FU.

	Clinically stable MS patients (n=146)	Clinically worsened MS patients (n=86)	Clinically improved MS patients (n=16)	p	Cognitively stable MS patients (n=59)	Cognitively worsened MS patients (n=22)	p
Sex (M/F)	57/89	33/53	4/12	0.54^{++}	26/33	9/13	0.80^{++}
Mean age [years] (range)	39.7 (17.3-69.7)	45.0 (17.3-67.9)	40.0 (22.1-56.1)	0.002**	38.7 (18.9-62.1)	48.5 (25.4-68.4)	<0.001*
Clinical phenotype (RRMS/SPMS/PPMS/BMS)	90/24/8/24	28/35/9/14	12/0/1/3	<0.001^{++}	39/10/1/9	8/7/3/4	0.03*
Median EDSS (range)	2.0 (1.0-9.0)	4.0 (0.0-8.5)	2.0 (1.0-5.0)	<0.001§	2.0 (0.0-6.5)	4.5 (1.0-6.5)	0.01^{+}
Median disease duration [years] (range)	12.0 (0.0-44.0)	15.1 (0.3- 31.3)	12.0 (2.6-18.6)	0.14**	12.0 (0.8-26.0)	15.3 (1.0-32.6)	0.01*
Mean T2 LV [ml] (SD)	9.3 (10.6)	14.0 (12.7)	6.1 (7.1)	0.02§	8.8 (8.8)	16.4 (14.0)	0.02^{+}
Mean T1 LV [ml] (SD)	6.4 (8.0)	10.1 (11.5)	4.4 (5.7)	0.02§	6.1 (6.9)	12.5 (11.0)	0.01^{+}
Mean NBV [ml] (SD)	1504 (111)	1460 (104)	1557 (81)	0.001**	1506 (95)	1449 (115)	0.03*
Mean GMV [ml] (SD)	686 (82)	647 (79)	719 (52)	<0.001**	690 (68)	633 (82)	0.002*
Mean WMV [ml] (SD)	818 (48)	814 (50)	839 (39)	0.10**	816 (42)	815 (45)	0.95*
Mean deep GM volume [ml] (SD)	33 (6)	31 (6)	34 (4)	0.13**	32 (5)	29 (4)	0.01*
Mean Hipp Volume [ml] (SD)	4.6 (0.6)	4.4 (0.7)	4.8 (0.5)	0.05§	4.6 (0.6)	4.3 (0.7)	0.05^{+}

*Two-sample t test; **ANOVA model; $^{+}$Mann-Whitney test; §Kruskal and Wallis test $^{++}$Chi-square test.

Differences at baseline between clinically worsened *vs* clinically stable/improved MS patients:

Diffusion MRI analysis:
- ↓ FA NAWM (p=0.004),
- ↑ GM MD (p=0.02),
- ↑ MD posterior limb of the internal capsule (p=0.04) and superior cerebellar peduncle (p=0.04),
- ↓ FA posterior thalamic radiation (p=0.03), cingulum (p=0.003) and CC (p=0.04).

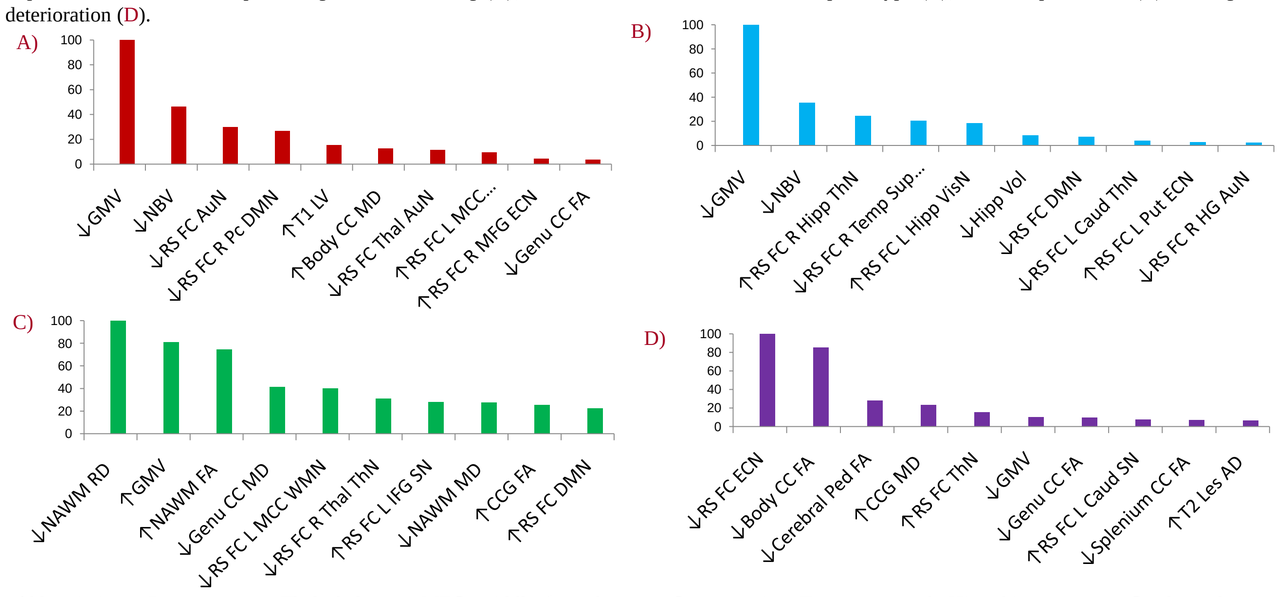
RS FC analysis:
- ↓ Global RS FC AuN (p=0.03),
- ↓ RS FC Cuneus of the Sensorimotor Network II (p=0.05).

