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NULL HYPOTHESIS CLASS OF EVIDENCE

Safety, tolerability, and efficacy of fluoxetine as an antiviral for acute flaccid myelitis

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Abstract

Objective
To determine the safety, tolerability, and efficacy of fluoxetine for proven or presumptive enterovirus (EV) D68–associated acute flaccid myelitis (AFM).

Methods
A multicenter cohort study of US patients with AFM in 2015–2016 compared serious adverse events (SAEs), adverse effects, and outcomes between fluoxetine-treated patients and untreated controls. Fluoxetine was administered at the discretion of treating providers with data gathered retrospectively. The primary outcome was change in summative limb strength score (SLSS; sum of Medical Research Council strength in all 4 limbs, ranging from 20 [normal strength] to 0 [complete quadriparesis]) between initial examination and latest follow-up, with increased SLSS reflecting improvement and decreased SLSS reflecting worsened strength.

Results
Fifty-six patients with AFM from 12 centers met study criteria. Among 30 patients exposed to fluoxetine, no SAEs were reported and adverse effect rates were similar to unexposed patients (47% vs 65%, $p = 0.16$). The 28 patients treated with >1 dose of fluoxetine were more likely to have EV-D68 identified (57.1% vs 14.3%, $p < 0.001$). Their SLSS was similar at initial examination (mean SLSS 12.9 vs 14.3, $p = 0.31$) but lower at nadir (mean SLSS 9.25 vs 12.82, $p = 0.02$) and latest follow-up (mean SLSS 12.5 vs 16.4, $p = 0.005$) compared with the 28 patients receiving 1 (n = 2) or no (n = 26) doses. In propensity-adjusted analysis, SLSS from initial examination to latest follow-up decreased by 0.2 (95% confidence interval [CI] −1.8 to +1.4) in fluoxetine-treated patients and increased by 2.5 (95% CI +0.7 to +4.4) in untreated patients ($p = 0.015$).

Conclusion
Fluoxetine was well-tolerated. Fluoxetine was preferentially given to patients with AFM with EV-D68 identified and more severe paralysis at nadir, who ultimately had poorer long-term outcomes.

Classification of evidence
This study provides Class IV evidence that for patients with EV-D68-associated AFM, fluoxetine is well-tolerated and not associated with improved neurologic outcomes.

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Glossary

AFM = acute flaccid myelitis; **CI** = confidence interval; **EC_{50}** = % effective concentration; **EV** = enterovirus; **FDA** = Food and Drug Administration; **IQR** = interquartile range; **IVIg** = IV immunoglobulin; **MRC** = Medical Research Council; **QTc** = corrected QT interval; **SAE** = serious adverse events; **SLSS** = summative limb strength score; **WLSS** = weakest limb strength score.

In 2014, clusters of acute flaccid paralysis cases with distinctive imaging changes in the gray matter of the spinal cord, termed acute flaccid myelitis (AFM), were noted in the United States in association with a widespread outbreak of enterovirus (EV) D68 respiratory disease.[1–3] Various therapies, including IV immunoglobulin (IVIg), corticosteroids, plasmapheresis, and antivirals, were administered, but no obvious acute clinical improvement or deterioration as a result of these therapies was noted.[4,5] One year later, few patients had completely recovered, with most continuing to show functional impairments, muscle weakness, and atrophy.[4,6] Current recommendations from the US Centers for Disease Control and Prevention conclude that there is insufficient evidence to recommend any available treatment for AFM.[7]

Accumulating evidence supports that EV-D68 may be a cause of AFM.[8,9] A role for antiviral therapy in EV-D68-associated AFM could therefore be postulated; however, testing of a wide variety of compounds for activity against the circulating 2014 strains of EV-D68 demonstrated that none of the anti-EV drugs in development (including pocapavir, vapendavir, and pleconaril) had consistent in vitro activity.[10–12] Fluoxetine, a selective serotonin reuptake inhibitor, was identified as the only available Food and Drug Administration (FDA)–approved medication with in vitro antiviral activity against circulating 2014 EV-D68 strains.[10,11] Fluoxetine inhibits replication of group B and D EVs by targeting viral protein 2C.[13] The drug concentrates 20-fold in the CNS compared to serum, which makes it feasible to reach concentrations that exceed the 50% effective concentration (EC_{50}) for EV-D68 at that site.[14,15] A single published case report of fluoxetine administered to a child with X-linked agammaglobulinemia and chronic EV encephalitis described that it was well-tolerated and potentially efficacious.[16] Given the long-term, potentially permanent paralysis associated with AFM, the lack of effective alternative therapies, and the possibility of antiviral activity against EV-D68, fluoxetine was proposed as a possible therapeutic agent for AFM.[17]

