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12-months prospective Pentraxin-3 and metabolomic evaluation in multiple sclerosis patients treated with glatiramer acetate

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ABSTRACT

Background: Pentraxin-3 (PTX-3) is involved in acute immunological responses and it is a pro-inflammatory protein and a novel biomarker of inflammatory diseases. It is demonstrated that PTX-3 is higher in cerebrospinal fluid (CSF) of aggressive Multiple Sclerosis (MS). Metabolomics, the identification of small endogenous molecules, offers a molecular profile of MS. Glatiramer acetate (GA) is a widely used treatment for (MS) but its mechanism of action is not completely defined. The aim of our study is to analyze PTX-3 and metabolomic profile in MS patients compared to controls and to investigate the effect of GA on PXT-3 and metabolic molecules during treatment in responder and not responder MS patients.

Methods: 28 unrelated MS patients and 27 age-and sex-matched controls were recruited. In serum, PTX-3 levels were measured by ELISA and Metabolomic panel was evaluated trough Nuclear Magnetic Resonance (NMR). According to clinical practice patients started GA treatment; PTX-3 and metabolomic identification were performed before and during treatment. Responders to treatment were identified if no evidence of instrumental, clinical relapses and disability progression (NEDA) occurred during follow up.

Results: Serum PTX-3 levels were higher in MS patients compared to matched controls (7,85 ± 2,19 vs 6,20 ± 1,63 ng/ml) (p = 0,03); metabolomic evaluation shows higher levels of lactate and lower levels of valine, tyrosine and tryptophan in MS patients compared to controls. During therapy, PTX-3 levels have been reduced statistically significant (p = 0,001) at six months and one year of treatment. After one year, of the twenty patients that completed the study, 55% were considered fully responders to treatment; in these patients the mean reduction of PTX-3 at one year was higher respect to not responders (−3,82 ± 1,24 ng/ml vs −2,32 ± 1,03 ng/ml p = 0,02) and we observed a higher reduction of lactate, tyrosine and hypoxanthine and an increase of hydroxyproline and ADP as well as of three oxidative phosphorylation markers, citrulline, ornithine and tryptophan approaching the metabolic profile of healthy subjects.

Discussion and conclusions: We demonstrated a metabolomic imbalance with mitochondrial dysfunction detected by higher levels of lactate and lower levels of tryptophan, tyrosine and valine in MS patients compared to healthy controls. The reduction of PTX-3 levels and the restoring of mitochondrial function, reducing oxidative stress by GA, allows to identify responder patients. Further and larger studies are needed to understand the predictive role of PTX-3 and metabolomic pattern in the identification of responder patients to GA.

1. Introduction

MS is an inflammatory demyelinating disease of the Central Nervous System (CNS) characterized by an autoimmune attack targeting myelin into the brain and spinal cord. The clinical disease course consists of relapses and remissions of neurological deficits (RRMS) followed in some cases and without treatments, by progressive disability (SPMS) (Sadiq, 2005). Since the introduction of disease modifying treatments (DMTs) the disease course has been modified reducing the occurrence of relapses, new lesions observed by magnetic resonance and therefore long-term disability (Hart and Bainbridge, 2016). Until now, clinical parameters as relapses, disability and MRI outcomes are used to assess disease activity and therapeutic response. Identification of predictive biomarkers of treatment response that serve as surrogate markers for

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assessing disease activity are needed to help an optimal treatment management strategy in patients with MS.

Pentraxin-3 (PTX-3) belongs to a superfamily of phylogenetically conserved multimeric proteins, that play a critical role in innate immunity and are generally considered acute phase inflammatory proteins (Fornai et al., 2016). Depending on the size of their primary structure, pentraxins are sub-classed into long and short forms (Agrawal et al., 2009). Between the short pentraxins, the most important are serum amyloid P component (SAP) and C reactive protein (PCR).

In contrast to the short pentraxins, PTX-3 is not produced and secreted by the liver, but locally at the site of inflammation. In response to several inflammatory stimuli, including toll like receptor (TLR) engagement or exposure to pro-inflammatory cytokines, PTX-3 is rapidly released by various cell types, as neutrophils, macrophages, microglia, dendritic cells, fibroblasts, and endothelial cells (Liu et al., n.d.). Apart from fulfilling the above antibody-like functions, PTX-3 plays also a role in regulating inflammatory pathways (Ummenthum, 2015).

Plasma PTX-3 levels are very low during physiological conditions (< 2 ng/ml in humans, < 25 ng/ml in mice), but rapidly increase during pathological conditions, reaching in humans 100–1000 ng/ml depending on the severity of inflammation (Rajkovica et al., 2016). Also, in autoimmune diseases Serum/plasma levels of PTX-3 seems to be higher than in normal control (Huang et al., 2016). Few studies explore the role of PTX-3 in MS; increasing levels were found in relapsing stage of MS with a slight correlation with Expanded disability status scale (EDSS) (Wang et al., 2020). Recently Magliozzi et al., found PTX-3 in the CSF of MS patients correlated with higher levels of gray matter (GM) damage at diagnosis (Magliozzi, 2018).

