Brain tissue volumes and neuropsychological status in early Relapsing-Remitting Multiple Sclerosis

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Introduction: Cognitive dysfunctions occur in approximately 40% patients with multiple sclerosis (MS) in community samples (Rao, 1991) and are already present in the very early stages of the disease (Callanan, 1989). Although studies of MRI correlates of cognitive impairment in MS have reported in most cases a correlation with total lesion load (LL) area, this correlation appeared weaker when only RR-MS patients were studied (Fulton, 1999; Rovaris, 2002).

In principle, the mechanism(s) underlying NP impairment may be limited to WM damage within MS lesions, or include the loss of normal brain tissues (i.e. normal-appearing WM and GM), whose loss has been recently demonstrated in RR-MS using automated MRI segmentation (Chard, 2002; Ge, 2001; Quarantelli, 2003).

However, to the best of our knowledge only one study (Edwards, 2001) has investigated the relationship between separate tissue volumes (i.e. normal and abnormal WM, GM and CSF) and cognitive status in patients with mixed types of MS course (both RR MS and SP MS), finding a correlation between overall NP status and WM volume.

Aim of the present study was to assess the relationship between brain tissue volumes (both abnormal and normal) measured by MRI segmentation and the NP status in a population of patients with RR MS in the early stages of the disease.

NP status was assessed using a comprehensive battery of tests including the Brief, Repeatable Battery of Neuropsychological Tests in Multiple Sclerosis (Rao, 1990), the Stroop color/Word interference test, premorbid IQ assessment and the Hamilton test for depression (Hamilton, 1967).

Multiple regression analysis was used to control for the effect of demographic variables, as well as for education, depression and premorbid-IQ.

Material and Methods: Patients: 88 patients (38/88 males) with clinically definite MS were enrolled. Relapsing-remitting course (Lublin and Reingold, 1996) and a DD of less than 5 years were inclusion criteria.
Demographic and clinical variables of the patients are reported in Table 1.

MRI studies: MRI protocol (1.5T Intera, Philips Medical Systems) included conventional spin-echo sequences providing T1w (500/10) and PD/T2w (2400/10-80) 3mm-thick axial images (25cm FOV, 256x256 acquisition matrix), sampling the entire brain at 48 levels.

MRI triplets were segmented into GM, WM, aWM, and CSF with a fully automated procedure (Alfano, 2000; Quarantelli, 2003). To avoid aWM-related segmentation bias of normal tissues (Quarantelli, 2003), the segmentation procedure was modified by introducing a gaussian fitting routine to steady the identification of GM and WM clusters (Fig. 1). Testing of the segmentation results using a data-set of simulated MS MRI studies, as previously described (Quarantelli, 2003), did not demonstrate any effect of the presence of aWM onto GM/WM volume measurements.
For subsequent analysis, tissue volumes were normalized by intracranial volume.

