Variations of uric acid levels and their clinical correlates during cladribine treatment

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INTRODUCTION

Experimental evidence supports an important role of oxidative stress in the pathogenesis of multiple sclerosis (MS) (Ljubisavljevic, 2014; Ortiz *et al.*, 2013; Pasquali *et al.*, 2015). In particular, in the initial relapsing MS phase, oxidative stress seems strictly associated with inflammatory activity, whereas in the progressive phase, neurodegenerative aspects of MS can further amplify oxidative damage (Ljubisavljevic, 2014; Martínez-Lapiscina *et al.*, 2014; M Moccia *et al.*, 2015; Pasquali *et al.*, 2015). Therefore, the measurement of oxidative biomarkers in accessible body fluids (i.e. serum) seems particularly promising in tracking the disease course.

In our recent study (Moccia *et al.*, 2019), supplementation with coenzyme Q10, a natural anti-oxidant (de Bustos *et al.*, 2000), along with Interferon-β1a 44mcg treatment, was associated with an improved oxidative balance and with related clinical benefits on fatigue and cognition. Oxidative balance was evaluated with a combination of markers of free radical scavenging activity (uric acid and bilirubin), markers of oxidative damage (8-hydroxydeoxyguanosine and protein carbonyls), and markers of neuroinflammation (IL-2, IL-4, IL-6, IL-10, TNF, Interferon-γ, and IL-17A). However, this study presented was limited by its short duration (6 months), not allowing conclusions on the impact of modifying the oxidative balance on disease evolution. Therefore, future observational studies should preliminary explore a limited set of biomarkers, and, afterwards, this set can be expanded towards a more comprehensive evaluation determining a more thoughtful understanding of disease mechanisms. We aim to test this hypothesis on retrospective data already collected in our database.

OBJECTIVES

We aim to explore retrospectively variations of uric acid levels in cladribine- and placebo-treated MS patients derived from clinical trials conducted in our centre, and their clinical associations. In our previous studies, uric acid resulted the biomarker being more suitable to detect meaningful variations in the oxidative balance (Moccia *et al.*, 2018, 2019), and is strongly associated with clinical measures (M Moccia *et al.*, 2015; Marcello Moccia *et al.*, 2015; Moccia *et al.*, 2019). Thus, our specific goals for this retrospective research project are:

1) measuring variations of uric acid levels during cladribine and placebo treatment;

2) evaluating how variations of uric acid levels are associated with variations in clinical outcomes during study duration.

METHODS

Study Design

This is a retrospective analysis on prospectively collected data recorded in the database of our MS centre. The "Federico II" ethical standards committee on human experimentation approved the study and written informed consent was obtained from all participants. Patients and assessor were blind to the use of cladribine during trial conduction.

Population

All patients for this analysis (cladribine and placebo) were derived from the CLARITY, OWARD, and ORACLE trials. At the Federico II MS Centre of Naples, we included 27 patients exposed to cladribine (or placebo) in ONWARD (n=7), CLARITY (n=10), and ORACLE-MS (n=10) (**Table 1**). Allocation to active treatment or placebo was performed centrally by clinical trial CROs (Contract Research Organization). Details on the trial design and results are fully reported elsewhere (Cook *et al.*, 2011; Giovannoni *et al.*, 2010; Leist *et al.*, 2014). Briefly, ONWARD and CLARITY are phase 2 and 3 clinical trials, respectively, including RRMS patients; ORACLE-MS is a phase 3 clinical trial including patients on their first clinical demyelinating event (either early RRMS or clinically isolated syndrome [CIS]). At the time of inclusion in the ORACLE-MS, patients already met 2010 criteria for MS diagnosis, as already described for 36.2% included patients (Freedman *et al.*, 2017; Leist *et al.*, 2014).

Table 1. Demographic and clinical features of included population. Follow-up duration and the reaching of different disease outcomes after study completion is reported.

	Placebo *(n=14)*	*Cladribine* *(n=13)*	p-values
Age, *years*	39.6 ± 10.9	39.4 ± 6.6	0.947
Sex, *female*	6 (42.8%)	9 (69.2%)	0.168
Disease duration, *years*	6.4 ± 8.2	7.7 ± 4.7	0.601
EDSS, *median (range)*	3.5 (1.5-5.5)	3.5 (1.5-5.5)	0.822
CLARITY/ONWARDS/ORACLE-MS	5/3/6	5/4/4	0.776

We did not perform sub-analyses on different cladribine regimens (3.5mg/kg or 5.25mg/kg) considering that they showed similar efficacy in clinical trials (Giovannoni *et al.*, 2010), and in light of sample size constraints. Allocation to active treatment or placebo was derived from unblinding procedures after trial termination or, when this was not available, on absolute lymphocyte count at two months after start of treatment (Comi *et al.*, 2019).

Primary and secondary endpoints

To explore retrospectively variations of uric acid levels in cladribine- and placebo-treated MS patients derived from clinical trials conducted in our centre, we extracted 3-month laboratory measurements of uric acid (using the UA2 with the COBAS® c501 analyser, Roche Diagnostic, Mannheim, Germany) from our database (**aim 1**).

