CSF biomarker profiles of MS patients treated with intrathecal methotrexate (ITMTX)

MSRCNY

Amir Touray, Violaine K. Harris, and Saud A. Sadiq
Multiple Sclerosis Research Center of New York, New York, NY USA

INTRODUCTION

Methotrexate is a dihydrofolate reductase inhibitor with anti-metabolite and anti-inflammatory properties. We have previously shown that intrathecal methotrexate (ITMTX) is a safe and well tolerated therapy in treatment unresponsive MS patients with progressive forms of the disease. In our initial study of 121 patients, ITMTX was administered every 2 months. Following 8 treatments, disease stabilization (EDSS stable or improved) was seen in over 80% of patients with both secondary progressive and primary progressive MS. In a separate ongoing study, we have seen disease stabilization over a period of 3 to 6 years during which patients have continued to receive ITMTX (16-42 treatments) (see poster # 515). The mechanism of action of ITMTX in MS patients who are unresponsive to other disease modifying agents is currently unknown.

OBJECTIVE

To analyze changes in CSF biomarkers in MS patients before and after pulsed ITMTX therapy.

DESIGN AND METHODS

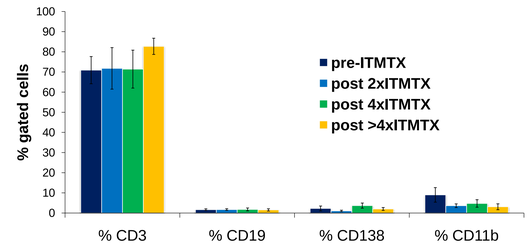
CSF was obtained from 15 patients prior to and after 5 to 9 ITMTX treatments. In addition, we also obtained CSF from 5 patients after 25 ITMTX treatments to determine long term effects of ITMTX therapy. CSF was immediately centrifuged at 200g for 15 min at 4ºC. Cells were analyzed by flow cytometry. Supernatant was quick frozen and stored in aliquots at -80ºC until assayed. All analytes were measured by Luminex or ELISA immunoassays.

Category	Biomarker
Cell populations	CD3+ (T cells), CD19+ (B cells), CD138+ (plasma cells), CD11b+ (macrophages)
Cytokines	IL-1β, IL-1Rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-16, IL-17, IL-21, IL-33, IFN-*g*, TNF-*a*, RANTES, G-CSF, GM-CSF, MCP-1, MIP-1b, LIF, GRO
Chemokines	CXCL12, CXCL13
Disease activity	Fetuin-A, osteopontin
Nitrosative stress	Nitric oxide (NO)
Axonal degeneration	Neurofilament heavy (NF-H) and light (NF-L)
Astrocyte markers	GFAP, S100B
Growth factors	PDGF-AA/BB, VEGF

Statistical significance was determined by paired student's t test.

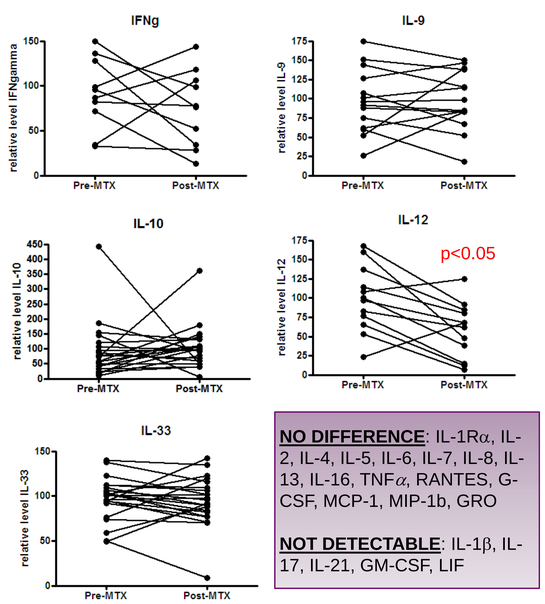
RESULTS

Figure 1. Flow cytometry analysis of CSF cell populations before and after ITMTX



➢***Percentage of CD11b+ cells are slightly reduced in the CSF after ITMTX treatments.***