Metabolomic is a relatively new field that investigate the concentrations of multiple small molecules (< 1500 Da) in various biological matrices (serum, plasma, cerebrospinal fluid (CSF), urine, and tissue) represented by a vast number of molecules belonging to different classes of compounds such as amino acids, lipids, organic acids, nucleotides etc. (Duarte et al., 2014). Since metabolites are the products of different physiological and pathological processes, metabolomics could give more information also about the inflammatory processes and the damage caused by the inflammation itself. In this direction, nuclear magnetic resonance (NMR) spectroscopy-based metabolomics represents an important quantitative and highly reproducible metabolomic tool. Applications of NMR based metabolomics have increased in recent years and it is now widely used in toxicology, ecology, and epidemiology. To date, in MS, difference in metabolomic pattern between different forms of disease and between MS patients and controls have been reported: higher glucose and lower valine have been observed in MS group (Mehrpour et al., 2013) and higher lactate levels could differentiate active or inactive MS patients (Villoslada, 2017).

Glatiramer acetate (GA) (Copaxone®) is one of the first line treatment in MS, varyingly effective to reduce clinical and instrumental activity of disease (Martinelli et al., 2003). Actually, the exact mechanisms of action of GA is still unknow; it is a heterogeneous mixture of random-sized peptides composed of the four amino acid found in myelin basic protein, able to generate GA-specific immune responses binding to MHC molecules and consequent competition with various myelin antigens for their presentation to T cells (Fridkis-Hareli et al., 1999). Other mechanisms have been hypothesized involving the induction of T regulatory cells (Dhib-Jalbut et al., 2003) and expression of anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF-β) together with brain-derived brain immunomodulatory activity (Sarchielli, 2007).

To date there are no validated predictive biomarkers of response to GA treatment. Analyzing treatment responder patients, Venezuela et al. reported high IL-18 level at baseline and reduction of TNF-alpha over time as factors associated with a response to GA.

The aim of our study is to analyze PTX-3 and metabolomic profile in MS patients compared to controls and to investigate the effect of GA on PXT-3 and metabolomic molecules during treatment in responder and not responder MS patients.

2. Methods

This is a prospective study involving patients with a diagnosis of relapsing MS (RR-MS) recruited at MS Center of University of Campania "Luigi Vanvitelli".

The study was approved by the Hospital ethics committee, and all patients gave their informed consent. Inclusion Criteria were MS diagnosis according to the revised Mc Donald Criteria (Polman et al., 2011); age ≥ 18 and ≤ 65 years; treatment-free period of 1 month with immunoglobulins and/or steroids; need to start GA (Copaxone®) treatment as for clinical practice and AIFA criteria. Exclusion criteria were chronic disease of the immune system, other than MS; presence of metabolic or vascular diseases; active systemic bacterial, viral or fungal infections; pregnant or nursing (lactating) women. As controls, age-sex-matched healthy volunteers, were recruited among healthcare personnel, if they met inclusion criteria as age ≥ 18 and ≤ 65 years and if they did not have exclusion criteria as chronic disease of the immune system, infections, metabolic or vascular diseases and were not pregnant or nursing women. They gave their informed consent and were recruited for analysis on sera samples at basal time, after six months and after one year from basal time. For the patients included in the study, basal demographic and clinical data were collected. For clinical data were collected relapses (defined as the occurrence of new or recurrent neurological symptoms not associated with fever or infection lasting for at least 24 h (Hawkes and Giovannoni, 2010)) one year before starting treatment, disability status with expanded disability status scale (EDSS), and at baseline the patients were considered active if they had any relapses and activity on MRI (presence of contrast enhancement); the Bayesian Risk Estimate for MS (BREMS) score were evaluated (Bergamaschi, 2007). At baseline were collected data of lesions load in MRI (high if > 9 T2 lesions or low if < 9 T2 lesions) and presence of spinal cord lesions. MS course was defined as relapsing-remitting (RR) or progressive (including primary and secondary progressive) (Lublin, 2014). During the follow up of one year, as for clinical practice, patients were clinically evaluated every six months; clinical and radiological outcomes were assessed collecting relapses, MRI data as new or enlarging T2 lesions or occurrence of active lesions with gadolinium enhancement after six months and after one year of treatment, and disability status with EDSS every six months. Responder patients were considered if no evidence of disease activity occurred after one year (NEDA-3) expressing as no clinical relapses, no MRI activity and no EDSS progression after one year (Giovannoni et al., 2017). We collected sera of patients before starting GA therapy, after six months and after one year of treatment to evaluate PTX-3 value and metabolomic pattern.