In 2016, a resurgence of AFM in the United States was noted concurrent with EV-D68 circulation.[8] Several centers administered fluoxetine off-label as an antiviral in proven or presumptive EV-D68-associated AFM cases, in addition to other therapies such as IVIg, corticosteroids, and plasmapheresis. Though fluoxetine is FDA-approved for psychiatric indications, it has not been studied as an antiviral medication in humans.[18] This study retrospectively analyzed the safety, tolerability, and efficacy of fluoxetine for proven or presumptive EV-D68-associated AFM.

Methods

This multicenter retrospective observational cohort study compared serious adverse events (SAEs), adverse effects, and outcomes between AFM cases treated with fluoxetine to those not receiving the medication. The study is rated Class IV because of the nonrandomized, open-label design. Inclusion criteria included patients with (1) clinical criteria of acute onset limb weakness or cranial nerve dysfunction and (2) MRI criteria of lesions in the gray matter of the spinal cord or motor nuclei of the brainstem with onset between January 1, 2015, and November 1, 2016. Cases were included regardless of proven or presumptive etiologies identified. Patients transferred to another facility during the course of their acute illness without records available from the transferring or accepting facility were excluded. US sites that treated patients with AFM in 2015–2016 were identified via infectious disease and neurology listservs and networks with eligible cases identified via an emailed survey.

All therapies, including fluoxetine, were administered to patients at the discretion of treating providers for clinical care. De-identified data were retrospectively gathered by chart review and entered into a standardized data collection tool in the REDCap system.

An intention-to-treat analysis of fluoxetine safety and tolerability was conducted with fluoxetine-exposed cases defined as patients receiving at least one dose of fluoxetine during their treatment course and unexposed controls never receiving fluoxetine. Suspected SAEs occurring after the start of fluoxetine were categorized using the FDA Adverse Events Reporting System.[19] Corrected QT intervals (QTc) were recorded from fluoxetine-exposed patients who underwent ECG monitoring. Adverse effects identified in the fluoxetine package insert in the categories of gastrointestinal symptoms, psychiatric symptoms, allergic symptoms, and laboratory toxicities were compared between fluoxetine-exposed cases and controls to assess tolerability.[18]

A per-protocol efficacy analysis was conducted with fluoxetine-treated cases defined as patients receiving >1 dose of fluoxetine, regardless of dosing utilized; all other AFM cases (including those receiving 1 dose of fluoxetine) were considered untreated controls. Strength outcomes were assessed at (1) initial examination (first documented neurologic examination), (2) nadir (maximal documented limb weakness), and (3) latest follow-up (latest documented neurologic examination as of the time of data

collection). Strength of the weakest muscle group in each limb by Medical Research Council (MRC) grade from 0 (no twitch) to 5 (normal strength) as documented in the medical record was recorded.[20] MRC scores of 4– and 4+ were assigned numerical scores of 3.5 and 4.5, respectively. A summative limb strength score (SLSS) was calculated by adding the lowest MRC score in each of the 4 limbs in each patient at each time point (e.g., for R bicep 3/5, L wrist flexor 4/5, L lower extremity 5/5, R lower extremity 5/5, SLSS = 3 + 4 + 5 + 5 = 17). A weakest limb strength score (WLSS), defined as the lowest documented MRC score in any limb, was also calculated for each patient at each time point. The primary efficacy outcome was defined as the change in SLSS from initial presentation to latest follow-up, and a sensitivity analysis was conducted using WLSS. Secondary outcomes included categorical change in strength classification (improved strength, no change, worsened strength), length of intensive care unit stay, length of hospital stay, and the proportion requiring and duration of respiratory and feeding support. Additional subanalyses compared outcomes between fluoxetine-treated patients who did and did not have EV-D68 identified, between patients treated with fluoxetine prior to nadir and untreated patients, and, among all patients, between those who did and did not receive IVIg and corticosteroids.