Figure 2. Cytokine profiles in CSF before and after ITMTX

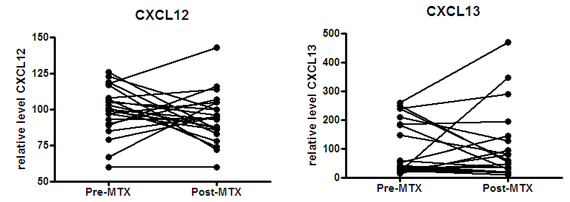


NO DIFFERENCE: IL-1Rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-13, IL-16, TNFα, RANTES, G-CSF, MCP-1, MIP-1b, GRO

NOT DETECTABLE: IL-1β, IL-17, IL-21, GM-CSF, LIF

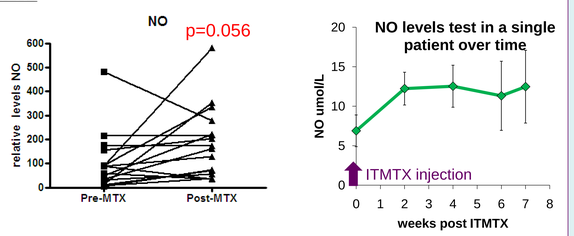
➢ ***CSF IL-12 levels were reduced in most patients following ITMTX treatment.***
➢***All other cytokine levels in CSF were largely unaffected after ITMTX therapy.***

Figure 3. Chemokine levels in CSF before and after ITMTX



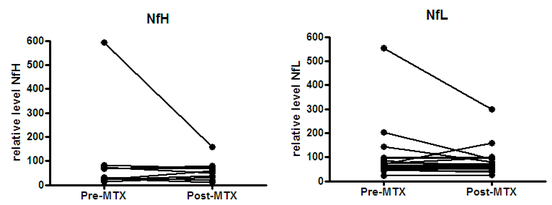
➢***No difference in CXCL12 or CXCL13 CSF levels in response to ITMTX.***
➢***ITMTX is unlikely to target lymphocyte chemotaxis.***

Figure 4. Nitric oxide levels increase slightly in CSF after ITMTX



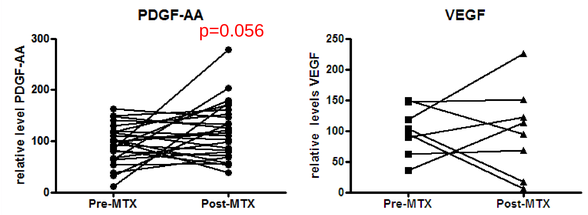
➢***There was a slight increase in CSF levels of NO after ITMTX treatment.***
➢ ***Weekly CSF analysis in a single patient also showed increased CSF NO levels after ITMTX treatment.***

Figure 5. No significant difference in CSF neurofilament levels after ITMTX treatment



➢***One outlier patient had very high levels of both heavy and light neurofilament in CSF that dropped after ITMTX.***
➢ ***In all other patients, we found no consistent trend in CSF neurofilament levels after ITMTX treatment.***

Figure 6. ITMTX results in slight increase in CSF PDGF-AA levels



➢***PDGF-AA levels in CSF increased (marginally significant) after ITMTX treatment. In the CNS, PDGF-AA plays a role in oligodendrocyte survival.***
➢ ***There was no difference in CSF levels of VEGF or PDGF-BB (data not shown) after ITMTX treatment.***

TESTED BUT NO DIFFERENCE: GFAP, S100B, Fetuin-A, osteopontin

CONCLUSIONS

- **Cytokine and chemokine levels in CSF were unaltered in response to ITMTX treatment (with the exception of IL-12). This result indicates that ITMTX affects progressive disease through non-immunosuppressive mechanisms.**
- **Preliminary experiments show that nitric oxide levels in CSF were increased in response to ITMTX. The beneficial contribution that elevated NO might have on progressive disease remains to be determined.**
- **In some patients, ITMTX may exert it's therapeutic benefit by slowing down axonal degeneration, as evidenced by changes in CSF neurofilament levels.**
- **ITMTX treatment results in increased PDGF-AA CSF levels, which may be a biomarker for oligodendrocyte survival.**
- **Overall, these findings identify candidate CSF biomarkers of ITMTX therapy, and suggest possible disease pathways by which ITMTX may benefit progressive MS.**

Amir Touray has nothing to disclose. Violaine Harris has nothing to disclose. Saud Sadiq has nothing to disclose. This work was supported by the Damiel Foundation, and by unrestricted MSRCNY funds.