3. Determination of PTX-3 levels in serum samples by ELISA

The quantitative determination of PTX-3 was performed using the Enzyme-Linked Immunosorbent Assay kit for PTX-3 (DIESSE research) based on pre-coated microplates with monoclonal rat anti-PTX-3 antibody MNB10. Each sample (20 µl) was added to the wells in which 100 µL of a dilution buffer (0.5%) of bovine serum albumin (BSA) in phosphate buffered saline (PBS) was previously dispensed. After two hours of incubation at 37 °C, the plates were washed four times with 300 µL of a washing solution (PBS), 150 µL of biotinylated rabbit anti-PTX-3 antibody was added. After another hour of incubation at 37 °C, four washings were performed, and 150 µL of streptavidin conjugated to horse radish peroxidase was added. After incubation for another hour at 37 °C, another four washings were made. Then, the absorbance was measured at 450 nm after 15 min of incubation at room temperature with 150 µL of substrate, blocking the enzymatic reaction with 100 µL

of stop solution (H2SO4 0.3 mol L). The sample determinations were performed in duplicate.

4. Serum ^{1}H NMR spectroscopy

The serum samples were prepared for NMR analysis by mixing 330 µl of serum with 300 µL of PBS (containing 10% *v*/v D2O) and 70 µL of reference standard D2O solution containing 0.1 mM sodium 3-trimethylsilyl [2,2,3,3-2H4] propionate (TSP). They were, then, inserted in a NMR tube. All the spectra were recorded using a BrukerAvance 600 NMR spectrometer operated at a 600.13 MHz 1H resonance frequency. To attenuate the broad NMR signals from slowly tumbling molecules due to lipids and proteins, a standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to record the 1D spin-echo spectra. To suppress the water peaks, the CPMG pre-saturation pulse sequence was used. In our experiment, the data points were acquired using 256 transients.

5. Data analysis

All of the ^{1}H NMR spectra were manually phased and baseline-corrected and referenced to the CH3 resonance of TSP at 0 ppm. The spectral 0.50–8.60 ppm region of ^{1}H NMR spectra was integrated in buckets of 0.04 ppm by AMIX package (Bruker, Biospin, Germany) excluding the water resonance region (4.5–5.2 ppm) during the analysis and normalized the bucketed region to the total spectrum area using Pareto scaling by MetaboAnalyst v4.0 tool [Xia, *J*. Sinelnikov, I. Han, B. &Wishart, D.S. (2015). MetaboAnalyst 3.0 - making metabolomics more meaningful. Nucleic Acids Research, 43, 251–257].

The principal component analysis (PCA) algorithm was applied to explain the maximum separation between the samples in the data. Score and loading plots were used to highlight and assess the role of X-variables (NMR signals) in the classification models and, hence, to prioritize the discriminating peaks for identification.

6. Statistical analyses

Data are presented as means ± standard deviation. Differences in the levels of PTX-3 between different subgroups were analyzed using the Mann-Whitney *U* test. The Wilcoxon signed-rank test was used to compare PTX-3 levels in the relapsing/progressive patients, active/inactive patients, presence or absence of spinal cord lesions and high or low lesions load in brain MRI. Correlations between serum PTX-3 levels and EDSS scores, disease duration, BREMS and relapses in the previous year were analyzed using Spearman's rank test. An analysis of variance for repeated measures was used to compared trend of PTX-3 in patients and controls during GA treatments. A Mann-Whitney *U* test was used to compare PTX-3 levels at six months and one year of patients NEDA + / NEDA- and the reduction delta of PTX-3 levels at six months in these two population. A p value < 0.05 was considered statistically significant. All statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

7. Results

7.1. Pentraxin-3 were increased in patient compared to controls

We included 28 patients and 27 matched age and sex controls; patients and controls did not differ for reactive C protein but as shown in Table 1 they have a statistically different PTX-3 levels resulting higher in patients with MS compared to controls.

Analyzing the MS populations, no difference in the PTX-3 levels were found in patients with high lesions load in MRI, spinal cord lesions and between active or inactive patients. We didn't find any differences between relapsing and progressive form of disease and for gender (Table 2). We didn't find correlations between PTX-3 and EDSS (p = 0,08), relapses in the previous year (0,91), disease duration (0,35) and BREMS (0,21). Then we investigated PTX-3 during GA therapy, and we found a significant reduction of PTX-3 levels for all MS patients during the first year of treatment, compared to controls; in particular the PTX-3 was 8,46 ng/ml at baseline and after six months of treatment decrease to 5,35 ng/ml and at one year remains stable at 5,38 ng/ml; this reduction was significative and the trend resulted different compared to controls (p = 0,001) (Fig. 1). After one year of GA treatment, the patients were divided in responder or not responder to treatment if no evidence of disease activity (NEDA-3) were detected at the end of follow up (one year); out of 20 patients that completed the year of treatment, 11 resulted NEDA + and 9 NEDA- but only 8 patients

Table 1
Demographic characteristics of patients and controls. Patients had significant increase of PTX-3 levels compared to controls (p = 0,03).

