High-dose methotrexate with leucovorin rescue: For monumentally severe CNS inflammatory syndromes

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ABSTRACT

Background: At sufficiently high doses, methotrexate (HDMTX) achieves substantial CNS penetration, whereas other tissues can be rescued from the effects of HDMTX by leucovorin rescue (LR), which does not penetrate the blood-brain barrier.

Objectives: To report on the efficacy and safety of HDMTX with LR (HDMTX-LR), in the treatment of acute demyelinating inflammatory CNS syndromes refractory to conventional immunotherapy.

Methods: We performed a retrospective chart review of 12 patients treated (6 multiple sclerosis [MS], 4 neuromyelitis optica [NMO], and 2 Sjogren's syndrome myelopathy [SSM]) with HDMTX-LR after failing to improve, or exhibiting worsening following conventional immunotherapy. 11 patients were followed for a total of 6 months following HDMTX-LR (one was lost to follow up after 1 month); and clinical findings were documented at 1 month, 3 months, and 6 months following HDMTX-LR therapy.

Results: Ten patients demonstrated both clinical and radiologic evidence of near, if not complete, abolishment of disease activity, in conjunction with impressive reconstitution of neurologic function in the 6-month period following HDMTX-LR. Mean Kurtzke Expanded Disability Status Scale (EDSS) prior to HDMTX-LR was 8.1 ($\pm$1.4). Following HDMTX-LR, mean EDSS was 6.6 ($\pm$2.4) at 1 month, 5.8 ($\pm$2.3) at 3 months, and 5.7 ($\pm$2.3) at 6 months.

Conclusions: In this retrospective assessment of treatment-recalcitrant fulminant inflammatory CNS syndromes, HDMTX-LR was observed to be a safe and highly effective treatment, producing the rapid and near complete cessation of disease activity, in conjunction with an important corresponding and 'durable remission' in the majority of our small treatment cohort.

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1. Introduction

Acute relapses of inflammatory demyelinating central nervous system (CNS) diseases such as multiple sclerosis (MS) and neuromyelitis optica (NMO) often result in lost productivity (missing work or school days), disability, emotional distress, reduced quality of life, adverse psychosocial effects, and risk of precocious mortality, as well as an increased utilization of healthcare costs, often over a highly protracted period; for some patients the remainder of their lives [1].

Acute exacerbations of inflammatory demyelinating CNS disorders can be treated with high-dose corticosteroids [2–8], or corticotrophin (ACTH) [9,10]. However, there are patients who do not respond to these typically utilized first-line therapies. In fact, only just over half of MS relapses show clinical (albeit typically incomplete) or radiologic improvement (reduced lesions, lesion size, and promoting the cessation of gadolinium enhancement) with high-dose corticosteroid monotherapy [3,8], strongly suggesting that more intensive preemptive treatment regimens should be considered for many if not most patients presenting with clinical and/or neuroradiologic characteristics indicative of a severe syndrome of inflammatory demyelination; particularly when the process is widely disseminated and impacting zones of neuroanatomic

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eloquence (e.g. optic nerve, spinal cord, cerebellum, and the brainstem tegmentum).

For the population afflicted with one of the monumentally severe inflammatory syndromes of the CNS, several alternative therapies exist, most typically plasmapheresis (PLEX) [11–13], and IVIG [14,15]. Plasmapheresis has been utilized in order to promote improvement in approximately 44% of patients who fail initially to respond to corticosteroid monotherapy [12,13,16].

Low-dose oral MTX has been used for long-term immunosuppression, with modest success in patients with NMO [17–19], and with unclear lasting benefits in MS [20–25]. In a small retrospective, open-label, chart review analysis, low-dose intrathecal MTX showed a modest benefit in progressive MS [26]. HDMTX has been used safely for the treatment of various malignancies, including lymphoma, acute lymphoblastic leukemia, and osteosarcomas in adults and children [27].

A major theoretical advantage of HDMTX is its potential to eradicate disease in immunologically-privileged sites, such as the CNS (which is in keeping with the use of HDMTX to treat CNS lymphoma and leptomeningeal leukemia) [28]. The steady-state blood:cerebrospinal fluid (CSF) ratio of MTX is about 30:1, and therefore, high serum levels of MTX (requiring IV administration of doses exceeding 1 g/m^2) are required to achieve therapeutic CSF MTX concentrations [29]. As such, HDMTX could theoretically eradicate not only circulating activated lymphocytes and immune cells, but also those within the CNS compartment itself.

To our knowledge, there have been no peer-review published studies to date on the application of high-dose intravenous methotrexate (HDMTX; which is defined as methotrexate (MTX) doses $>1 \text{ g/m}^2$) to treat acute inflammatory demyelinating CNS disorders that fail to adequately respond to conventional immunosuppressive therapies, whether employed as monotherapy, or as part of a multicomponent treatment regimen (e.g. high-dose corticosteroids, plasmapheresis, IVIG). Given the potential of HDMTX to deplete activated lymphocytes within the CNS, we investigated the impact of HDMTX-LR on patients with CNS demyelinating diseases that were refractory to traditional first-line therapies.

2. Methods

Institutional review board approval was obtained to conduct a chart review of all patients who were treated with HDMTX with LR (HDMTX-LR) in our institution. These were patients with acute inflammatory demyelinating CNS syndromes refractory to treatment with conventional immunotherapy (e.g. high-dose corticosteroids, PLEX).

2.1. Patient demographics

A total of 12 patients (11 females, 1 male) with a mean age of 43.3 (ranging from 31 to 53) years were included in this study. The study population consisted of 6 patients with MS, 4 with NMO, and 2 with Sjogren's syndrome-related myelopathy (SSM). The details of these patients are summarized in Table 1.

