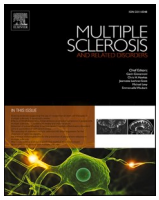
Multiple Sclerosis and Related Disorders 57 (2022) 103431

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard



Commentary

Changes in lymphocytes, neutrophils and immunoglobulins in year-1 cladribine treatment in multiple sclerosis

Antonio Luca Spiezia [a], Vincenza Cerbone [b], Eduardo Alberto Molinari [c], Nicola Capasso [a], Roberta Lanzillo [a], Antonio Carotenuto [a], Maria Petracca [a], Federica Novarella [a], Bianca Covelli [c], Giulia Scalia [b], Vincenzo Brescia Morra [a], Marcello Moccia [a,*]

[a] Multiple Sclerosis Clinical Care and Research Centre, Department of Neuroscience, Reproductive Sciences and Odontostomatology, Federico II University of Naples, Naples, Italy
[b] Centre for Advanced Biotechnology (CEINGE), Naples, Italy
[c] Department of Translational Medical Sciences, Federico II University of Naples, Naples, Italy

ARTICLE INFO

Keywords
Multiple sclerosis
Cladribine
Lymphocytes
Immunoglobulin

1. Introduction

Cladribine is a synthetic purine nucleoside analogue, approved for the treatment of active multiple sclerosis (MS). Cladribine mechanism-of-action encompasses selective cytotoxicity on circulating autoreactive B and T lymphocytes, which are thought to drive inflammatory demyelination and neuro-axonal loss in MS (Compston and Coles, 2008). Looking at clinical trial results, during cladribine treatment, there is a marked and long-lasting CD19 B-cell depletion and a rather modest T-cell depletion (Baker et al., 2017; Stuve et al., 2019; Comi et al., 2019). Ideally, cladribine only targets memory B cells (Moser et al., 2020), while plasma cells should remain unaffected (Jacobs et al., 2018), with normal immunoglobulin (Ig) levels, though never explored.

In our real-world study, we aim to evaluate changes in lymphocytes, neutrophils and immunoglobulins over the first 12 months of cladribine treatment.

2. Methods

2.1. Study design and population

This observational retrospective study has been conducted at the MS Clinical Care and Research Centre of the Federico II University Hospital of Naples, Italy, on prospectively collected data from Apr 2018 to Jun 2021. The study was approved by the Federico II Ethics Committee (355/19 and subsequent amendments). All patients signed informed consent authorizing the use of anonymized data collected routinely as part of clinical practice, in line with data protection regulations (GDPR EU2016/679). The study was performed in accordance with good clinical practice and the Declaration of Helsinki.

Inclusion criteria were: 1) diagnosis of MS; 2) year-1 dosing of cladribine tablets during the study period (as for clinical practice); 3) availability of clinical and laboratory data at baseline and after 2, 6 and/or 12 months.

Exclusion criteria were: 1) concomitant conditions affecting clinical and laboratory variables.

2.2. Clinical variables

At treatment start (baseline), we collected age, sex, disease duration, expanded disability status scale (EDSS), and previous disease modifying treatments (DMTs). For patients with previous DMTs, wash-out period was in line with clinical practice. During follow-up assessments (2, 6 and 12 months) we collected severe side effects, relapses and EDSS.

* Corresponding author at: Multiple Sclerosis Clinical Care and Research Centre, Department of Neuroscience, Reproductive Science and Odontostomatology, Federico II University, via Sergio Pansini 5, 80131 Naples, Italy.
E-mail address: marcello.moccia@unina.it (M. Moccia).

https://doi.org/10.1016/j.msard.2021.103431
Received 30 October 2021; Received in revised form 15 November 2021; Accepted 27 November 2021
Available online 29 November 2021

2.3. Laboratory variables

Laboratory variables were collected at baseline, 2, 6 and 12 months (before year-2 cladribine dosing). Laboratory procedures were performed in accordance with UK-NEQAS quality standards (https://ukneqas.org.uk/).

For serum immunoglobulins, we used nephelometry with a wavelength of 840 nm (BN™ II System, Siemens Healthcare, Erlangen, Germany), in accordance with manufacturer instructions. Reference curves were generated by multi-point calibration. Serum samples were automatically diluted 1:400 (IgG), 1:20 (IgA) or 1:5 in the low concentration assay (IgAs and IgMs).