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC) with propensity-adjusted analyses performed in STATA 13 (StataCorp, College Station, TX). Descriptive statistics were reported in percentages for categorical variables, medians with interquartile ranges for most continuous variables, and means with SD for unadjusted and adjusted strength outcomes. Tests for differences among treatment, exposure, or etiology groups were performed with χ^2/Fisher exact test for categorical variables, Wilcoxon (Mann-Whitney)/Kruskal-Wallis tests for most continuous variables, and *t* tests/analysis of variance for strength outcomes. Adjusted SLSS/WLSS comparisons were performed using doubly robust regression and propensity-weighted ATE models controlling for age, sex, administration of corticosteroids, IVIg, plasmapheresis, and SLSS/WLSS at initial examination. Adjusted subanalyses of strength outcomes by EV-D68 detection, administration of IVIg, and corticosteroids were performed similarly, controlling for age, sex, and strength score at initial presentation. Pearson correlation between the time to fluoxetine initiation (days from neurologic onset to first dose) and the primary outcome (change in SLSS from initial examination to latest follow-up) was analyzed among fluoxetine-treated patients as well as the subgroup of fluoxetine-treated patients with EV-D68 identified.

Standard protocol approvals, registrations, and patient consents

The Colorado Multiple Institutional Review Board provided central ethical approval of the study with waiver of consent for retrospective collection of de-identified data. Each site obtained appropriate local ethical approval for data collection.

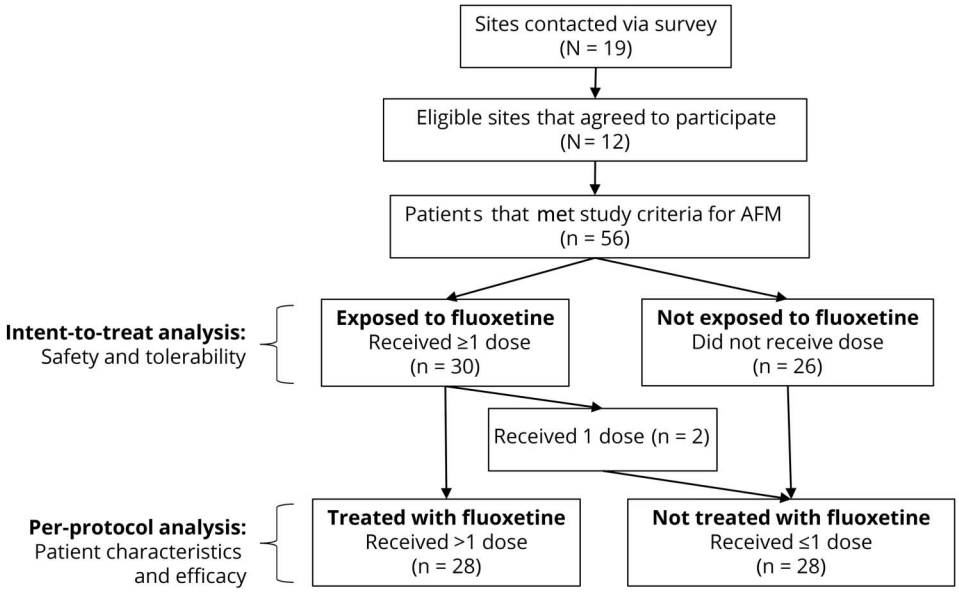
Data availability

Individual participant data will not be made publicly available due to potential confidentiality concerns related to a rare condition and small study population for whom a waiver of consent was obtained.

Results

From 19 sites contacted via survey, 12 sites identified eligible patients and agreed to participate (figure 1). Fifty-six patients with AFM meeting study criteria were included. Thirty patients received at least 1 dose of fluoxetine and were

Figure 1 Study inclusion flowchart



Flowchart of study site and study population inclusion with intention-to-treat analysis of safety and tolerability conducted comparing 30 fluoxetine-exposed patients to 26 fluoxetine-unexposed patients and per protocol analysis of patient characteristics and efficacy comparing 28 fluoxetine-treated patients (receiving >1 dose) to 28 fluoxetine-untreated patients.

considered fluoxetine-exposed, including 2 patients who received a single dose. Twenty-eight patients received >1 dose of fluoxetine and were considered fluoxetine-treated cases, and 28 patients were considered untreated controls.