Almost all the patients were treated with at least 5 days of intravenous methylprednisolone (IVMP) at 1 g daily, and five treatments of PLEX; except for Patient 1 (who did not receive IVMP due to a history of corticosteroid-induced hip avascular necrosis two years prior), and Patient 2 (who received IVMP but not PLEX). Further, Patients 1, 8 and 11 also received IVCP and Patient 6 received IVIG (0.4 mg/kg/day for 5 days) (Table 1). Patient 10 was also treated with mitoxantrone.

All the patients were treated with HDMTX-LR because they failed to exhibit a satisfactory response (clinical and/or radiographic) to the aforementioned immunotherapies (Table 1). The mean duration between the time the patient received the final treatment of immunotherapy, and the time of administration of HDMTX-LR was 17.9 (ranging from 2 to 38) days.

2.2. Treatment protocol

Prior to administration of HDMTX-LR, urine specific gravity was documented to be < 1.1010, active infections were ruled out, and serum creatinine (SCr) was documented to be below 1.4 mg/dL. Complete blood count (CBC), SCr, and liver function tests (LFTs) were likewise assessed prior to the administration of HDMTX-LR.

In order to avoid MTX crystalline deposition in the kidneys, D5W and sodium bicarbonate 100 mEq were administered intravenously at a rate of $150 \text{ mL/m}^2/\text{h}$ for at least 8 h prior to administration of HDMTX-LR to ensure that urine pH was at least 8.0 before and during the entire HDMTX-LR infusion. MTX 2500 mg/m^2 in 250 mL of normal saline was administered intravenously over 2 h. Precisely 12 h after the start of the MTX infusion, leucovorin 80 mg was given intravenously; subsequently, leucovorin 35 mg was administered intravenously every 6 h for a total of 12 doses. Serum MTX concentrations were checked at 24 h, 48 h, and 36 h after administration of HDMTX. The patient could be discharged only when the serum MTX concentration fell below 0.05 μmol/L (usually requiring a period of 24–72 h).

Nausea and vomiting tend to occur during or shortly after HDMTX-LR administration [28]. Anti-emetics (e.g. ondansetron) were given 30 min before the infusion and as needed thereafter. Dextromethorphan was administered as needed to treat infusion-related headaches. CBC, SCr, and LFTs were checked one week and four weeks after HDMTX-LR; with identified derangements henceforth mandating more frequent monitoring and further investigation where indicated.

Penicillin derivatives, probenecid, fluoroquinolones, sulfonamides, aspirin, and non-steroidal anti-inflammatory drugs decrease renal excretion of MTX and were avoided during HDMTX-LR treatment [29]. Vancomycin and trimethoprim/sulfamethoxazole were avoided immediately prior to, and during HDMTX administration to avoid potential additive or synergistic nephrotoxicity, and possible myelosuppression [29].

2.3. Patient assessment

Neurological examinations were documented prior to HDMTX-LR administration, and at 1 month, 3 months, and 6 months after HDMTX-LR. Kurtzke Expanded Disability Status Scale (EDSS) scores were derived from these examinations (Table 2). Magnetic resonance imaging (MRI) findings, specifically gadolinium-enhancing lesions and volume distribution of vasogenic edema, were documented before and after HDMTX-LR treatment.

The patients were followed for 6 months following HDMTX-LR therapy, except for patient 4 who did not return to our clinic following her 1-month post-HDMTX-LR visit. Patient 11 deteriorated one month after receiving HDMTX-LR and was advanced to alemtuzumab, as part of an effort to further control her disease. Patient 12 refused post-HDMTX-LR MRI due to financial reasons.

2.4. Adverse effects

Patients 1, 11, and 12 developed nausea that resolved within 24 h of HDMTX-LR infusion. Patient 3 described a transient tingling in her extremities that occurred during the infusion. Patient 7 experienced nausea and headaches that resolved two days after the infusion. Patient 5 developed pneumonia one week after HDMTX-LR. However, since she had been intubated and mechanically ventilated for a prolonged period (3 weeks) prior to our treatment intervention, we believe that this was most consistent with ventilator-associated pneumonia, rather than being associated with HDMTX-LR treatment.

3. Results

The mean EDSS prior to HDMTX-LR was 8.1 (5–9.5) (Table 2). All of the patients (except Patients 2 and 7 who had SSM) demonstrated

Table 1
Patient demographics, clinical history & physical findings, and immunosuppressive therapy prior to HDMTX-LR.