For lymphocyte counts, we incubated an aliquot (50 μL) of anticoagulated ethylenediaminetetraacetic acid (EDTA) whole fresh blood (within 12 h) at 4 °C for 30 min in the presence of appropriate amounts of monoclonal antibodies. The mixtures were then diluted 1:20 in ammonium chloride lysing solution, incubated at room temperature for 10 min and finally washed prior to flow cytometric analysis (FACSCanto II flow cytometer, Becton Dickinson, San Jose, CA, USA). Samples were analysed on FACSDiva software (BD Bioscience, San Jose, CA, USA). The following antigens were analysed: CD4 PE (from BD San Diego, CA, USA), CD8 FITC (from BD San Diego, CA, USA), and CD19 APC (from BD San Diego, CA, USA). B and T-lymphocytes were gated on forward scatter (FSC) and side scatter (SSC) parameters, identifying 50,000 events. The lower level of detection was 10^{-4} (as such, zero corresponds to a level below 1/10,000 cells). For lymphocyte absolute count, we coupled cytometry to complete blood count on haematological counter (double platform). The same applied to neutrophil count.

2.4. Sample size

Considering a normal distribution of laboratory variables to be analysed on paired *t*-test, a two-sided tail, a 50% effect size (based on previous studies showing changes of lymphocytes during cladribine treatment) (Comi et al., 2019) and a 5% α error, our sample ($n = 62$) provides 97% power (Faul et al., 2007).

2.5. Statistical analyses

Results are reported as mean (±standard deviation), number (%), or median (range), as appropriate. Changes in laboratory variables between baseline and follow-ups were evaluated using paired *t*-test. Statistical analyses were performed with Stata 15.0. Results were considered statistically significant for $p<0.05$.

3. Data availability

Data available on request due to privacy/ethical restrictions.

4. Results

Demographics and clinical features are reported in **Table 1**. At descriptive level, over 12-months' follow-up, we observed relapses in 2 patients and EDSS increase in 4 patients. No severe side effects were reported.

Laboratory variables at baseline and follow-ups are reported in **Fig. 1**. Using baseline as reference, total lymphocyte count was lower after 2 ($p<0.01$), 6 ($p<0.01$), and 12 months ($p<0.01$) (**Fig. 1A**). Neutrophils were lower after 2 ($p = 0.01$) and 6 months ($p = 0.02$), but not after 12 months ($p = 0.91$) (**Fig. 1B**). We observed no changes in IgG, IgM and IgA over 12 months (**Fig. 1C, 1D, 1E**). CD19 B-cell count was lower after 2 ($p<0.01$) and 6 months ($p<0.01$), but not after 12 months ($p = 0.50$) (**Fig. 1F**). CD8 T-cell count was lower after 2 ($p = 0.01$) and 6 months ($p<0.01$), but not after 12 months ($p = 0.07$) (**Fig. 1G**). CD4 T-cell count was lower after 2 ($p<0.01$), and 6 months ($p<0.01$), but not after 12 months ($p = 0.08$) (**Fig. 1H**).

Table 1
Demographics and clinical features. Table shows baseline demographics, clinical features and previous DMT.

	MS patients treated with cladribine *(n = 62)*
Age, *years*	41.3 ± 12.7
Female, *number (%)*	40 (64.5%)
Disease duration, *years*	11.9 ± 9.6
Baseline EDSS	2.5 (1.5–4.5)
Previous DMTs	
None	7
Interferon beta/Peg-interferon beta/ Glatiramer acetate	6
Dimethyl-fumarate/Fingolimod/ Teriflunomide	22
Alemtuzumab/Natalizumab/Ocrelizumab	27

5. Discussion

In our real-world study, we observed a significant decrease in total lymphocyte count from 2 months after cladribine treatment start until the end of year 1. In particular, after 2 months, CD19 B-lymphocytes were reduced by −85%, CD4 T-lymphocytes by −50%, and CD8 T-lymphocytes by −40% of baseline levels. After 12 months (before year-2 dosing), we observed complete reconstitution of CD19 B-lymphocytes, while CD4 and CD8 T-lymphocytes remained depleted at −40% and −30% of baseline levels, respectively. On the contrary, immunoglobulin levels remained unchanged during year-1 cladribine treatment. The large majority of patients (90%) remained free from relapses and disability progression. Overall, our study provided similar, but not identical, lymphocyte profiles to those that have arisen from the trials, and also showed the lack of hypogammaglobulinemia, as in the emerging data from the MAGNIFY study (Wiendl et al., 2021).