	Patients = **28**	Controls = **27**	
Mean age (ds)	41,67 (12,4)	40,03 (16,03)	P = 0,67
Sex (female)	78,5%	66,6%	P = 0,15
Mean PTX-3 (ng/ml) (ds)	7,85 (2,19)	6,20 (1,63)	P = 0,03
Mean PCR (mg/dl) (ds)	0,37 (0,57)	0,08 (0,07)	P = 0,14
Basal EDSS	1,6 (2,19)		
EDSS after one year	1,9 (2,18)		

Table 2
Clinical and instrumental features based on basal PTX-3 in evaluated patients.

Patients = 28	PTX-3	
Active (=8)	8,45 (1,86)	
inactive (=20)	7,61 (2,30)	P = 0,37
High lesion load (=18)	7,87 (1,52)	
Low lesion load (=10)	7,53 (1,52)	P = 0,49
+ spinal cord (=20)	7,99 (2,26)	
-spinal cord (=10)	7,50 (2,11)	P = 0,59
Relapsing (=22)	7,56 (2,20)	
Progressive (=6)	9,55 (1,16)	P = 0,07
Female (=22)	7,27 (1,89)	
Male (=6)	6,50 (2,43)	P = 0,25

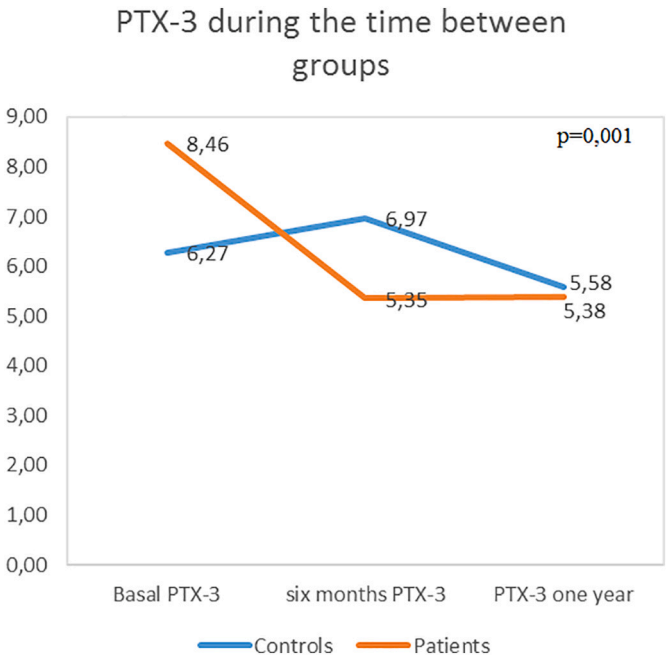

Fig. 1. GA effect on mean PTX-3 levels in patients compared to controls, we observed a reduction over the time and between groups with a significative interaction (p = 0,001).

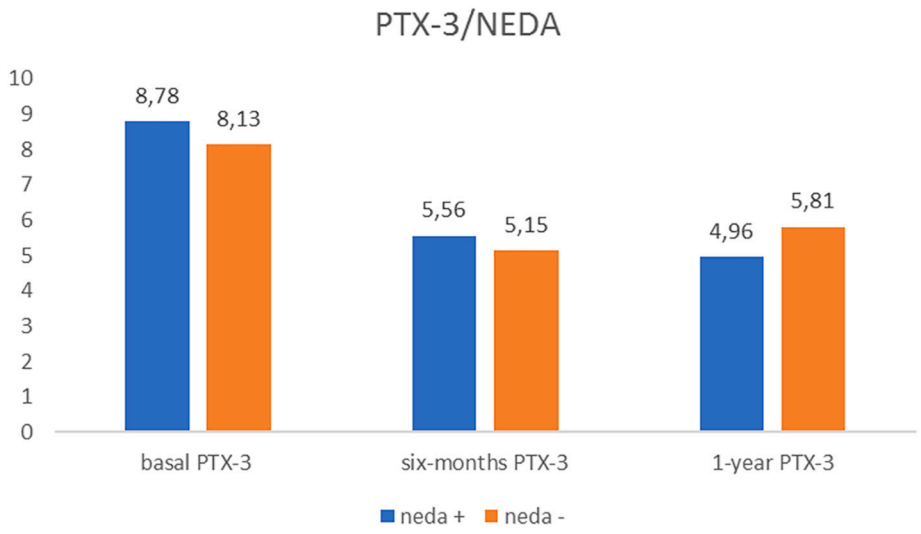

Fig. 2. Mean PTX-3 at baseline, six-months and one year of treatment in NEDA + and NEDA- patients. Delta reduction of PTX-3 after one year was higher in NEDA + compared to NEDA- (−3,82 vs −2,32 p = 0,02).