Patient	Age & sex	Disease	Clinical history & physical findings	Immuno-suppressive treatment prior to HDMTX-LR	Duration between final immune-suppressive treatment & HDMTX-LR
1	31 M	MS	One year after stopping IFNB on his own, he presented with paraplegia, right upper extremity weakness, and urinary and bowel incontinence. In the past, he was treated with IVMP for a relapse but developed bilateral hip avascular necrosis and used a cane to ambulate.	5 treatments of PLEX followed by IVCP 1 mg/m^2	27 days
2	50 F	SSM	Patient with Sjogren's syndrome on MMF presenting with two years of progressive lower extremity weakness, numbness, paresthesia. She had intermittent treatments with IVMP without any improvement.	IVMP 1 g daily for 5 days	38 days
3	34 F	NMO	She was admitted with progressive quadriparesis, bilateral vision loss, dysarthria, dysphagia, urinary incontinence, and severe constipation; a diagnosis of NMO was made after a thorough workup. She developed respiratory failure and had to be intubated.	IVMP 1 g daily for 5 days followed by 5 treatments of PLEX	3 days
4	53 F	MS	She had stopped using GA in anticipation for escalating to NTZ (due to breakthrough relapses). However, she subsequently decided to remain off disease-modifying therapies. Four months later, she was admitted for quadriplegia and visual loss.	IVMP 1 g daily for 5 days followed by 5 treatments of PLEX	14 days
5	45 F	MS	During the three-month "washout" period of transitioning from fingolimod to DMF, she developed quadriparesis, diplopia, and sphincteric dysfunction. She continued to deteriorate despite treatment with IVMP and PLEX.	IVMP 1 g daily for 5 days followed by 6 treatments of PLEX	15 days
6	32 F	NMO	Four months prior to presentation to our institution, she developed complete paraplegia and received IVMP. She partially improved but a week later, was paraplegic again. IVMP was repeated and she partially improved. A month later, she became paraplegic and underwent PLEX followed by IVIG but deteriorated and progressed to quadriplegia, dysarthria, and encephalopathy. She was then transferred to our institution for HDMTX.	IVMP 1 g daily for 5 days (twice) followed by 8 treatments of PLEX, and subsequently IVIG 0.4 mg/kg/day for 5 days	24 days
7	49 F	SSM	Four-year history of progressive lower extremity paresthesia, pain, and weakness despite being on AZA. During this duration, she had been treated multiple times with IVMP without any improvement. She continued to deteriorate despite being put monthly IVCP 1 g/m^2 (total of 6 months). Due to continued deterioration, a decision was made for HDMTX.	IVCP 1 g/m^2 monthly pulse therapy	30 days
8	49 F	NMO	Three months prior to admission her vision deteriorated, and subsequently she developed dysarthria, tremors, left trigeminal neuralgia, left INO, ataxia, bilateral numbness, urinary incontinence, severe constipation, and quadriparesis. Despite treatment with IVMP, PLEX, and IVCP, she developed dysphagia, quadriplegia, aphasia, and respiratory failure (requiring mechanical intubation).	IVMP 1 g daily for 5 days followed by 7 treatments of PLEX, and subsequently 2 infusions of IVCP 750 mg/m^2	5 days
9	42 F	MS	She was on IFNB and 3 months before presentation, she developed right hemiparesis, ataxia, bilateral upper extremity numbness, right visual impairment, and dysarthria. She partially improved after treatment with IVMP and PLEX but worsened two weeks later and became wheelchair bound.	IVMP 1 g daily for 5 days followed by 5 treatments of PLEX	16 days
10	46 F	MS	A year prior to her current admission, she had a large tumefactive right hemispheric lesion that resulted in left hemiparesis. A brain biopsy demonstrated findings consistent with MS (Fig. 2). She was treated with IVMP and PLEX, and recovered except for mild left hemiparesis. During her current admission, she developed global aphasia, confusion, and worsening left hemiparesis. Despite treatment with IVMP, PLEX, and mitoxantrone her clinical and radiologic findings worsened.	IVMP 1 g daily for 5 days followed by 5 treatments of PLEX, and subsequently mitoxantrone (12 mg/m2) for a single dose, given that there was no effect upon the lesion or its enhancement pattern after a period of almost 4 weeks.	7 days
11	35 F	NMO	Three months prior to presentation, she suffered subacute paraplegia with painful leg paresthesia, which progressed to bladder dysfunction, constipation, left eye blindness, and intractable vomiting and hiccupping.	IVMP 1 g daily for 5 days followed by 5 treatments of PLEX, and subsequently 2 infusions of IVCP 1 mg/m^2	34 days
12	53 F	MS	She had been stable on NTZ for several years, but suffered a relapse resulting in left upper extremity weakness, and paraparesis, ataxia, and falls.	IVMP 1 g daily for 5 days followed by 5 treatments of PLEX	2 days

AZA – azathioprine; DMF – dimethyl fumarate; F – female; GA – glatiramer acetate; IFNB – interferon-beta; INO – internuclear ophthalmoparesis; IVCP – intravenous cyclophosphamide; IVIG – intravenous immunoglobulin; IVMP – intravenous methylprednisolone; M – male; MMF – mycophenolate mofetil; MS – multiple sclerosis; NMO – neuromyelitis optica; NTZ – natalizumab; PLEX – plasmapheresis; SSM – Sjogren's syndrome-related myelopathy.

Table 2
EDSS prior to, and 1, 3, and 6 months following HDMTX-LR.

Patient	Disease	Pre-HDMTX-LR	Post-HDMTX-LR			Post-HDMTX-LR-months disposition
			1 month	3 months	6 months	
1	MS	7.5	7.5	6.5	6.5	12-Stable
2	SSM	6.5	2.5	2.5	2.5	7-Exhibiting insidious progression in gait decline
3	NMO	9	7.5	6.5	6	12-Stable
4	MS	9	7.5	Lost to follow-up		Lost to follow-up
5	MS	9.5	9.5	9.5	9.5	12-Stable
6	NMO	9	8	4.5	4.5	12-Stable
						On Cellcept
7	SSM	7.5	6.5	6.5	6.5	8-Exhibiting insidious progression in gait decline
8	NMO	9.5	7.5	7.5	7.5	12- Relapse at 1 year post Rx
9	MS	7.5	4.5	4.5	4.5	8-Relapse despite transition from MTX to Tysabri.
10	MS	9.5	7.5	7.5	7.5	12 Months-Stable
11	NMO	8	9	Given alemtuzumab		Alemtuzumab administered 1 month after MTX; exacting remission for >2 years
12	MS	5	2	2	2	12-Stable on Gilenya
Mean EDSS		8.1 ± 1.4	6.6 ± 2.4	5.8 ± 2.3	5.7 ± 2.3	

multiple gadolinium-enhancing lesions on brain and/or spine MRI prior to receiving HDMTX-LR.

Ten patients (83.3%) demonstrated improvement in their EDSS at 1, 3, and 6 months following HDMTX-LR. The mean EDSS was 6.6 at 1 month, 5.8 at 3 months, and 5.7 at six months following HDMTX-LR therapy (Table 2). Improvements in ambulation were also observed. Those who were *bed-bound* (Patients 3, 6, 8, and 10) were eventually able to use a wheelchair, rolling walker, or cane; Patient 6 was quadriplegic before HDMTX-LR but was able to walk without any aids six months after being treated (Supplementary Table).

Wheelchair-bound patients (Patients 1, 7, and 9) were eventually able to use rolling-walkers, or even ambulate without aids (Patient 9). Patients 2 and 12 demonstrated objective improvements in the timed 25-foot walk. Notably, Patient 10, who had global aphasia prior to HDMTX-LR, had completely normal language function 1 month after being treated. Other neurological deficits that improved following HDMTX-LR included corrected visual acuity, muscle strength, ataxia, mental status, and sensory loss. These findings are summarized in the Supplementary Table.