Our results confirmed previous clinical trial data showing greater but more transient B-cell depletion, compared with T-cells (Baker et al., 2017; Comi et al., 2019). However, in our sample, T-cell depletion was more pronounced after 2 and 6 moths (up to −60% for CD4 T-lymphocytes; and up to −45% for CD8 T-lymphocytes), when compared with clinical trial patients (up to −45% for CD4 T-lymphocytes; and up to −30% for CD8 T-lymphocytes) (Baker et al., 2017; Comi et al., 2019), suggesting that the use of previous immunodepleting DMTs might cause more significant immunodepletion within first 6 months of cladribine treatment. Still, at 12 months (before year-2 dosing) results were largely comparable between our real-world data and clinical trials (Baker et al., 2017; Comi et al., 2019), suggesting similar immune-repopulation. We also confirmed a mild and quickly reversible effect on neutrophils (Moser et al., 2020).

The main novelty of our study is the assessment of immunoglobulins, which remained stable over year-1 cladribine treatment, notwithstanding changes in lymphocyte subsets, suggesting that memory B cells rather than plasma cells are preferentially susceptible to cladribine treatment. Indeed, cladribine active-metabolite is produced by deoxycytidine kinase and metabolized by 5′ nucleotidase, which are highly expressed by mature, memory and germinal centre B cells, but not by plasma cells (Baker et al., 2017; Jacobs et al., 2018; Ceronie et al., 2018). Interestingly, in a previous study, cladribine was showed to suppress intrathecal humoral response (oligoclonal bands), though this effect was exerted in the long term (10 years) (Rejdak et al., 2019), possibly as a result of depletion of new plasma cells' precursors. Our results are also in line with observed normal antibody production to COVID19 infection and vaccination in patients treated with cladribine (Achiron et al., 2021), further suggesting adequate humoral immune response.

A limitation of our study is the observational, retrospective and monocentric design. Furthermore, longer follow-up is needed to look at long-term laboratory changes. Our sample was quite heterogeneous (e.g., disease duration, previous DMTs), and sub-analyses would have been