In addition, to explore clinical correlates of variations of uric acid levels in cladribine- and placebo-treated MS patients, we extracted demographic (age, gender) and clinical features (disease duration, annualized relapse rate -ARR- in the previous 2 years and during the extracted period, expanded disability status scale -EDSS- recorded at different study points) (**aim 2**).

Statistics

Stata 15.0 has been used for data processing and analysis. Results have been considered statistically significant if $p<0.05$. Comparisons between placebo and cladribine treated groups were performed with χ^2 test, Fisher's exact test or t-test, as appropriate.

First, differences between treatment groups (cladribine vs placebo) in laboratory measures were explored using linear regression models including the variation in uric acid levels between baseline (before clinical trial inclusion) and average level while on treatment (**aim 1**). Results are presented as coefficient (Coeff) and 95% confidence intervals (95% CI). All the variables included in the model will be tested for multicollinearity (variance inflation factor [VIF] smaller than 2.5). Covariates included in the statistical models were age, gender, disease duration, baseline EDSS, creatinine, and clinical trial of inclusion.

Then, differences in reaching different clinical outcomes (e.g. 1-point EDSS progression, SP conversion, EDSS 6.0) in relation to laboratory measures were explored using linear regression models including the variation in uric acid levels between baseline (before clinical trial inclusion) and average level while on treatment (**aim 2**). Results were presented as coefficient (Coeff) and 95% confidence intervals (95% CI). All the variables included in the model were tested for multicollinearity (variance inflation factor [VIF] smaller than 2.5). Covariates included in the statistical models were age, gender, disease duration, baseline EDSS, creatinine, clinical trial of inclusion, and randomization to placebo or cladribine.

RESULTS

Demographic and clinical characteristics are reported in **Table 1**. At clinical trial inclusion, placebo and cladribine-treated patients were similar in age, sex, disease duration and EDSS, and were equally distributed between clinical trials.

Uric acid variations

Overall, we collected 166 uric acid measurements. All patients had at least one measurement before clinical trial inclusion and one at trial completion (6.1 measurement per patient on average. Placebo and cladribine-treated groups were similar in uric acid levels at baseline and follow-up (**Table 2**). A not-significant increase of uric acid levels was observed in the cladribine treated group (Coeff=0.631; 95%CI=-0.091/1.353; p=0.084) **(Figure 1).**

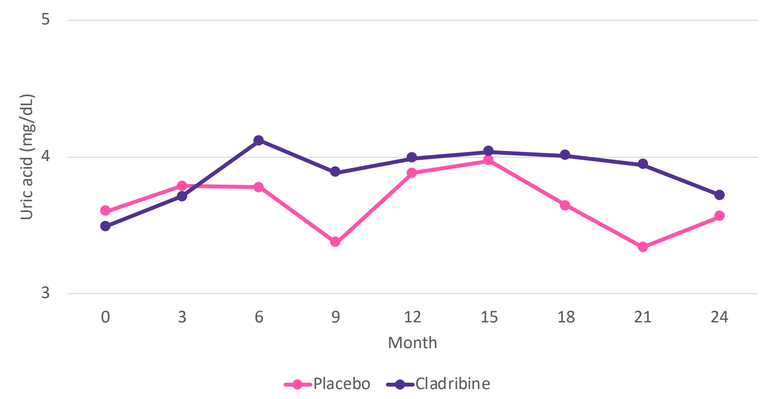
Table 2. Uric acid levels before clinical trial inclusion, at the end of the clinical trial and variation between baseline and average level while on treatment.

Uric acid *(mg/dL)*	*Placebo* *(n=14)*	*Cladribine* *(n=13)*	p-values
Before clinical trial inclusion	3.6 ± 0.9	3.5 ± 0.7	0.735
Average value during trial	3.5 ± 0.8	3.9 ± 0.9	0.329
Average variation during trial	-0.3 ± 1.2	0.3 ± 0.5	0.084

Clinical correlates

From a clinical point of view, no associations were found between uric acid variations and relapse occurrence (Coeff=-0.078; 95%CI=-0.943/0.786; p=0.851), ARR (Coeff=0.645; 95%CI=-1.084/2.375; p=0.443),1-point EDSS progression (Coeff=0.665; 95%CI=-0.240/1.570; p=0.140), SP conversion (Coeff=-0.258; 95%CI=-1.318/0.801; p=0.614), and reaching of EDSS 6.0 (Coeff=-0.258; 95%CI=-1.318/0.801; p=0.614).

Figure 1. Uric acid variations during clinical trials.



CONCLUSIONS

An improvement in the oxidative balance during cladribine treatment could have been hypothesized. Cladribine has a strong effect on inflammation and, subsequently, could reduce the release of oxidants, contributing to restoring anti-oxidant reserves (as measured by uric acid) (Giovannoni *et al.*, 2010; Giovannoni *et al.*, 2017; Leist *et al.*, 2014).

In conclusion, we failed to reach study outcomes. We noticed a trend towards increased uric acid levels in cladribine-treated patients, as from improved oxidative balance, but this did not reach statistical significance. Variations of uric acid levels were not associated with clinical features, independently from allocation to placebo or cladribine. This result would suggest the sample size is insufficient for the purpose of the study.

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