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Short communication

# Relation between plasmatic and cerebrospinal fluid oxidative stress biomarkers and intrathecal Ig synthesis in Multiple Sclerosis patients

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ABSTRACT

The aim of this study was to evaluate if cerebrospinal fluid (CSF) oxidative stress biomarkers were related to plasmatic levels and to intrathecal Ig synthesis in 51 patients with Multiple Sclerosis (MS) or clinically isolated syndrome (CIS).
Plasmatic and CSF ferric reducing ability (FRA) showed a significant positive correlation ($\rho$ 0.28, $p = 0.04$), while advanced oxidation protein products (AOPPs) did not. A negative correlation was found between IgG synthesis index and CSF FRA levels. No difference in CSF AOPPs or FRA was observed between patients with and without intrathecal IgM synthesis.
Our results indicate that plasmatic and CSF FRA are strictly linked, while CSF oxidative stress biomarkers are not related to intrathecal Ig synthesis.



## 1. Introduction

Multiple Sclerosis (MS) is a chronic autoimmune inflammatory disease of the Central Nervous System (CNS), histopathologically characterized by demyelination, axonal damage and reactive gliosis (Trapp et al., 1998).

The inflammatory reaction is the leading cause of CNS tissue damage in MS. However in the progression of tissue pathology an important mechanism, that occurs in all lesions at all disease stages, is represented by oxidative damage and mitochondrial dysfunction, which are mediated by activated macrophages and microglia (Lassmann and van Horssen, 2011). The purpose of our study was to evaluate the correlation between plasmatic and CSF oxidative stress biomarkers, such as advanced oxidation protein products (AOPPs), which are a stable marker of oxidative damage to proteins (Witko-Sarsat et al., 1996) and ferric reducing ability (FRA) which express the "antioxidant power" of biologic fluids measuring the combined effect of non-enzymatic antioxidants (Benzie and Strain, 1996), and between CSF oxidative stress biomarkers and intrathecal immunoglobulin synthesis, in CIS and MS patients.

Plasmatic AOPPs have been found at significantly higher levels in MS patients compared to healthy controls (Karlík et al., 2015; Pasquali et al., 2015), while FRA levels were lower in MS patients compared to healthy controls (Pasquali et al., 2015), this reflecting a mechanism of oxidative damage and reduced antioxidant capability in MS.

## 2. Materials and methods

### 2.1. Patients

The study included 51 patients consecutively admitted to the Neurology Clinic, who underwent CSF analysis for standard diagnostic evaluation and fulfilled McDonald criteria (McDonald et al., 2001) and Polman revision for diagnosis of CIS or MS (Polman et al., 2011). CIS was defined by the presence of a clinical neurological attack and at least two T2 lesion on MRI and at least 2 oligoclonal Ig band at CSF examination. Clinical disease course was defined according to the classification of Lublin (Lublin and Reingold, 1996).

No patient was under disease modifying therapy for MS at the time of inclusion in the study; no patients were taking vitamin supplements, antioxidants or oral contraceptive therapy during the three months before the inclusion in the study. Patients with concomitant diseases like diabetes or renal failure were excluded ab initio.

This study was approved by the local ethical committee and all patients provided a written informed consent before the inclusion in the study.

CSF and blood samples were collected, immediately centrifuged at 3000 rpm for 10 min, and stored at −20 °C, for subsequent biochemical analysis.

### 2.2. Biochemical analysis

Standard procedures of routine CSF analysis, including leukocyte count by Fuchs–Rosenthal chamber within 2 h after CSF drawing, CSF cell staining with phenoptical staining and light microscopic examination,

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**Table 1**
Demographic characteristics of subjects.

| | CIS | RR-MS | PP-MS | SP-MS |
|---|---|---|---|---|
| Number of subjects | 19 | 22 | 9 | 1 |
| Age (mean ± SD) | 33 ± 9.6 | 38 ± 10.8 | 40.1 ± 12.6 | 68 |
| Sex (male/female) | 6/13 | 6/17 | 2/7 | 0/1 |
| Intrathecal IgM synthesis (number of subjects) | 4 | 4 | 4 | 0 |

glucose (CSF-to-serum ratio), total CSF protein, albumin CSF/serum ratio immunoglobulin G, A and M, oligoclonal IgG with CSF and serum run in parallel by isoelectric focusing and immunoblotting, were performed.

Intrathecal IgG and IgM synthesis was calculated as described by Reiber (Reiber and Peter, 2001).

In particular, the upper limit QLim (Ig) of the reference range in the CSF/serum quotient diagrams was calculated as follows:

$$\mathrm{QLim(IgG)} = 0.93\sqrt{(Q(Alb)^2 + 6 * 10^{-6}} - 1.7 + 103$$

$$\mathrm{QLim(IgM)} = 0.67\sqrt{(Q(Alb)^2 + 120 * 10^{-6}} - 7.1 + 103.$$

The amount of locally synthesized immunoglobulins released into CSF was expressed as the intrathecal fraction, IgIF, referring IgLoc to the total Ig concentration in CSF (IgLoc/IgCSF), and rearranged with QIg = IgCSF/Igserum

$$\mathrm{IgIF} = [1 - \mathrm{QLim(Ig)}/\mathrm{QIg}] * 100.$$

AOPPs were determined according to Witko-Sarsat and collaborators (Witko-Sarsat et al., 1996). Briefly, plasma or CSF was mixed with $H_2O$, acetic acid and potassium iodide. The absorbance was read spectrophotometrically at 340 nm and compared with a solution of chloramine T dissolved in the same buffer. Data were expressed as mmol/L of chloramine equivalents.

