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Letter to the Editor: New Observation

Long-Term Tofersen and CSF Macrophage Inclusions in Superoxide Dismutase 1 Amyotrophic Lateral Sclerosis

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Antisense oligonucleotides (ASOs) are a growing class of therapeutics that modulate gene expression in patients with varied neurogenetic disorders. Tofersen, an intrathecally delivered ASO that reduces superoxide dismutase 1 (SOD1) protein expression, was recently approved by Health Canada (March 2025) under a Notice of Compliance with Conditions for adults with amyotrophic lateral sclerosis (ALS) due to a pathogenic *SOD1* variant. As use of this therapy increases among Canadian neurologists, understanding its immunologic effects, particularly those observed inCSF, becomes increasingly important. Here, we describe a patient with familial *SOD1* ALS receiving long-term intrathecal tofersen who developed atypical CSF macrophage inclusions. This report aims to raise awareness of this finding and contribute to our understanding of the evolving cytological landscape associated with long-term ASO therapy.

A 49-year-old female with a family history of ALS (one paternal aunt, two paternal uncles) and a heterozygous pathogenic variant in *SOD1* (c.341T > C (p.I114T)) (confirmed in one uncle) presented with 2 years' progressive muscle cramping. Examination demonstrated mild symmetric lower limb weakness (4/5 Medical Research Council grade in hip flexors, 4+/5 in toe extensors), hyperreflexia with bilateral Hoffman's and crossed adductor signs and bilateral Achilles areflexia. Sequencing of *SOD1* identified the same pathogenic variant, and she was diagnosed with familial limb-onset ALS. She declined treatment with riluzole but displayed minimal clinical progression over the following two years and subsequently enrolled in a clinical trial of intrathecal tofersen. She was assigned to the treatment arm and has since received 92 injections over 7 years, with ongoing therapy, and has remained ambulatory and functionally independent, with stable examination findings, unchanged ALS Functional Rating Scale-Revised score of 45 and no adverse events.

Baseline CSF studies pretreatment were normal (3 cells/μL, protein 0.33 g/L). Pre-injection CSF profile has consistently demonstrated a stable lymphocytic-predominant pleocytosis and elevated protein (Figure 1). While routine CSF microscopy was unremarkable in the majority of studies, microscopy at treatment doses 30, 65 and 92 demonstrated atypical macrophage morphology (Figure 2). The majority of macrophages observed were unremarkable; however, a subset exhibited cytoplasmic vacuoles and granular inclusions of various sizes (Figure 2A-B). Most strikingly, one macrophage featured large, rounded azurophilic granular inclusions resembling phagocytosed microorganisms (Figure 2C). For each of these three incidents, clinical review was done for concern of central nervous system (CNS) infection; however; the patient was asymptomatic and neurologically stable and had negative CSF gram stain, Venereal Disease Research Laboratory and cultures.

Tofersen and other ASOs such as nusinersen (approved for spinal muscular atrophy) and tominersen (under investigation for Huntington's disease) are disease-modifying therapies that require intrathecal administration due to poor blood-brain barrier penetration and act by modulating mRNA of disease-associated genes.[1,2] Tofersen specifically reduces SOD1 protein expression by inducing *SOD1* mRNA degradation.[3] Both nusinersen and tofersen are associated with inflammatory CSF changes including lymphocytic pleocytosis, protein elevation and rarely reported macrophage inclusions.[4,5] The majority of patients in nusinersen studies did not experience any clinically significant inflammatory side effects; however, inflammatory adverse events have been observed with tofersen, including myelitis, aseptic meningitis, radiculitis and associated papilledema from intracranial hypertension.[3,6] These adverse events corresponded to rises in CSF pleocytosis and protein from previous baselines; however, similar CSF fluctuations were observed in asymptomatic patients, limiting the predictive value of changes in CSF parameters alone.[6]