3.1. Characterizing neuroimaging features of fulminant CNS inflammatory syndromes

Of the patients who had gadolinium-enhancing lesions on MRI, 8 had complete or near complete resolution of these lesions, confirmed upon post-HDMTX-LR MRI investigations. Specifically, Patients 3, 6, 9, and 10 exhibited *complete resolution of all enhancing lesions*, whereas Patients 1, 4, 5, and 8 had near complete resolution of the enhancing lesions following treatment with HDMTX-LR (Fig. 1). Patient 2 had no gadolinium-enhancing lesions on her pre-HDMTX-LR MRI; nonetheless, an MRI performed 6 months after receiving HDMTX-LR demonstrated complete resolution of the T2-hyperintense cervical cord lesions seen on the MRI before treatment. Patient 7 had areas of abnormal T2 signal in the cervical spinal cord with no areas of gadolinium-enhancement; she demonstrated remarkable clinical improvements following HDMTX-LR treatment, and her radiologic findings remained stable. Large areas of vasogenic edema surrounding the lesions in Patients 9 and 10 also resolved following HDMTX-LR treatment (Fig. 1). Alternately, we observed radiologic worsening in Patient 11, despite the application of our intensive treatment regimen. MRI findings for all patients are summarized in Table 3.

3.2. A putative histopathologic prognostic signature of fulminant CNS inflammatory syndromes?

A brain biopsy (of a right fronto-parietal lesion) of Patient 10 was performed before HDMTX-LR treatment to rule out a possibly underlying neoplastic process. Representative biopsy slides are presented in Fig. 2. The H&E sections showed confluent macrophage infiltration suggesting either organizing necrosis (as seen in patients with an infarct) or active demyelination. Application of CD68 stains, were employed to highlight the cytoplasm of the infiltrating macrophages (to assess for ingestion of tissue injury fragments; such as myelin). The finding of greatest histopathological conspicuity however, was that of the broad landscape of intact axons designated pathologically as 'relative sparing' of axons', and whose molecular identification was confirmed with the application of neurofilament protein-stained sections. This stereotyped observation, across different sections was particularly salient in that such axonal preservation was identified despite their intimate juxtaposition to a myriad of activated macrophages. If replicated in other patients with similar syndromes, this may support the utility of a strategy of systematic biopsy assessment of tissue injury, for the purpose of characterizing inter-patient, histopathological heterogeneity, and to correspondingly elucidate individualized prognostic signatures, with implications for the formulation of commensurately intensive treatment in order to promote neuroprotection and potentially even repair and clinical restoration.

3.3. Variable response characteristics

For Patient 5, her clinical findings and EDSS stabilized but did not improve following HDMTX-LR therapy; however, there was resolution of all of her gadolinium-enhancing lesions except for a single lesion in her left postcentral gyrus. Only Patient 11 exhibited unmistakable worsening in her clinical and radiological findings one month after HDMTX-LR treatment, and was henceforth treated with alemtuzumab.

4. Discussion

For the first time, to our knowledge, we report in a retrospective analysis, that HDMTX-LR was effective in producing remission induction, with respect to both clinical and neuroradiologic features of disease activity; in conjunction with subsequent improvements in neurologic status for the majority of patients harboring monumental acute inflammatory demyelinating syndromes (specifically secondary to MS, NMO, and SSM), and who were designated as treatment-recalcitrant to conventional immunotherapy by the treating neurologist. Further, the HDMTX-LR protocol was very well tolerated, with no cases of significant morbidity or mortality attributable to this treatment strategy.

Almost all of our patients had considerable disability (EDSS equal to or >6) that failed to improve with IVMP and PLEX (and some even receiving IVCP and mitoxantrone). Alternately, following treatment with HDMTX-LR, the majority of our small cohort, demonstrated accelerated objective clinical and radiological evidence of remission-exacting effects of this intensive treatment strategy. As such, it may be preferable that
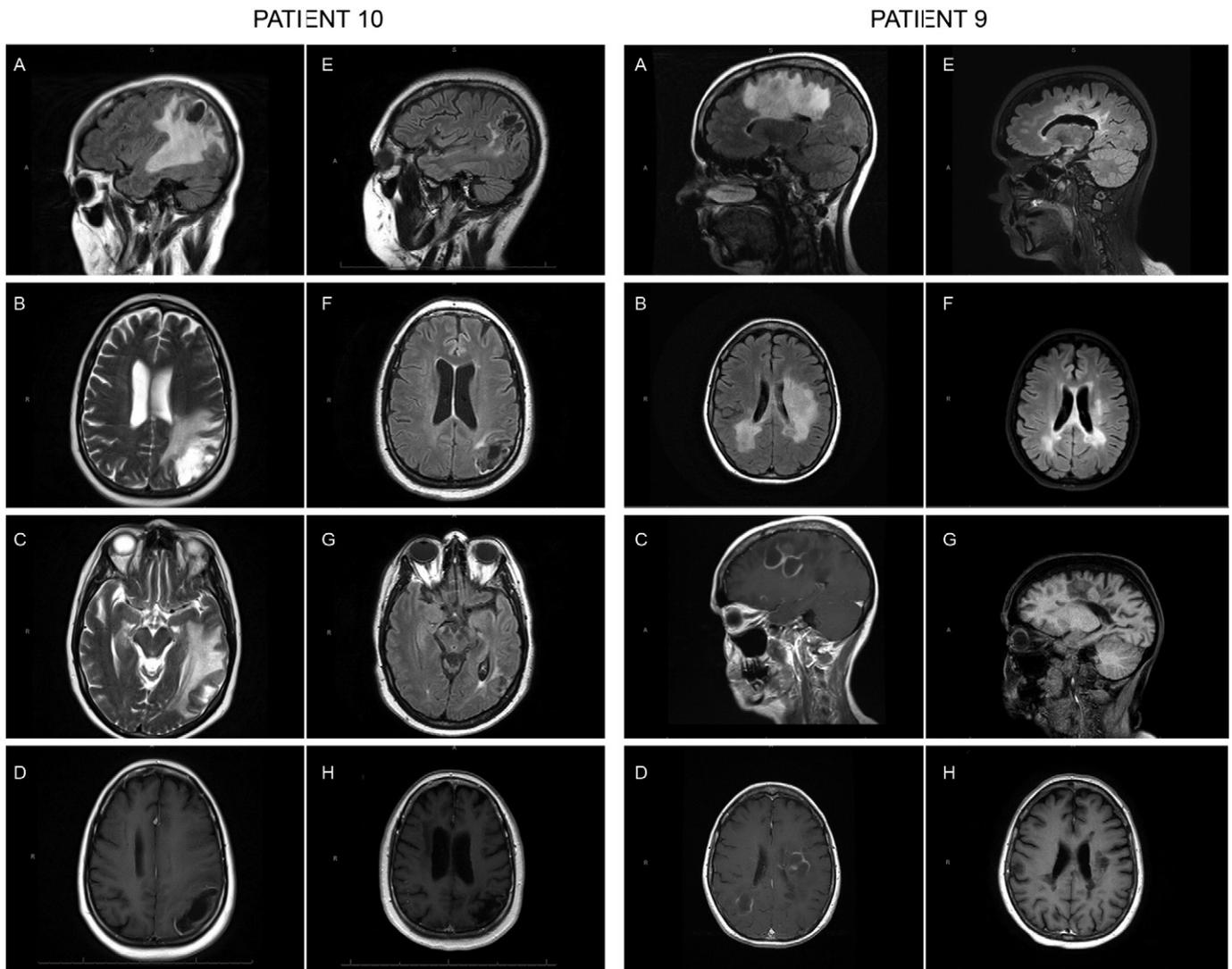
PATIENT 10   PATIENT 9