NEDA + and 8 patients NEDA- had a complete evaluation of PTX-3 at every time point (basal, six-months and one year). No differences in PTX-3 levels were detected at six months and at one year (Fig. 2), but the NEDA + patients had a higher reduction of PTX-3 at one year (−3,82 ± 1,24) compared to NEDA– patients in which we registered an increase of PTX-3 levels (−2,32 ± 1,03) (p = 0,02). Three out of the eight patients that no complete the study, withdrawals the treatment for adverse events, the remaining because no adherence.

7.2. Serum metabolomic analysis in MS patients respect to matched controls at baseline, after six months and one-year treatment

^{1}H NMR spectra were acquired on sera of 15 MS patients and 9 matched controls and they were analyzed statistically as reported in the Methods section. At baseline, PCA plot evidenced that MS patients compared to controls grouped into two different clusters, suggesting the presence of statistically different expression in some metabolites between the two groups (Fig. 3A). As evidenced by the loading plot that shows the significant metabolites selected by the PCA model (Fig. 3B), in MS patients the levels of lactate were higher whereas the levels of isoleucine, hydroxyproline, phenylalanine and 2-hydroxybutyrate as well as of three oxidative phosphorylation markers, valine, tyrosine and tryptophan were lower compared to those in controls. After six months, and after the immunomodulating treatment with GA the metabolomic profile of the patients change compared to baseline; we registered an increase of tyrosine, 2-hydroxybutyrate and valine similar expression of lactate, ornithine and lysine and a reduction in tryptophan expression (Fig. 4A and B).

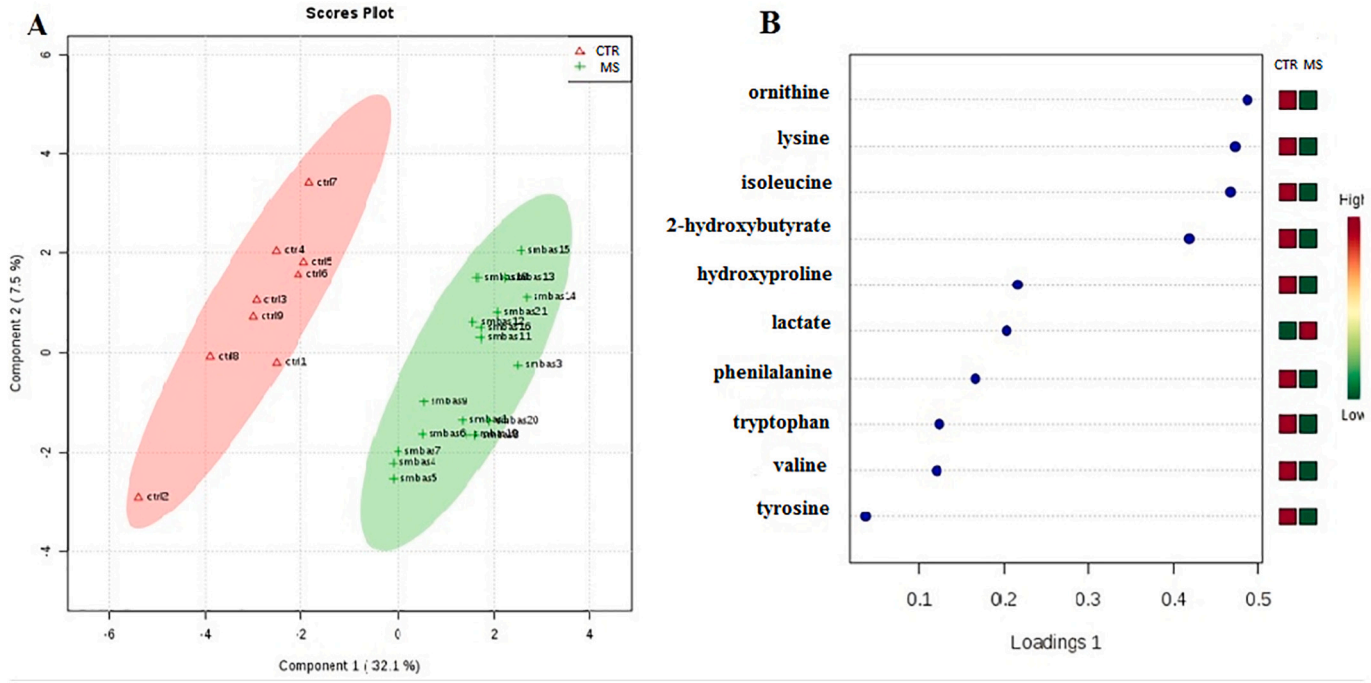

Fig. 3. Score plot (A) and loading plot (B) related to metabolomic profiling on sera of MS patients and matched controls. (A) An orthogonal partial least squares (OPLS) model plot of metabolomics data from a cohort of patients with MS and healthy controls. The plot shows separation of the two groups using multivariate analysis. (B) The Loading plot which shows the metabolites that are most important in driving the separation of the two groups.