Macrophage inclusions are recognized in the CSF of patients receiving intrathecal nusinersen and, to date, in only two patients treated with tofersen.[5,7] These inclusions typically appear as basophilic bodies within intracytoplasmic vacuoles of varying size.[4,5] The contents of these inclusions remain unknown[7] but may reflect an activation of the innate immune system in response to ASOs or their excipients. With lymphocytes in close proximity to activated macrophages (Figure 2C), an antigen-presenting interaction may also be taking place. Importantly, these rounded inclusions resemble those seen in an immune response to microbial pathogens (such as mycobacteria) by macrophages and

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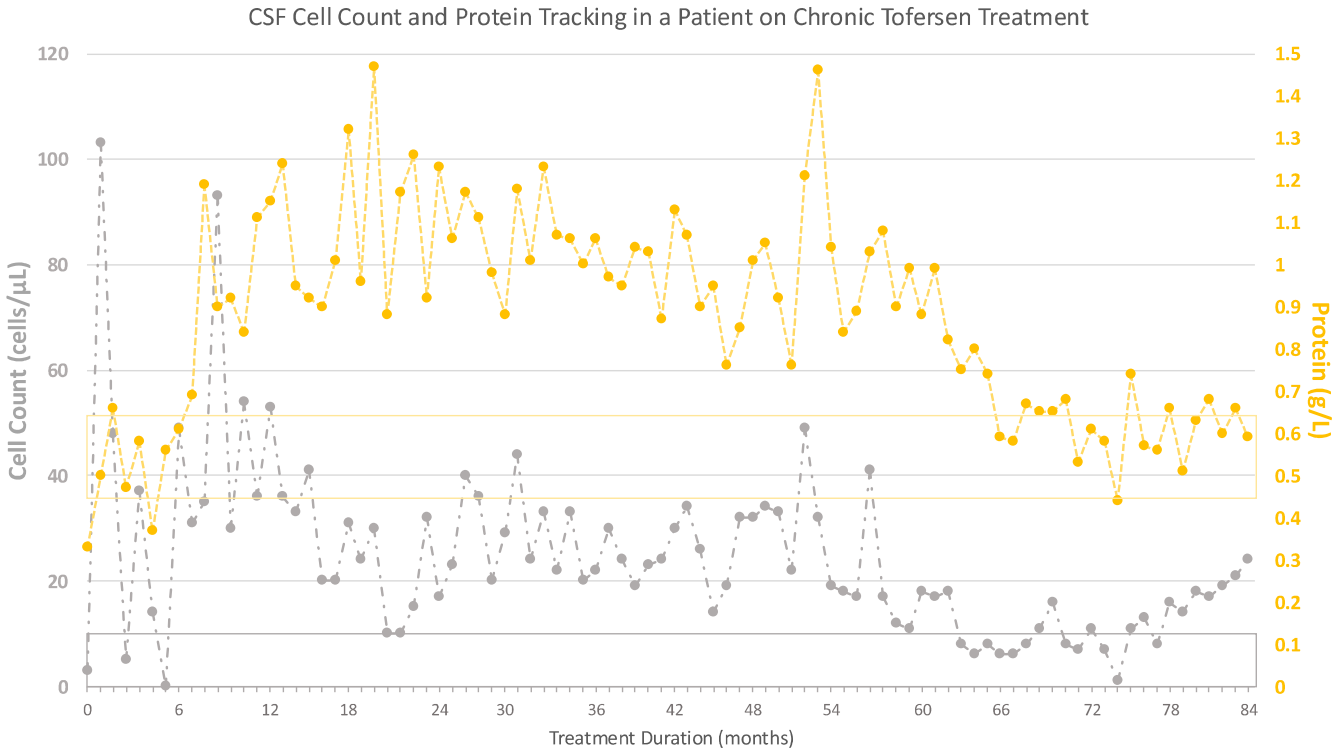

Figure 1. CSF cell count (gray) and protein (yellow) monitoring values in a 49-year-old patient with superoxide dismutase 1-amyotrophic lateral sclerosis, from initiation of tofersen treatment to most recent follow-up (84 months total treatment duration, 92 total injections), showing chronic lymphocytic pleocytosis and elevated protein. Laboratory normal reference ranges shown as pale gray (cell count, 0–10 cells/µL) and pale yellow (protein, 0.45–0.65 g/L) boxes.