Neuropsychological Testing: All patients underwent the following tests:
1) Bushke Verbal Selective Reminding Test (SRT) (a measure of verbal learning and delayed recall of a 12 word list).
Subitems of the SRT are:
• the long-term Storage (LTS) score (sum of words recalled on 2 consecutive trials without reminding)
• the Consistent Long Term Retrieval (CLTR) score (sum of words recalled on all subsequent trials without reminding)
• the Total Delay (number of words recalled after 10 minute delay).
2) 10/36 Spatial Recall Test measuring visuospatial learning and delayed recall using a checkerboard pattern. Three learning trials and one delayed trial are scored for total number of correct responses. Total and delayed correct answers are recorded.
3) Symbol Digit Modalities Test (SDMT) assessing sustained attention and information processing speed. The written form of the test was used to score the number of correct pairs.
4) Paced Auditory Serial Addition Task (PASAT) is a measure of complex attention and concentration. Single digit numbers are presented every 3 ("easy" condition) or 2 ("hard" condition) seconds using a pre-recorded tape. The patient is asked to add each number to the digit immediately preceding it. The percentage of correct additions is recorded.
5) Word List Generation, a measure of verbal fluency and sustained attention. Total number of generated admissible words that belong to a specific category (e.g. " vegetables") in 60 seconds are recorded. The number of intrusions (words beginning with the wrong letter) and perseverations (repetitions of the same word commencing with the correct letter) are also recorded.
Additionally, the following tests were administered:
7) Stroop color/Word interference test (a measure of visual attention). The added time due to Stroop effect interference was used in the present analysis.
8) The TIB ("Test di Intelligenza Breve") (Colombo, 2000), an Italian version of the National Adult Reading Test, providing estimates of premorbid IQ.
9) Hamilton test for depression
Statistical analysis: Stepwise multiple regression analysis was performed separately for each NP score.
TIB results were entered as first block, to preliminarily adjust for the possible effects of premorbid IQ onto NP test results, followed by age, years of education, EDSS and Hamilton scores.
ICV-normalized LL, normal-appearing GM and normal-appearing WM were then entered as third block.
CSF was entered as final block to check for possible additional role of overall atrophy.
Significance level for multiple regression model was set to 0.05.
Results: All the tests, with the exception of visuospatial memory (both early and delayed scores at 10/36 checkerboard test) correlated to pre-morbid IQ. Age added a significant contribution to SDMT and PASAT ("easy" condition only) results, and was the only variable correlated to the performance at 10/36 spatial recall test.
No other demographic or clinical variable correlated significantly with any of the NP test results.
Among the brain tissue volumes, WM volume inversely correlated to the Stroop interference (P<0.05) and to the number of correct answers at PASAT under the hard condition (P<0.05), while GM loss correlated to the WLG perseverations (P<0.005).
It is of note that among the excluded variables in multiple regression, PASAT (hard condition only) and Stroop test results correlated significantly with CSF (P<0.05 and P=0.005, respectively), although this correlation was lost once WM was included preliminarily (i.e. global atrophy did not add to the WM loss in influencing the NP), while no significant correlation was present in any case between LL volume and any of the NP tests.

Discussion: We have found, already in the early stages of RR MS, a moderate correlation between normal-appearing WM volume loss and NP performance as measured by PASAT and Stroop test and between GM loss and sustained attention at a verbal fluency task as measured by perseverations during WLG test.

Unlike other studied, in our case the was no significant correlation between LL and any of the NP test scores in our patients. It is of note that such correlation did not emerge even if LL was the only brain tissue tested in the multiple regression analysis. We hypothesize that the lack of correlation between LL ad NP status in our patients is due to the fact that we focused our study on early RR-MS patients.

The finding of a correlation between WM loss and PASAT (at 2 seconds pace) scores in early RR-MS, integrates similar results from previous studies in MS patients with mixed progressive and RR disease courses (Edwards, 2001; Rao, 1989).

As in other studies (Rao, 1989), a correlation with MRI variables was not present in the "easy" version of PASAT (3 seconds pace). The emerging of this correlation under more demanding conditions (in the "hard" version of PASAT) may reflect a relationship between the degree of integrity of the CNS, and in particular of its interconnections as measured by the WM integrity, and the speed of information processing, a function particularly affected in MS as demonstrated by the effects of rapid processing demands seen in MS.

A previous study testing verbal fluency with a technique similar to WLG have not detected correlations with MRI variables (Edwards, 2001), although in that case the perseverations, which are inversely correlated to GM volume in our group of patients, were not assessed specifically.
The meaning of the correlation between GM volume and perseverations remains to be assessed.

Conclusion: The correlations of normal-appearing WM loss with Stroop and PASAT results and those between GM loss and perseverations at WLG test, suggest that brain tissue loss may play a role onto the damage of specific NP capacities, beyond the effects of simple disconnection due to the presence of MS plaques, in early RR MS.