Overall, the 56 included patients with AFM were a median 3.8 years of age; 17 (30%) had an underlying medical condition, most commonly asthma (n = 10, 18%) (table 1). A prodromal illness was identified in 51 (91%), most commonly with fever

Table 1 Patient characteristics

	Fluoxetine-treated (n = 28)	Fluoxetine-untreated (n = 28)	Overall (n = 56)	*p* Value
Demographics				
Age, y	3.4 (2.6–7.5)	4.5 (3.2–9.0)	3.8 (2.9–8.0)	0.10
Sex (% male)	14 (50)	16 (57)	30 (54)	0.59
Race (% nonwhite)	11 (39)	10 (36)	21 (38)	0.78
Ethnicity (% Hispanic or Latino)	9 (33)	6 (21)	15 (27)	0.32
Host characteristics				
Underlying medical condition	10 (36)	7 (25)	17 (30)	0.38
Asthma	6 (21)	4 (14)	10 (18)	0.49
Immunocompromised	3 (11)	1 (4)	4 (7)	0.61
Neurologic condition	2 (7)	1 (4)	3 (5)	>0.99
Psychiatric condition	0 (0)	2 (7)	2 (4)	0.49
Prodrome				
Preceding illness	27 (96)	24 (86)	51 (91)	0.35
Fever	22 (79)	18 (64)	40 (71)	0.24
Respiratory symptoms	23 (82)	18 (64)	41 (73)	0.13
Gastrointestinal symptoms	6 (21)	7 (25)	13 (23)	0.75
Neurologic onset				
Days from prodromal illness onset	8 (3–14)	9 (5.5–14.5)	8.5 (4–14)	0.65
Fever	18 (64)	15 (54)	33 (59)	0.42
Meningeal signs[a]	8 (29)	12 (43)	20 (36)	0.27
Limb pain	5 (18)	9 (32)	14 (25)	0.22
Altered mental status or seizures	2 (7)	0 (0)	2 (4)	0.49
CSF pleocytosis[b] **(% of those with lumbar puncture)**	26 (93)	23 (82)	49 (88)	0.42
AFM presentation				
No. of limbs with weakness	2 (1–4)	2 (1–3)	2 (1–4)	0.31
Lower extremity involvement	17 (61)	14 (50)	31 (55)	0.42
Upper extremity involvement	24 (86)	23 (82)	47 (84)	>0.99
Cranial nerve involvement	13 (46)	7 (25)	20 (36)	0.09
AFM time course				
Days from onset to initial examination	2 (0–4)	1 (0–4)	2 (0–4)	0.52
Days from onset to nadir	5 (3–9)	4 (2–6)	4 (3–7)	0.05
Days from onset to latest follow-up	229.5 (121–304)	184 (119–266)	210 (121–280)	0.40

Abbreviation: AFM = acute flaccid myelitis.
[a] Includes headaches, photophobia, and stiff neck.
[b] Defined as >5 white blood cells/μL.
Values are n (%) or median (interquartile range).

(n = 40, 71%) and respiratory symptoms (n = 41, 73%). Neurologic onset of weakness began a median of 8.5 days after prodromal illness onset, frequently accompanied by fever, meningeal signs, and limb pain. Weakness involved a median of 2 limbs with upper extremities affected in 47 patients (84%) and lower extremities in 31 patients (55%). Cranial nerve dysfunction was present in 20 patients (36%). There were no significant differences in demographics, host characteristics, or illness presentation between treatment groups. Initial examination coincided with nadir in 19 participants (33%). Fluoxetine-treated patients had a median of 1 day longer between neurologic onset and nadir than untreated patients (5 days vs 4 days, $p = 0.05$). The median time from neurologic onset to latest follow-up was 210 days (interquartile range [IQR] 121–280 days).

An EV was identified in 24 of 56 (43%) patients, most commonly EV-D68 (n = 20, 36%), from respiratory or stool specimens (table 2). Fluoxetine-treated patients were significantly more likely than untreated patients to have EV-D68 identified (57% vs 14%, $p < 0.001$). Patients were treated with a variety of therapies, including corticosteroids (n = 33, 59%), IVIg (n = 46, 82%), and plasmapheresis (n = 8, 14%), but rates of treatment with these therapies did not differ significantly between groups.