Fig. 1. MR images in patients 9 and 10 before and after HDMTX-LR. Brain MRI for patient 10 before (A–D) and 1 month after (E–H) HDMTX-LR. A large tumefactive lesion with vasogenic edema in the left parieto-temporo-occipital lobe demonstrates abnormal FLAIR (A) and T2 (B & C) signal, and gadolinium-enhancement (D) with mass effect. One month after HDMTX-LR, resolution of vasogenic edema and improvement of abnormal FLAIR/T2 signal (E, F & G), as well as resolution of gadolinium-enhancement (H) is observed. Brain MRI for patient 9 before (A–D) and 3 months after (E–H) HDMTX-LR. A tumefactive lesion with vasogenic edema in the left and right parieto-temporo-occipital lobes demonstrates abnormal FLAIR signal (A & B) and gadolinium-enhancement (C & D). Three months after HDMTX-LR, resolution of vasogenic edema and improvement of abnormal FLAIR signal (E & F), as well as resolution of gadolinium-enhancement (G & H) is observed.

immunosuppression and HDMTX-LR be considered for administration as close as possible to the inception of monumentally inflammatory demyelinating syndromes.

4.1. Characterizing therapeutic and safety mechanisms of HDMTX-LR treatment

The predominant mechanism by which HDMTX-LR effectively treats acute inflammatory demyelinating CNS syndromes is by disrupting folate-dependent DNA and RNA synthesis, and thereby rapidly eliminating activated mononuclear cells (in the circulation and within the CNS). However, MTX also has additional anti-inflammatory effects. By promoting intracellular accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), MTX promotes adenosine release at sites of inflammation, which exerts a potent anti-inflammatory effect [33].

Upon entering the cell via the chemically reduced folate carrier, MTX undergoes polyglutamation (catalyzed by folyl-polyglutamate synthetase). Once polyglutaminated, MTX is retained intracellularly for a prolonged period [27,28]. MTX and its polyglutamates competitively

inhibit dihydrofolate reductase (which reduces folic acid to tetrahydrofolate), blocking the formation of tetrahydrofolate, which is a critical cofactor for the de novo synthesis of purines and pyrimidines (making this agent an ideal cell-cycle synthesis phase inhibitor; S-phase), the formation of polyamines, and the transmethylation of phospholipids and proteins [27–29].

Rapidly dividing cells spend a greater proportion of their cell cycle within the S-phase, and as such this particular pool of cells is especially vulnerable to the MTX-induced deprivation of nucleoside precursors required for DNA and RNA synthesis. The vast majority of the body's cells are capable of synthesizing DNA bases of either family by de novo synthesis (enzymatically contingent upon inosine monophosphate dehydrogenase for purines, and dihydroorotate dehydrogenase for pyrimidines), or through the utilization of the salvage pathways. Alternately, the rapidly dividing cell pools (such as lymphocytes that responsible for transplant rejection, or the organization of the repertoire of mononuclear cells that orchestrate autoimmune disorders) are wholly dependent upon de novo biosynthetic pathways.

Given that HDMTX by itself is indiscriminant with respect to the blockade of folate dependent pathways, a method has been developed

Table 3
MRI findings before and after HDMTX-LR.