Our results are not in keep with a direct role of LL in the NP impairment in the early stages of RR MS.

Differences between our results and previous studies, which pooled together progressive and RR- MS patients, may reflect different mechanisms involved in the pathogenesis of NP impairment among different MS courses, adding o the clinical heterogeneity of MS subtypes.
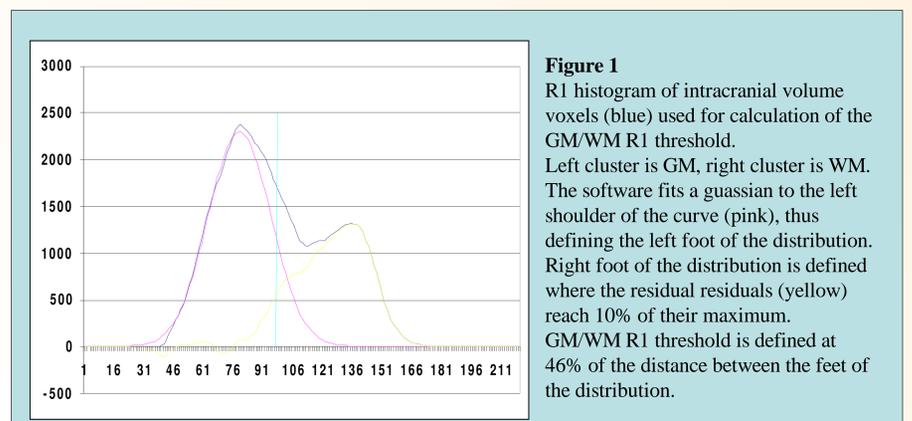
Figure 1
R1 histogram of intracranial volume voxels (blue) used for calculation of the GM/WM R1 threshold.
Left cluster is GM, right cluster is WM. The software fits a guassian to the left shoulder of the curve (pink), thus defining the left foot of the distribution. Right foot of the distribution is defined where the residual residuals (yellow) reach 10% of their maximum.
GM/WM R1 threshold is defined at 46% of the distance between the feet of the distribution.

Table 2

Test	Variable	R	P
Stroop (interference)	IQ	-0.242	0.023
	WM	-0.232	0.031
SRT LTS	IQ	0.245	0.022
SRT CLTR	IQ	0.302	0.004
SRT Delayed	IQ	0.314	0.003
10/36 Total	Age	0.299	0.005
10/36 Delayed	Age	0.282	0.008
SDMT	IQ	0.369	<0.001
	Age	-0.229	0.033
PASAT "easy"	IQ	0.397	<0.001
	Age	-0.261	0.015
PASAT "hard"	IQ	0.370	<0.001
	WM	0.234	0.029
WLG appropriate	IQ	0.580	<0.001
WLG perseverations	GM	-0.323	0.002
WLG intrusions	-	n/a	n/a

Effects of demographic and MRI variables onto NP test results.
Only significant correlations at multiple regression analysis are reported.
R: partial correlation coefficient between NP test score and the explanatory variable in the multiple regression model
IQ: estimate of premorbid IQ as derived from an Italian version of the National Adult Reading Test
SRT: Selective Remaining Test
LTS: Long-Term Storage
CLTR: Consistent Long Term Retrieval
SDMT: Symbol Digit Modalities Test
PASAT: Paced Auditory Serial Addition Task. Results are reported for both the 3 ("easy") and 2 ("hard" condition) second pace
WLG: Word List Generation

TABLE 1

Demographic and clinical variables of the patients

	Mean	Standard Deviation	Mode	Min	Max
Age	33.9	8.6	34.6	17.2	53.4
DD	1.0	1.1	0.1	0.1	4.7
EDSS	2.23	0.7	2.0	1.0	4.0
Education (years)	12.3	3.9	13.0	5.0	18.0

DD: disease duration; EDSS: Expanded Disability Status Scale

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