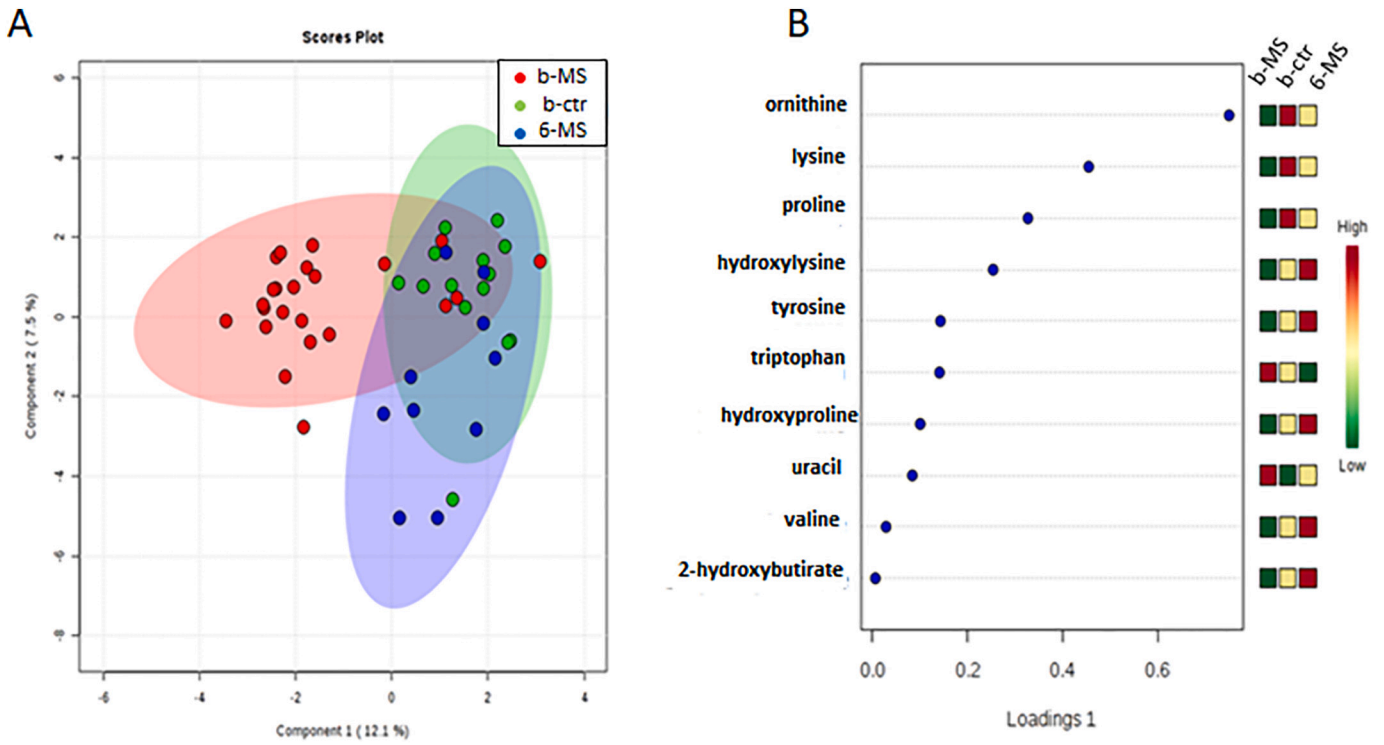

Fig. 4. Score plot (A) and loading plot (B) related to metabolomic profiling on sera of MS patients at baseline (b-MS), after six months (6-MS) and controls (ctr). (A) An orthogonal partial least squares (OPLS) model plot of metabolomics data from MS patients at baseline compared to controls and MS patients after six months. The plot shows separation of the two groups using multivariate analysis. (B) The Loading plot which shows the metabolites that are most important in driving the separation of the three groups.