Patient	MRI findings before HDMTX-LR	MRI findings after HDMTX-LR
1	Multiple foci of increased FLAIR signal from the centrum semiovale, extending into the periventricular, subcortical and periatrial white matter. The left optic tract, left cerebral peduncle and right superior cerebellar peduncle, bilateral pons, bilateral medulla and right cerebellum were also involved. The lesions demonstrate corresponding low T1 signal and peripheral enhancement. Multiple foci of abnormal signal on all sequences and gadolinium-enhancement were noted throughout the cervical spinal cord. Multiple large lesions seen throughout the thoracic spinal cord, with focal abnormal cord enlargement and enhancement at the level of T3–T4, and T6–T8. Smaller lesions are identified at the level of T10 and T12.	MRI performed one and three months after HDMTX-LR showed resolution of all the gadolinium-enhancing lesions seen on the previous MRI, except for one left corona radiate lesion that continued to demonstrate minimal enhancement.
2	T2/STIR hyperintense lesions at C1, C3–4, C5–6 and C7 without any enhancement.	MRI performed 6 months after HDMTX-LR showed resolution of these lesions.
3	T2 hyperintense signal and gadolinium-enhancement of bilateral optic nerves, ventral medulla, and entire spinal cord.	MRI performed a month after HDMTX-LR demonstrated resolution of all the gadolinium-enhancing lesions seen on the previous MRI.
4	Periventricular T2 hyperintense lesions without any gadolinium-enhancing lesions on brain MRI. Increased T2 signal from C2 to T6 with gadolinium-enhancement from C4 to C6 on spine MRI.	MRI performed one month after HDMTX-LR demonstrated resolution of all the gadolinium-enhancing lesions seen on the previous MRI except for a single, small lesion at C5.
5	Multiple T2 hyperintense foci in the brain and spinal cord with a single enhancing lesion in the left middle cerebellar peduncle were noted. After IVMP and PLEX, multiple new and enhancing lesions in the brain and spinal cord developed.	MRI performed one month after HDMTX-LR demonstrated resolution of all the gadolinium-enhancing lesions seen on the previous MRI except for a single lesion in the left postcentral gyrus.
6	Brain MRI demonstrated extensive, confluent FLAIR signal abnormality within the bilateral parieto-occipital white matter, with increased involvement of the corpus callosum. Spine MRI revealed abnormal T2 hyperintense signal extending from the cervicomedullary junction to T6 with cord swelling and patchy areas of enhancement, most prominently from T1-T4.	MRI performed a month after HDMTX-LR demonstrated resolution of all the gadolinium-enhancing lesions seen on the previous MRI.
7	Abnormal T2 signal extending from C4–C5 through C7 without any areas of gadolinium enhancement.	MRI performed three months after HDMTX-LR was unchanged.
8	Brain MRI revealed abnormal T2/FLAIR signal in the right medial frontal lobe, periventricular white matter, corpus callosum, left parietal lobe, left midbrain, left pons, and bilateral medulla. Abnormal enhancement was seen in right medial frontal, midbrain, pons, cerebellar peduncles, and bilateral optic nerves. Abnormal T2 signal, cord swelling, and enhancement was observed in the entire cervical cord and from T2-T6.	MRI performed one month after HDMTX-LR demonstrated resolution of all the gadolinium-enhancing lesions seen on the previous MRI except for a single, small lesion in the right middle cerebellar peduncle.
9	Brain MRI revealed large,	MRI performed three months after

Table 3 (*continued*)

Patient	MRI findings before HDMTX-LR	MRI findings after HDMTX-LR
	tumefactive lesions in the periventricular and subcortical white matter; the largest lesion was in the left parieto-temporo-occipital region resulting in midline shift, as well as lesions in the left frontal lobe, left external capsule, and left basal ganglia. An incomplete peripheral rim of enhancement was present around all these lesions.	HDMTX-LR demonstrated resolution of all the gadolinium-enhancing lesions and vasogenic edema seen on the previous MRI.
10	Brain MRI revealed an enhancing, tumefactive left parieto-temporal mass measuring 7.7 cm AP × 5.4 cm transverse and 6.9 cm craniocaudal, with surrounding vasogenic edema. There was mass effect on the left lateral ventricle with a left-to-right midline shift of 8 mm. Right frontal encephalomalacia was stable compared to prior MRIs.	Brain MRI performed 6 months after HDMTX-LR revealed previously seen encephalomalacia with residual T2 hyperintensity involving the right frontal lobe. The left parieto-temporal tumefactive lesion was no longer enhancing. T1 shortening along the periphery of this left parieto-temporal lesion indicative of laminar necrosis.
11	Brain MRI revealed T2/FLAIR hyperintense lesions within the splenium of the corpus callosum, bilateral frontal white matter, left hypothalamus extending into the left optic tract and chiasm, thalamus, lower midbrain and pons. Gadolinium-enhancement was seen in the left optic nerve, left hypothalamus, and corpus callosum. Spine MRI revealed T2/STIR hyperintense signal and enhancement of C3 to C6. Abnormal cord signal extending from T9/10 to the conus medullaris with enhancement of the conus medullaris also present.	Brain and spine MRI performed one month after receiving HDMTX-LR showed new lesions seen within the right thalamus, pons, and right middle cerebellar peduncle. Enhancement is seen in the right basal ganglia, and left optic nerve. Abnormal cord signal and enhancement has worsened and extended into the cervicomedullary junction superiorly and inferiorly through T3. Abnormal cord signal and enhancement also extended from T8 to the conus medullaris.
12	Innumerable foci of T2 hyperintensity within bilateral cerebral hemispheres, including centrum semiovale, corona radiata, periventricular white matter, subcortical white matter, and left pons. Multiple enhancing lesions within the right frontal and parietal lobes were seen. New lesions were noted in the right thalamus, right basal ganglia, right cerebellar hemisphere and periaqueductal area of the midbrain showing restricted diffusion and post-contrast enhancement.	No followup MRIs were available for review. The patient had declined followup MRIs due to financial constraints.

to 'rescue' the peripheral folate stores, when folinic acid (Leucovorin rescue; LR) is administered at precise time intervals following the infusion of methotrexate. The rescue of peripheral folate by LR is hypothesized to circumvent the antifolate metabolic blockade of MTX outside of the CNS, and thus serves to strategically protect the peripheral tissues from HDMTX toxicity [28].

Toxic effects of HDMTX include myelosuppression (leading to sepsis and hemorrhagic complications), nephrotoxicity (from precipitation of MTX and 7-OH-MTX crystals in the kidney), and stomatitis [27–29]. Idiosyncratic toxicities from HDMTX include hepatotoxicity dermatologic reactions (ranging from mild erythematous eruptions to exfoliative dermatitis), transient neurologic deficits, and chemical conjunctivitis [28].