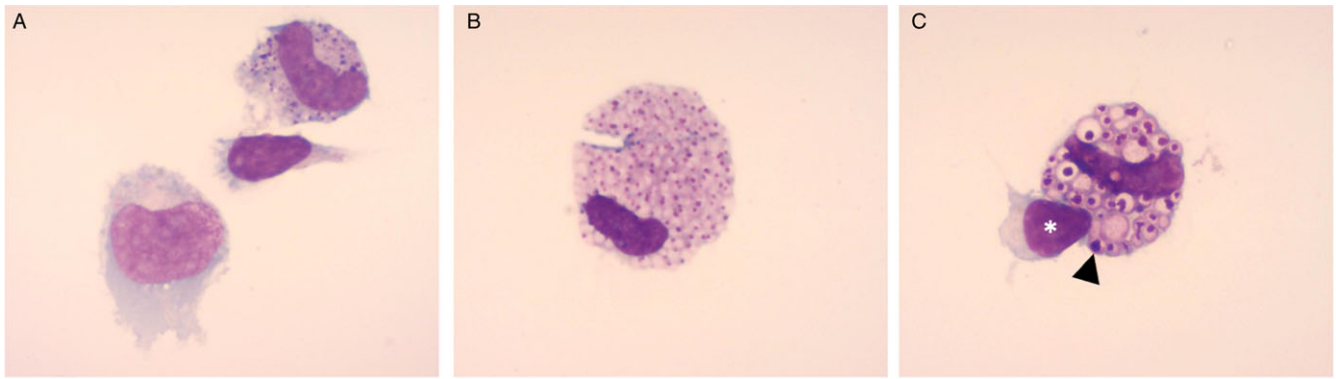

Figure 2. Macrophages in the CSF of a 49-year-old patient treated with tofersen for superoxide dismutase 1-amyotrophic lateral sclerosis. (A) Macrophage at top-right contains numerous inclusions of various sizes, alongside a lymphocyte (middle) and an unremarkable-appearing macrophage (bottom-left). (B) Macrophage with many vacuoles and inclusions of uniform size. (C) Macrophage with numerous vacuoles of varying sizes, containing unusually large round basophilic inclusions (arrowhead); it is abutted by a lymphocyte (asterisk). Images captured on a Leica DM3000 microscope (1000×) and brightened and sharpened using ImageJ.

neutrophils[8] and thus may prompt concern for CNS infection when viewed outside of the clinical context of ASO therapy.

Our patient provides new insights into the persistence of these abnormal macrophage inclusions in patients receiving tofersen, with their presence noted even after 92 injections. Two prior patients reported with similar CSF cytology findings were noted to develop macrophage inclusions after the second tofersen injection; however, follow-up was limited to 4 and 9 months, respectively. Therefore, long-term persistence of the finding was unknown. In comparison, macrophage inclusions are observed in adults treated with nusinersen up to 14 months after treatment initiation[4] and in one patient after 54 months.[7] Our patient represents the longest ASO treatment duration associated with persistent macrophage inclusions reported to date. The clinical significance of persistent macrophage inclusions during extended therapy is unclear. Our patient has remained clinically stable, without adverse events during this period; therefore, this cytological abnormality does not necessarily correlate with adverse therapy outcomes or lack of response to therapy.

As intrathecal ASO therapies expand in scope and duration of use, novel immune responses – such as macrophage inclusions reported here – are likely to emerge. This case highlights the importance of contextual interpretation of CSF abnormalities in patients receiving ASO therapies and the evolving landscape of

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monitoring in this population. With recent conditional approval of tofersen in Canada, Canadian neurologists should be familiar with these atypical cytological findings.

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TN performed CSF microscopic analysis and edited the manuscript.

GBB provided conception of the case report and edited the manuscript.

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