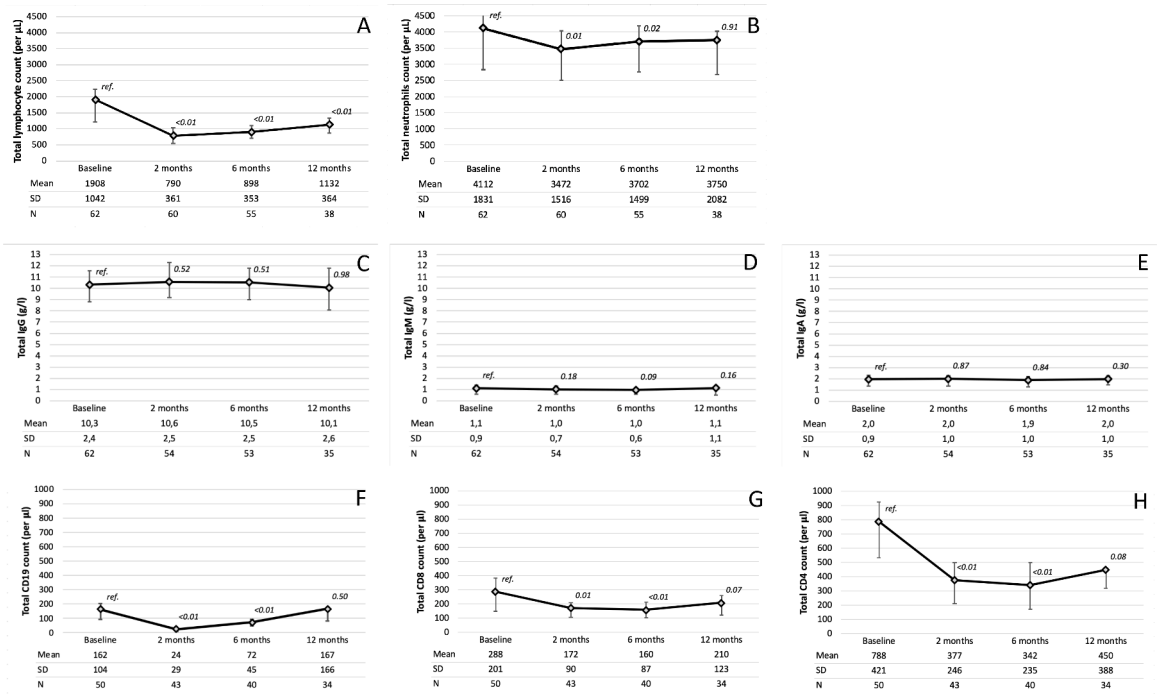


Fig. 1. Profile plots show changes in total lymphocyte count (**A**), neutrophils (**B**), IgG (**C**), IgM (**D**), IgA (**E**), CD19 lymphocytes (**F**), CD8 lymphocytes (**G**), and CD4 lymphocytes (**H**). Mean, standard deviation (SD), and number of observations (N) are reported for each time point. P-values are related to paired *t*-test using baseline as reference.

interesting, but not feasible due to sample size constraints. Also, we did not analyse relapses and EDSS statistically due to the small number of observations.

In conclusion, we showed similar but more pronounced depletion of B and T cells during year-1 cladribine treatment, when compared with clinical trial results, in the absence of changes in immunoglobulin levels. These results are relevant to the clinical practice in relation to the risk of infection and vaccine response.

Declaration of Competing Interests

The authors declare that they have no conflict of interest.

Funding

No funds, grants, or other support was received.

References

Achiron, A., Mandel, M., Dreyer-Alster, S., et al., 2021. Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. Ther. Adv. Neurol. Disord. 14, 1–8.

Baker, D., Herrod, S.S., Alvarez-Gonzalez, C., et al., 2017. Both cladribine and alemtuzumab may effect MS via B-cell depletion. Neurol. Neuroimmunol. NeuroInflamm. 4, e360.

Ceronie, B., Jacobs, B.M., Baker, D., et al., 2018. Cladribine treatment of multiple sclerosis is associated with depletion of memory B cells. J. Neurol. 265, 1199–1209.

Comi, G., Cook, S., Giovannoni, G., et al., 2019. Effect of cladribine tablets on lymphocyte reduction and repopulation dynamics in patients with relapsing multiple sclerosis. Mult. Scler. Relat. Disord. 29, 168–174.

Compston, A., Coles, A., 2008. Multiple sclerosis. Lancet 372, 1502–1517. https://doi.org/10.1016/S0140-6736(08)61620-7.

Faul, F., Erdfelder, E., Lang, A., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191.

Jacobs, B.M., Ammoscato, F., Giovannoni, G., et al., 2018. Cladribine: mechanisms and mysteries in multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 89, 1266–1271.

Moser, T., Schwenker, K., Seiberl, M., et al., 2020. Long-term peripheral immune cell profiling reveals further targets of oral cladribine in MS. Ann. Clin. Transl. Neurol. 7, 2199–2212.

Rejdak, K., Stelmasiak, Z., Grieb, P., 2019. Cladribine induces long lasting oligoclonal bands disappearance in relapsing multiple sclerosis patients: 10-year observational study. Mult. Scler. Relat. Disord. 27, 117–120.

Stuve, O., Soerensen, P., Leist, T., et al., 2019. Effects of cladribine tablets on lymphocyte subsets in patients with multiple sclerosis: an extended analysis of surface markers. Ther. Adv. Neurol. Disord. 12, 1–6.

Wiendl, H., Schmierer, K., Hodgkinson, S., et al., 2021. Characterization of Peripheral Immune Cell Dynamics and Repopulation Patterns in the First 12 Months of Cladribine Tablets Treatment: MAGNIFY-MS Study (2235). Neurology 96 (15 Supplement), 2235.