Transient neurologic alterations can range from headaches and malaise to mood alterations, and rarely focal neurologic deficits and seizures [27–30]. Among the posited mechanisms to explain such changes has been the folate-dependent remethylation of homocysteine
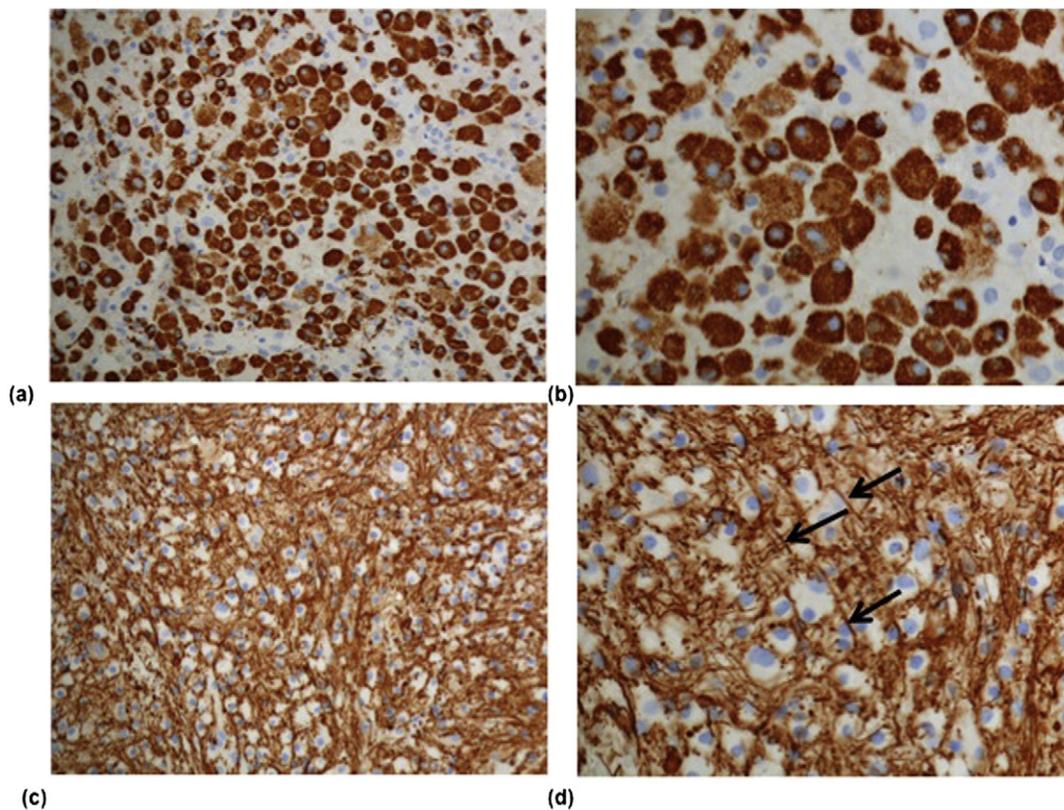
Fig. 2. Brain biopsy findings from a right fronto-parietal lesion from patient 10. 200× (a) and 400× (b) CD68-stained sections demonstrate fragments of white matter with abundant macrophage infiltration. 200× (c) and 400× (d) neurofilament-stained sections demonstrate intact residual axons (as highlighted by arrows) amidst the macrophages (i.e. "relative axonal sparing").

to methionine (the two principal pathways by which homocysteine level are maintained at physiologic levels include the tetrahydrofolate reductase/vitamin B-12 dependent enzyme, methionine synthetase; and the vitamin B-6 dependent enzyme, cystathionine synthetase; the latter of which ultimately converts homocysteine to cysteine, and then to cysteine; which can subsequently access to the tricarboxylic acid cycle) [30].

MTX is well known to promote elevations, in both plasma and CSF levels of homocysteine, a biochemical intermediate with potential toxicity to vessel endothelium, in addition to acting as an agonist at the excitatory amino acid receptor, *N*-methyl-D-aspartate (NMDA) [30]. A salient 'pearl' related to the tolerability of the HDMTX-LR regimen, is that the not infrequently associated headaches, can be reliably and effectively aborted with dextromethorphan [30].

HDMTX toxicities are mitigated by only administering it to patients with normal renal function (a minimum glomerular filtration rate of 60 mL/min), and by ensuring vigorous hydration, urine alkalinization (pH ≥ 8), and utilizing LR [27,29]. Since renal tubular precipitation of MTX and 7-OH-MTX crystals occurs in acidic urine environments, a urine pH of at least 8.0 is required to prevent nephrotoxicity [31,32].

In those deemed treatment responsive, improvement with PLEX typically occurs early; within the first two weeks of starting treatment [11,12]. More specifically, improvement typically occurs within three exchanges, and 5 days of treatment [13]. As such, we propose that HDMTX-LR should be considered in acute inflammatory demyelinating CNS syndromes that fail to improve within a week of being treated with IVMP and PLEX. Further, in those exhibiting characteristics in keeping with syndromes associated with a disposition of higher morbidity and mortality, as well as a highly protracted period of neurorehabilitation (increasing the risk of derivative complications and substantial costs), we suggest that HDMTX-LR treatment be considered as a *potential first-line* treatment intervention, given the prospect of its rapid

'remission-exacting' effects upon some of the most formidably complex and dangerous CNS inflammatory syndromes confronted by the neurologist.

Perhaps most crucially, HDMTX-LR can be used safely with low risk of toxicity, by ensuring vigorous hydration, urine alkalinization, and precisely timed administration of the leucovorin rescue.