Differences at baseline between cognitively worsened *vs* cognitively stable MS patients:

Diffusion MRI analysis:
- ↓ FA NAWM (p=0.05),
- ↑ lesional (p=0.02) and NAWM (p=0.03) MD,
- **Significantly different DT measures** in most of the WM tracts examined (p ranging from <0.001 to 0.05).

RS FC analysis:
- ↓ Global RS FC ECN (p>0.001), R WMN (p=0.01) and L WMN (p=0.02),
- ↓ RS FC L Cerebellum R WMN (p=0.05),
- ↓ RS FC R Mid Cingulate Cortex ECN (p=0.04),
- ↑ RS FC L Caudate SN (p=0.05).

Figure 3. Results of the RF analysis. Normalized variable importance, ranging from 0 (not important) to 100 (the most important), of the ten most important MRI variables in predicting EDSS worsening (A), evolution to a more severe clinical phenotype (B), EDSS improvement (C) and cognitive deterioration (D).



Abbreviations: Pc=precuneus; Thal=thalamus; MFG=middle frontal gyrus; Sup=superior; Temp=temporal; Hipp=hippocampus; Caud=caudate; Put=putamen; HG=Heschl gyrus; IFG=inferior frontal gyrus; CC=corpus callosum; Ped= peduncle; Les=lesional.

Multivariable analysis

Clinical worsening (C-index=0.66):
- ↓ baseline GMV (p=0.0003; β= -0.01),
- ↓ RS FC Precuneus of the DMN (p=0.0466; β=-0.29).

Clinical improvement (C-index 0.71):
- ↑ FA in the NAWM (p=0.0093; β=40.11).

Cognitive deterioration (C-index=0.84):
- ↓ Global RS FC of the ECN (p=0.004; β =-9.15),
- ↓ FA of the body of the CC (p=0.006; β =-13.6),
- ↑ Global RS FC of the ThN (p=0.02; β=4.44).

Evolution to a more severe disease phenotype (C-index= 0.90):
- ↓ baseline GMV (p=0.0005; β=.0.0154),
- ↑ RS FC of the R hippocampus in the ThN (p=0.0161; β=0.73),
- ↓ RS FC in the superior temporal gyrus in the DMN (p=0.002; β=-1.4365),
- ↑ RS FC of the L hippocampus of the visual network (p=0.0274; β=0.7399).

CONCLUSIONS

- **Baseline EDSS, disease clinical phenotype, disease duration and age** influence disease worsening after 4 years of FU in MS patients;
- Clinical disability and cognitive impairment at FU are predicted by measures of **structural and microstructural damage** as well as **RS FC** measures;
- **GM involvement** plays a critical role in MS-related clinical worsening and evolution to a more severe disease phenotype; **atrophy measures** resulted more important than focal WM lesions in predicting clinical worsening;
- Preserved **NAWM structural integrity** predicts clinical improvement;
- **Lower RS FC in the DMN** predicts clinical worsening and evolution to a more severe disease phenotype;
- Increased intrathalamic RS FC is likely to be a **maladaptive mechanism** in MS patients, while a preserved intrathalamic connectivity could represent an **adaptive response**;
- **Structural damage** of strategic CNS regions (e.g., the CC) might influence **functional system rewiring**;
- The combination of measures sensitive to **destructive**, but also **reparative** and/or **compensatory** mechanisms is a valid approach to identify the factors associated to a more **severe clinical status,** and might contribute to **optimize patients' treatment**.

REFERENCES

[1] Filippi M., Neurology 2013; [2] Biswal BB., PNAS 2010; [3] Kurtzke JF., Neurology 1983; [4] Rao SM., National Multiple Sclerosis Society 1991; [5] Mori S., Neuroimage 2008.

DISCLOSURES. F. Pirro, P. Valsasina, E. Pagani, A.Meani, M. Copetti, F. Martinelli-Boneschi, V. Martinelli, A. Falini have no conflicts of interest.
M.A. Rocca received speakers honoraria from Biogen Idec, Novartis and ExecMED and receives research support from the Italian Ministry of Health and Fondazione Italiana Sclerosi Multipla. **G. Comi** has received compensation for consulting services and/or speaking activities from Novartis, Teva Pharmaceutical Ind., Sanofi-Aventis Pharmaceuticals, Genzyme, Merck Serono, Biogen-Dompè, Bayer Shering, Actelion, Serono Symposia International Foundation, Almirall, Chugai and Receptos. **M. Filippi** serves on scientific advisory board for Teva Pharmaceutical Industries; has received compensation for consulting services and/or speaking activities from Biogen Idec, Excemed, Novartis, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Teva Pharmaceutical Industries, Novartis, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, Cure PSP, Alzheimer's Drug Discovery Foundation (ADDF), the Jacques and Gloria Gossweiler Foundation (Switzerland), and ARiSLA (Fondazione Italiana di Ricerca per la SLA).
This work has been partially supported by a grant from the FISM 2014/R/7.