Table 2 Enterovirus testing and therapies

	Fluoxetine-treated (n = 28)	Fluoxetine-untreated (n = 28)	Overall (n = 56)	*p* Value
Enterovirus testing				
Enterovirus identified (% of those tested; any site)	18 (64)	6 (21)	24 (43)	0.001
Specimen type positive for enterovirus				
CSF (% of tested)	1 (4)	0 (0)	1 (2)	0.49
Respiratory (% of tested)[a]	15 (63)	2 (10)	17 (39)	<0.001
Blood (% of tested)	3 (21)	0 (0)	3 (10)	0.09
Stool (% of tested)	10 (46)	4 (24)	14 (36)	0.16
Enterovirus typing				
EV-D68 (% of tested; any site)	16 (57)	4 (14)	20 (36)	<0.001
Coxsackie B4 (% of tested; any site)	1 (4)	0 (0)	1 (2)	>0.99
Coxsackie A10 (% of tested; any site)	1 (4)	0 (0)	1 (2)	>0.99
EV-A71 (% of tested; any site)	0 (0)	2 (7)	2 (4)	0.49
Therapies[b]				
Corticosteroids	16 (57)	17 (61)	33 (59)	0.79
Timing, days after onset	4 (1–5.5)	2 (1–3)	2 (1–5)	0.23
Duration, d	5 (3.5–5.5)	5 (4–20)	5 (4–6)	0.43
Cumulative dose, mg/kg methylprednisolone equivalents	111.1 (90–180)	135 (83.5–150)	120 (90–150)	>0.99
IV immunoglobulin	25 (89)	21 (75)	46 (82)	0.16
Timing, days after onset	4 (2–5)	5.5 (3–8.5)	4 (2–6)	0.06
Duration, d	3 (2–5)	3 (2–4)	3 (2–4)	0.48
Cumulative dose, g/kg	2 (2–2.2)	2 (2–2)	2 (2–2)	0.25
Plasmapheresis	6 (21)	2 (7)	8 (14)	0.25
Timing, days after onset	10 (7–12)	4.5 (4–5)	7 (5–12)	0.13
No. of treatments	5.5 (5–7)	3.5 (2–5)	5 (4–6.5)	0.27

Abbreviation: EV = enterovirus.
[a] Patients with respiratory specimens that were negative for EV/RVs were included in the denominator. Patients with respiratory specimens that were positive by EV-specific PCR were included in the numerator and denominator. Patients with respiratory specimens that were positive for EV/RVs but that did not have EV-specific PCR performed were not included in the numerator or denominator, as this testing did not differentiate EVs from RVs.
[b] See table 3 for fluoxetine treatment data.
Values are n (%) or median (interquartile range).

Among 30 patients exposed to fluoxetine, a dose of 0.75 mg/kg/d (maximum 40 mg/d) was administered in 21 patients (70%) and the median duration of treatment was 7 days (IQR 7–12 days) (appendix, table e-1, links.lww.com/WNL/A764). Fluoxetine was initiated a median of 5 days (IQR 3–7) after neurologic onset, prior to nadir in 11 (37%) and following nadir in 18 (60%). There were no SAEs reported, though treatment was stopped in 2 cases following a single dose: in 1 patient due to perceived anxiety and in another due to weakness not judged to be severe enough to warrant further treatment. QTc prolongation was noted in 2 of 17 patients (12%) with ECG obtained following fluoxetine initiation, though preinitiation ECGs were not obtained and ECGs were not obtained in untreated patients for comparison. While the most common adverse effects among fluoxetine-treated patients were psychiatric (primarily anxiety and agitation) and gastrointestinal, there were no significant differences in these rates or overall adverse effect rates compared to untreated patients (appendix, table e-2).