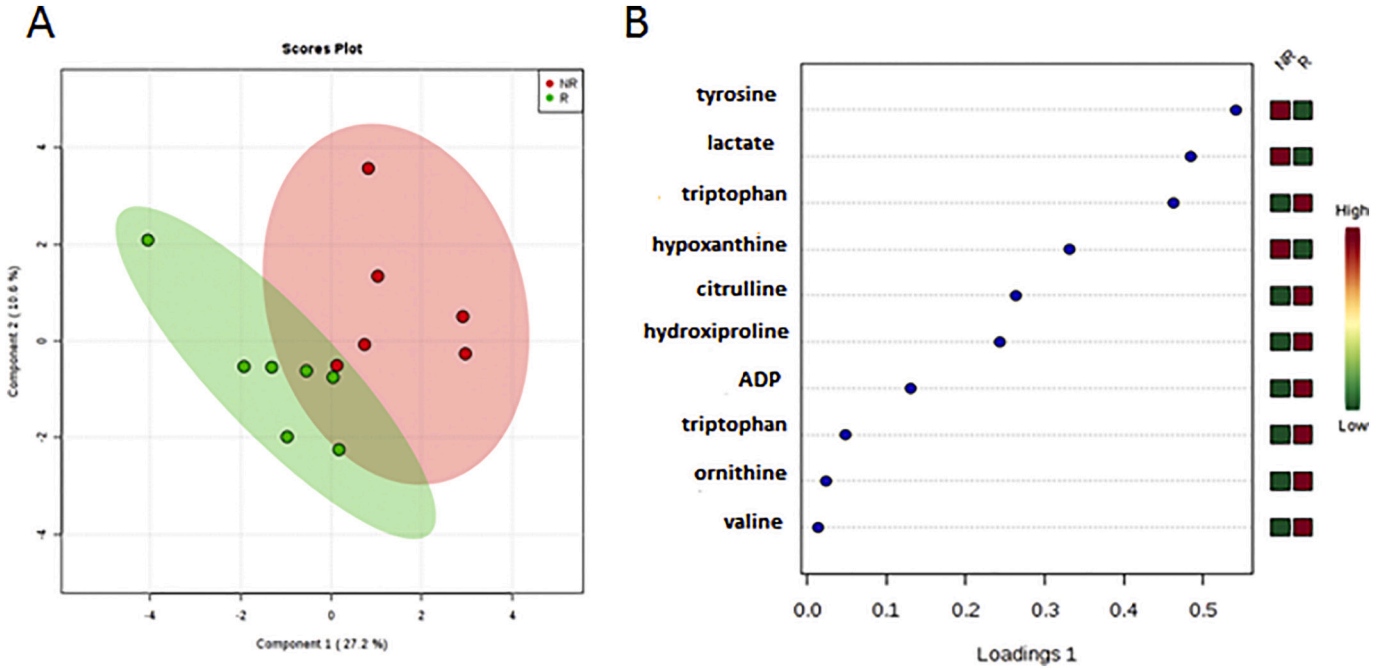

Fig. 5. Score plot (A) and loading plot (B) related to metabolomic profiling on sera of MS responder patients and not responder to treatment. (A) An orthogonal partial least squares (OPLS) model plot of metabolomics data from a cohort of responder patients with not responder patients. The plot shows separation of the two groups using multivariate analysis. (B) The Loading plot which shows the metabolites that are most important in driving the separation of the two groups.

After one year, 7 out of the 15 analyzed patients were considered responders to treatment; in these patients we observed a clear separation of the metabolomic profiling between responder and not responders patients (Fig. 5A): in particular, in responder patients was evident a higher reduction of lactate, tyrosine and hypoxanthine and an increase of hydroxyproline and ADP as well as of three oxidative phosphorylation markers, citrulline, ornithine and tryptophan approaching the metabolic profile of healthy subjects (Fig. 5B).

8. Discussion

PTX-3 is an inflammatory protein, key component of the humoral immunity (Bottazzi et al., 2010); few studies investigated the role of this protein and its potentially role in MS and the role in neuroinflammation is poorly understood; otherwise, it is well known that PTX-3 has the ability to modulates phagocytic activity of microglia, playing a role in adequate recovery into CNS (Jeon et al., 2010). Our study demonstrated and confirmed that PTX-3 levels are increased in patients with MS compared to healthy controls; other reports are in line with our results: Wang et al. reported higher levels of PTX-3 in patient with MS and neuromyelitis optica with a positive correlation with inflammatory activity of disease (higher levels were found in patients during relapses compared to remitting phase) (Wang et al., 2013) and a positive correlation during relapses with EDSS in MS patients. In our population, we found a higher mean value of PTX-3 in patients with baseline activity but this difference was not statistically significant; a positive but not significant trend were found also between PTX-3 and progressive form of disease and EDSS but probably the small cohort of patients did not allow us to find any difference. PTX-3 could be potentially be considered an inflammatory biomarker in MS participating to autoimmune process as demonstrated for other autoimmune diseases (Huang et al., 2016) but also a marker of CNS damage. Previous studies demonstrated that high plasma levels of PTX-3 are associated with increased mortality after stroke in humans (Van Horssen et al., 2012) and in acute Experimental autoimmune encephalitis (EAE), the animal model for MS, levels of PTX-3-encoding transcripts are increased in the spinal cord early in disease, also after recovery from neurological disease (Agnello et al., 2000); also in pre-active MS lesions increased levels of PTX-3 were prominently expressed by microglia/macrophages engaged in myelin phagocytosis in actively demyelinating lesions (Liu et al., 2007). In MS patients these data were confirmed by Magliozzi et.al, because PTX-3 were detected in the CSF of MS patients with higher levels of GM damage at diagnosis (Magliozzi, 2018).