In order to measure non-enzymatic anti-oxidant properties, FRA was assessed as previously reported (Mancuso et al., 2010). Briefly, the FRA reagent (sodiumacetate, tripyridyltriazine in hydrochloric acid and ferric chloride) pre-warmed to 37 °C was mixed with plasma or CSF; the absorbance was read after 3 min at 593 nm. A calibration curve was established by substituting the sample with a solution of iron sulfate in hydrochloric acid. Data were expressed as mmol/L (Benzie and Strain, 1996).

### 2.3. Statistical analysis

The statistical analysis was carried out using StatPlus2009® running on Windows (AnalystSoft Inc.) and included descriptive statistics of clinical and demographic features, comparisons with the nonparametric Mann–Whitney *U* test and Kruskal–Wallis test when appropriate, as well as correlation analysis with Spearman's rank correlation coefficient. P-values were considered significant for $p < 0.05$.

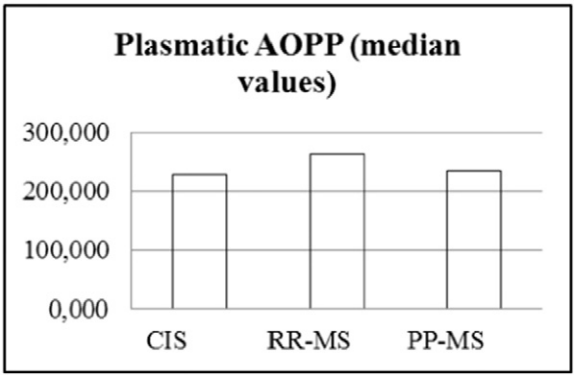


**Fig. 1.** Plasmatic AOPP median values in CIS, RRMS and PPMS patients are graphically reported.

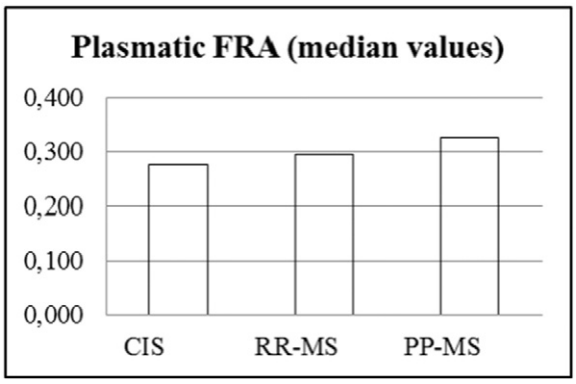


**Fig. 2.** Plasmatic FRA median values in CIS, RRMS and PPMS patients are graphically reported.

## 3. Results

### 3.1. Patients

The study included 51 patients (14 males, 37 females), aged between 18 and 70 years.

41 subjects were affected by CIS or RR-MS, while 10 patients were in the progressive disease course (PP-MS, SP-MS). Mean age of subjects was 38.7 ± 12.3 years, (35.0 years in CIS/RR-MS and 42.9 years in PP-MS/SP-MS groups). Demographic characteristics of patients are reported in Table 1.

### 3.2. Oxidative stress biomarkers

Plasmatic FRA levels showed a statistically significant positive correlation with CSF FRA levels ($\rho$ 0.28, $p = 0.04$), while AOPP plasmatic and CSF levels did not significantly correlate.

Plasmatic and CSF AOPPs and FRA levels are shown in Figs. 1 to 4 and no significant differences in CIS, RR-MS and PP-MS subjects were found (Table 2).

Furthermore, comparison of relapsing versus progressive MS patients, failed to detect statistically significant difference between plasmatic and CSF AOPPs and FRA in CIS/RR-MS compared to PP-MS/SP-MS patients (Table 3).

Oligoclonal intrathecal Ig bands were found in all patients. In 12 patients (23.5%) intrathecal IgM synthesis was detected (Table 1). The

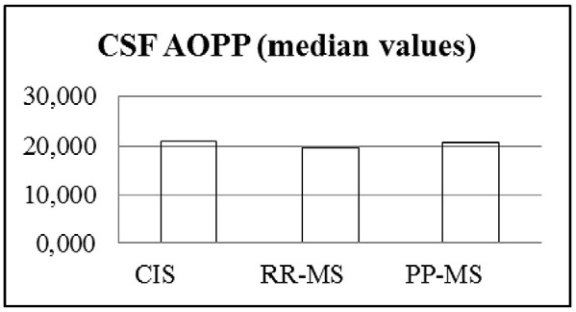


**Fig. 3.** The graph shows AOPP median values in the CSF of CIS, RRMS and PPMS patients.