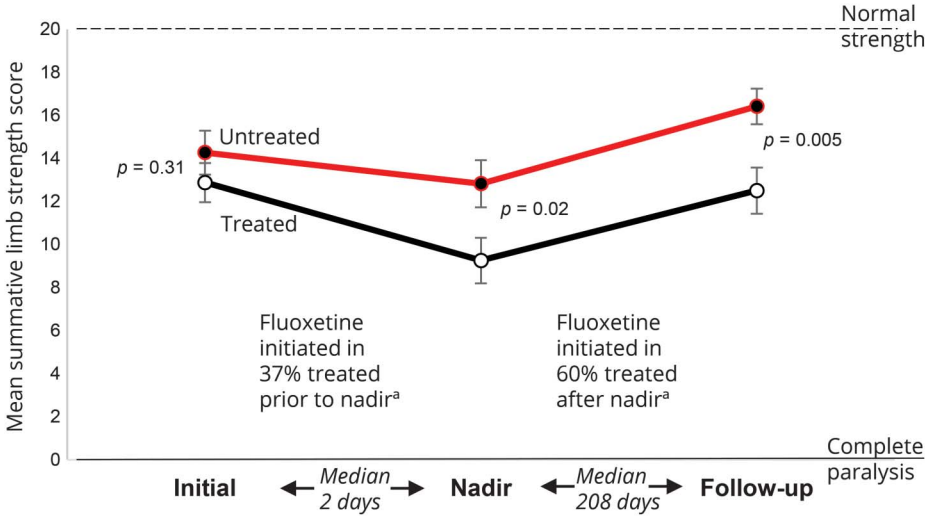
Fluoxetine-treated patients had similar strength on initial examination compared to untreated patients (mean SLSS 12.9 vs 14.3, $p = 0.31$), but more severe paralysis at nadir (mean SLSS 9.3 vs 12.8, $p = 0.02$) and latest follow-up (mean SLSS 12.5 vs 16.4, $p = 0.005$) (figure 2). The proportions of patients with improved, same, or worsened SLSS between initial examination and last follow-up did not differ significantly between groups ($p = 0.25$; table 3). The mean SLSS at latest follow-up compared with initial examination was 0.4 lower (95% confidence interval [CI] −2.5 to +1.8) in fluoxetine-treated patients vs 2.1 higher (95% CI 0 to +4.3) in untreated patients ($p = 0.097$; unadjusted analysis). After propensity-weighted adjustments for age, sex, additional therapies, and strength at initial examination, the mean SLSS change at latest follow-up compared with initial examination was 0.2 lower (95% CI −1.8 to +1.4) in fluoxetine-treated patients compared to 2.5 higher (95% CI +0.7 to +4.4) in untreated patients ($p = 0.02$). Sensitivity analysis using WLSS was consistent with these findings. The subset of 11 patients treated with fluoxetine prior to nadir also had a lower unadjusted mean SLSS change from initial examination to latest follow-up (−2.1 [95% CI −5.6 to +1.3]) compared to untreated patients ($p = 0.035$). Time from neurologic onset to fluoxetine initiation did not correlate with SLSS change overall ($r = -0.24$, $p = 0.21$), though a stronger, but not significant, inverse correlation was noted among the subgroup of fluoxetine-treated patients with confirmed EV-D68 infection ($r = -0.53$, $p = 0.09$). Compared to untreated patients, fluoxetine-treated patients had a longer length of stay (median 14 vs 7 days, $p = 0.007$) and were more likely to require intensive care unit care, rehabilitation services, and ventilatory and supplemental feeding support (appendix, table e-3, links.lww.com/WNL/A764). There was one death in the fluoxetine-treated group and no deaths in the untreated group.

IVIg and corticosteroids were not associated with significant treatment effects in unadjusted or adjusted analyses using SLSS or WLSS (appendix, table e-4, links.lww.com/WNL/A764). Limiting analysis to only fluoxetine-treated patients, those with EV-D68 were more likely to be male and to have underlying asthma and shorter time between neurologic onset and initial examination, and less likely to have cranial nerve involvement than those without EV-D68 (appendix, table e-5). Unadjusted and adjusted mean changes in SLSS and WLSS between initial and follow-up examinations did not differ by EV-D68 status among fluoxetine-treated patients.

Discussion

High-dose, short course fluoxetine as an antiviral in AFM was well-tolerated in this cohort. However, the available data did

Figure 2 Fluoxetine efficacy on limb strength outcomes



Unadjusted mean summative limb strength score with SE is depicted at the time of initial examination, nadir, and latest follow-up examination for the fluoxetine-treated group (n = 28, black line) and the fluoxetine-untreated group (n = 28, gray line). [a]Fluoxetine was initiated prior to nadir in 11 of 30 (37%) treated patients and on or after nadir in 18 of 30 (60%) treated patients; fluoxetine timing was unknown for one patient.