In our study, during GA treatment, mean levels of PTX-3 significant decrease at six months and one year (stable compared with six-months value) probably due to immunomodulatory effect of GA and the reduction of inflammation affecting innate immune cells including macrophages and dendritic cells and subsequently production of PTX-3 (Liu et al., 2007); GA could also influence monocyte/macrophage polarization by shifting the balance from pathological M1 toward the M2 regulatory phenotypes interfering with PTX-3 release from microglia (Weber et al., 2007).

After one year of treatment we identify responder patients to treatment as NEDA; although we didn't find any difference in PTX-3 levels after six months and one year of treatment, but patients reached NEDA condition after one year had a higher reduction of PTX-3 levels after one year (delta reduction −3,82 ng/ml) compared to NEDA negative patients (−2,32 ng/ml). These results could lead us to hypothesize that the modulation of the PTX-3 by immunomodulating drugs such as GA could make us to early identify the patient most responsive to treatment, but other larger and longer study need to confirm these results.

In the second part of our study, we investigate the metabolomic pattern of patients compared to controls at baseline and the modification of the metabolomic expression after six months and one year of therapy. At baseline we can separate patients from controls by metabolomic profile because we identify high levels of lactate, isoleucine, hydroxyproline, phenylalanine and 2-hydroxybutyrate in patients compared to controls and lower levels of three oxidative phosphorylation markers as valine, tyrosine and tryptophan compared to those in controls. The increase levels of lactate and 2-hydroxybutyrate together with lower levels of valine could indicate that in MS patients there is an elevated oxidative stress caused by impaired Glutathione (GSH) metabolism as demonstrated in previous studies by Kim H·H et al. (Kim et al., n.d.). After six months, the expression of some metabolites change compared to baseline approaching the pattern of controls, with an increase of valine but continue reduction of tryptophan and similar levels of lactate. At six months our MS populations include responder and not responder to treatment, so probably we could see an initial modifications of metabolites expression but only an initial restoring of mitochondrial dysfunction. After one year of treatment we observed, in responder patients, a higher reduction of lactate, tyrosine and hypoxanthine and an increase of hydroxyproline and ADP as well as of three oxidative phosphorylation markers, citrulline, ornithine assuming a recovery of normal mitochondrial function approaching the metabolomic profile of controls. As we know recently mitochondria had received a lot of attention in the aetiology of MS suggesting that dysfunction may contribute to the disease underlying neurodegenerative process (Mao and Reddy, 2010). As a part of immunomodulating and protective effect of GA, we could speculate, in line with previous studies, a direct effect on mitochondrial dysfunction affecting T activated cells. Infact De Riccardis et al. demonstrated that GA in vitro could restore mitochondrial activity of T activated cells and their response to oxidative stress (De, 2016). About tryptophan, it is important to remember that alterations in its metabolism are suspected to be involved in the pathogenesis and progression of neurological disorders such as multiple sclerosis (MS) (Lovelace et al., 2016). This metabolite can be degraded through the kynurenine (Kyn) pathway, and chronically elevated Kyn/Tryptophan ratios have been reported in persons with MS (Lim et al., 2017). How GA can affect this metabolite is not fully understood; it could be a direct effect of the treatment or consequence of of the drug-induced reduction of inflammation.

Recently, some authors studied the association between altered tryptophan metabolism and pediatric MS risk. They, using global metabolomics data, demonstrated that higher relative abundances of Tryptophan were associated with lower risk of MS, and that increase in serum Tryptophan level was associated with decrease in adjusted odds of having MS (Nourbakhsh et al., 2018). Considering these data, we can suggest that the decrease of Tryptophan levels after one year of treatment can be index of restoring of Tryptophan metabolism in our MS patients. The principal limit of our results is the small sample size of the sample and larger and longer study need to confirm our study.

In conclusion we confirm that PTX-3 could be a potential biomarker of inflammation underlying progression of disease in MS. Although the results here reported are quite robust, further and larger studies are needed to define the predictive role of PTX-3 to identify responder patients to Glatiramer Acetate (Copaxone®). Finally, confirming the mitochondrial disfunction that characterizes and differentiates MS patients from controls we emphasize the potential action of GA to recovery a normal mitochondrial function ameliorating the neurodegenerative impact on the MS.

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Declaration of Competing Interest

Giacomo Lus and Elisabetta Signoriello received speaker honoraria and/or consultancy from Biogen, Teva, Genzyme, Merck, Novartis, Almirall, Roche.

Sara Casertano, Emilio Chiosi, Patrizia Iardino, Gianfranco Puoti, Domenico De Lucia and Alessia Pucciarelli have nothing to disclose.

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