4.2. Study limitations

Our retrospective, small cohort study exploration of the application of HDMTX-LR in patients with acute inflammatory CNS syndromes is confounded by the patently obvious limitations of a class III investigation. As such, the necessary next steps going forward will be to carefully and systematically evaluate the safety and effectiveness of HDMTX-LR in patients presenting with acute inflammatory CNS syndromes, exhibiting those characteristics where risk versus benefit considerations favor the early application of this treatment, rather than mandating failure with other conventional strategies.

This particular issue is heuristically a focal point when justifying the application of aggressive chemotherapy regimens, including those used in stem cell transplantation, which have shown significant atrophy accumulation over time since the inception of the intervention.

While the majority of inflammatory exacerbations in clinical practice will still be treated with corticosteroids (followed by plasma exchange in those exhibiting an inadequate recovery response, or in those presenting with a monumentally severe syndrome), severe syndromes of the brain and spinal cord can be associated with rather remarkable vasogenic edema changes such that inflammatory demyelination, along with damage to axons in eloquent tract systems (associated with concomitant clinical disability), can foment another, and highly ominous facet of the pathology of such syndromes. Most specifically the ability of robust vasogenic changes to lead to compression

of the neuro-vascular apparatus (potentially involving both arteriolar delivery of vascular-derived requirements of CNS tissues, as well as the draining venous arborization, which could then result in tissue congestion and infarction.

The other end of the damage spectrum in such patients, involves the propensity of chemotherapeutic approaches to provide benefits that are potentially accompanied by toxicity within the same tissues we are hoping to rescue from the mechanisms of inflammatory demyelination, and the corresponding signatures of irreversible disability; axonal and neurodegeneration; ultimately atrophy.

In the case of our utilization of methotrexate, while we characterize the regimen as 'High Dose', in actual fact we are employing dosing sufficient to achieve remission of the current exacerbation, after which we then transition treatment to one of the FDA approved disease-modifying strategies. Further, our dosing scheme of 2.5 g/kg for either a single dose, or two doses administered at baseline and approximately 8 weeks later, constitutes a highly attenuated dosing scheme when compared to those employed in other settings. For instance, in children with sarcomas, the utilization of our regimen typically involves doses at 7.5 to 10 g/kg, making our particular treatment scheme extraordinarily modest. This critical difference, along with our highly systematic and precisely timed dosing of leucovorin rescue, are perhaps the two factors most germane as to why we have rarely observed methotrexate-associated toxicity in our treated patients.

Despite the application of substantially lower doses of MTX, our study is limited by its retrospective nature, particularly given that we employed our regimen as a rescue treatment in patients not responding sufficiently to more conventional treatments, and without the patent benefits of a prospective trial, where the systematic ascertainment of critical measures of treatment-related neurotoxicity can be rigorously detected and monitored. Such measures were clearly beyond the scope of our small, retrospective, uncontrolled, and open label study. Further, the broad heterogeneity across our small cohort; including different diagnostic entities, made rigorous imaging follow up investigations of dubious value, particularly given our intention to organize a prospective trial of HDMTX-LR in a larger cohort of candidate patients across multiple participating centers, where uniform application of brain atrophy imaging paradigms shall be utilized.

Notwithstanding the formidable challenge that would be involved in accurately differentiating the pathoetiologic underpinnings of brain atrophy subsequent to a fulminant inflammatory demyelinating syndrome and its correspondingly intensive treatment intervention, prospectively assessing for loss of CNS tissue does represent a principal goal for our future investigations utilizing MTX, as with other intensive agents that carry the risk of toxicity to the very same tissue we aim to protect.

4.2.1. Future directions

In planning a prospective, randomized, blinded, and comparator controlled study of high dose methotrexate with leucovorin rescue, we will include the systematic procurement of CSF both before as well as timed intervals following treatment inception, for the purpose of identification of neuroimmunological changes in CSF that may correlate with either efficacy, or other correlates in those patients who fail to achieve efficacy with this therapy.

While we do not recommend tissue biopsy in all such patients with fulminant CNS inflammatory syndromes, each patient should be carefully considered for tissue characterization, especially given that a variety of conditions may produce clinical and radiologic characteristics that are indistinguishable, and yet the choice of immunotherapy can be distinctive; and in some circumstances particular agents may be contraindicated for an individual patient's severe inflammatory syndrome (e.g. the application of immunotherapy that modulates TNF pathways may be rapidly remission-exacting for neurosarcoid, and yet the same class of agents may serve to ignite or exacerbate demyelinating disorders such as MS.

In the patient where biopsy was performed, we found the data it revealed to be very reassuring in terms of proceeding with yet another intensive treatment intervention, given that the examined tissue identified severe de and dysmyelination, albeit in the context of relative sparing of brain axons. This finding, we believed, was germane to our counseling of the family who had already been highly disappointed with the proceeding treatment, and were seriously considering withdrawal from future interventions on the basis of futility. The demonstration of 'axonal sparing' actually provided me with evidence to make compelling my recommendation for advancing therapy to high dose methotrexate with leucovorin rescue.

Fortunately I was able to convince the family to give me another shot at rescuing my patient. Her survival to this day, and the nearly miraculous recovery and stabilization of her course, came with considerable dividends; not the least of which was a potent revalidation of my (EMF) longstanding mantra in treating inflammatory disorders of the CNS; "nothing has worked yet, until something does … I cannot promise outcome, but can always promise to try, and never to quit … I care less about 'being right' than I do about 'getting it right' … the latter can be the product of extensive work, recurrent failure, and even at times having to reconcile treatment associated risks in order to keep going until we have established remission, optimized neurorehabilitation, and subsequently move on to discussions with our patient and their family concerning about our ultimate goal of achieving a durable longstanding remission on one of the MS disease modifying therapies.

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Author contributions

Shin Beh was involved in formulating the study concept and design, acquisition of the data, analysis and interpretation, and for the critical revision of the manuscript.
Eric Kildebeck contributed to the analysis and interpretation of the data, and for the critical revision of the manuscript.
Ram Narayan was involved in formulating the study concept and design, acquisition of the data, analysis and interpretation, and for the critical revision of the manuscript.

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