Table 3 Fluoxetine association with limb strength outcomes

	Change in summative limb strength score: initial examination to latest follow-up, n (%)			Change in weakest limb strength score: initial examination to latest follow-up, n (%)		
	Fluoxetine-treated (n = 28)	Fluoxetine-untreated (n = 28)	*p* Value	Fluoxetine-treated (n = 28)	Fluoxetine-untreated (n = 28)	*p* Value
Improved	14 (50)	19 (68)	0.25	11 (39)	17 (61)	0.10
Same	3 (11)	4 (14)		6 (21)	7 (25)	
Worsened	11 (39)	5 (17)		11 (39)	4 (14)	
Unadjusted mean change (95% CI)	−0.4 (−2.5 to +1.8)	+2.1 (0 to +4.3)	0.10	0 (−0.5 to +0.5)	+0.9 (+0.2 to +1.6)	0.04
Adjusted mean change (95% CI)[a]	−0.2 (−1.8 to +1.4)	+2.5 (+0.7 to +4.4)	0.02	+0.1 (−0.4 to +0.6)	+1.1 (+0.5 to +1.6)	0.01

Abbreviation: CI = confidence interval.
[a] Adjusted for age, sex, strength score at initial examination, administration of corticosteroids, immunoglobulin, and plasmapheresis using doubly robust, propensity score–weighted ATE model.

not reveal a signal of efficacy. Fluoxetine was administered preferentially to patients with EV-D68-associated AFM and more severe paralysis at nadir. Patients treated with fluoxetine required greater supportive care and had poorer strength outcomes than untreated patients at latest follow-up. Despite significant limitations, this study has important implications to inform future therapeutic trials in AFM.

There are several possible explanations for the apparent lack of fluoxetine efficacy in this cohort despite in vitro antiviral activity. AFM is a clinical syndrome that can likely be caused by a variety of infectious etiologies (including poliovirus, EV-A71, and West Nile virus) and potentially noninfectious etiologies.[5] However, fluoxetine would only have the potential for efficacy in cases of CNS infection with a type B or D EV infection,[13] which was confirmed in a minority of all patients studied. The stronger inverse correlation between time to fluoxetine initiation and improved strength outcomes in fluoxetine-treated patients with confirmed EV-D68 infection compared with the fluoxetine-treated group as a whole may suggest a differential treatment response in this subgroup. In addition, there was likely significant selection bias, with clinicians choosing patients with more severe AFM to receive off-label, experimental treatment. Though propensity adjustment was used to partially correct for severity of weakness at onset, potentially differing trajectories of illness associated with certain etiologies cannot be completely accounted for through statistical adjustment. Notably, fluoxetine-treated AFM cases were much more likely to be EV-D68-associated than untreated cases, suggesting clinicians preferentially administered fluoxetine to patients with suspected or confirmed EV-D68 infection. The association of increased severity of illness in the fluoxetine-treated group with greater prevalence of EV-D68 infection may reflect that EV-D68 infection produces a more severe clinical phenotype than other etiologies of AFM. Previous studies have suggested poor long-term outcomes of EV-D68-associated AFM more similar to those of poliovirus than EV-A71.[6,21]

Antiviral treatment targeting EV-D68 would only be expected to produce clinical benefit if neurologic damage is mediated by direct effects of viral infection. Further, if EV-D68 produces neurologic damage via direct effects of CNS infection and if fluoxetine has in vivo anti-EV activity, clinical benefit would only occur if therapeutic concentrations are achieved at the site of infection prior to irreversible damage. Pharmacokinetic data (using a volume of distribution = 37.4 L/kg,[22] target EC_{50} = 418 ng/mL,[13,17] and serum to brain concentration ratio = 1:20 μM[14,15]) suggest that the fluoxetine dosage of 0.75 mg/kg/d received by most of those treated has the potential to reach therapeutic concentrations in the CNS. However, published data on detectable CNS concentrations of fluoxetine have been conducted in patients receiving chronic dosing and therapeutic levels are likely dependent on drug accumulation.[23] It is unknown whether drug concentrations after acute dosing reach the EC_{50} in the CNS in a window for potential therapeutic efficacy. Patients with AFM in this study had neurologic onset a median of 8.5 days following onset of prodromal illness and had their first documented neurologic examination a median of 2 days after onset of weakness. Fluoxetine was initiated a median 5 days after neurologic onset when most patients had already reached their neurologic nadir. This may have been past the point of irreversible neurologic damage in the majority of patients. Of note, the subgroup of patients with fluoxetine initiated prior to neurologic nadir had poorer outcomes compared to untreated patients. This may suggest timing of initiation alone did not account for the lack of treatment efficacy (and potential harm) associated with fluoxetine administration, though it remains possible that even treatment prior to nadir in this subgroup was too late to affect the disease process.

This retrospective study was subject to limitations inherent in observational research. Because detection of EV-D68 in CSF and stool in AFM cases is relatively infrequent,[5,24] and respiratory specimens, for which the yield is greater, are not

consistently obtained early in the disease course when sensitivity is highest,[3] it is possible that some patients in this series (in both treatment groups) were infected with undetected B or D EVs, resulting in misclassification. In addition, retrospective collection of outcomes data relied on strength assessments documented for clinical purposes. While more detailed strength and function assessments including the Assisting Hand Assessment and Hammersmith Functional Motor Scale have been utilized in prospective outcomes studies in AFM,[6] MRC score was chosen in this study as it was the most widely documented strength assessment in the medical record among study sites. The MRC score is highly operator-dependent, with significant variations in interrater reliability. It assesses a single muscle group, not overall limb strength or function, and is subject to muscle selection bias. MRC score is also an ordinal scale with nonlinear differences between scores (i.e., the difference between 3 and 4 is different than 4 to 5). Therefore, the conversion of MRC scores to a continuous variable and summation of weakest MRC scores from each limb to generate the SLSS as an overall measure of strength provides an imprecise approximation of clinical outcomes. Finally, the sample size of the study, in particular in the post hoc subgroup analyses, was relatively small, warranting caution in making definitive conclusions.

Despite these limitations, the findings of this study are consistent with lack of efficacy, and potential harm, of fluoxetine observed in an EV-D68 AFM mouse model.[25] In the murine study, in which fluoxetine was administered at doses equivalent to those used in humans in the present study, motor scores and viral titers in muscle and spinal cord were identical in treated mice compared to control mice treated with inert vehicle. In addition, mortality was higher in animals receiving fluoxetine. The mechanism by which fluoxetine would lead to possible harm in animals (or humans) with AFM is unclear.

The limitations of and lessons learned from this study should be used to inform future studies of therapeutics in AFM. Placebo-controlled, double-blind randomized studies would provide optimal evaluation of therapies for EV-D68-associated AFM. Collaborative national or international networks would be necessary to adequately power such a trial and overcome logistical obstacles, such as the varying and unpredictable geographic localization and sporadic incidence of AFM. Any potentially efficacious treatment would need to be initiated before irreversible CNS damage. Increased awareness is needed to ensure prompt recognition of AFM with early sample collection, including respiratory specimens, for rapid EV-D68 testing. Standardized prospective assessments of functional outcome measures are needed to objectively evaluate treatment efficacy.

High-dose, short course administration of fluoxetine as an antiviral to patients with AFM was well-tolerated. Fluoxetine was preferentially administered to AFM cases with EV-D68 identified and with more severe paralysis at nadir. Treated patients required more acute supportive care and continued to have more severe paralysis at latest follow-up. After propensity adjustment, fluoxetine was associated with poorer strength outcomes compared to untreated patients. These data, coupled with recent animal model data, do not suggest a positive efficacy signal for fluoxetine as a potential antiviral therapy for AFM.

Author contributions

Dr. Messacar: designed and conceptualized the study, interpreted the data, drafted the manuscript. Dr. Sillau: analyzed the data, revised the manuscript for intellectual content. Dr. Hopkins: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Otten: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Wilson-Murphy: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Wong: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Santoro: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Treister: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Bains: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Torres: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Zabrocki: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Glanternik: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Hurst: interpreted the pharmacologic data, revised the manuscript for intellectual content. Dr. Martin: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Schreiner: assisted in study design, revised the manuscript for intellectual content. Dr. Makhani: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. DeBiasi: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Kruer: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Tremoulet: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Van Haren: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Desai: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Benson: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Gorman: major role in the acquisition of data, revised the manuscript for intellectual content. Dr. Abzug: assisted in the design and conceptualization of the study, interpreted the data, revised the manuscript for intellectual content. Dr. Tyler: assisted in the design and conceptualization of the study, interpreted the data, revised the manuscript for intellectual content. Dr. Dominguez: assisted in the design and conceptualization of the study, interpreted the data, revised the manuscript for intellectual content.

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