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Single versus multiple dose ivermectin regimen in onchocerciasis-infected persons with epilepsy treated with phenobarbital: a randomized clinical trial in the Democratic Republic of Congo

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Abstract

Background

There is anecdotal evidence that ivermectin may decrease the frequency of seizures in *Onchocerca volvulus*-infected persons with epilepsy (PWE).

Methods

In October 2017, a 12-month clinical trial was initiated in rural Democratic Republic of Congo. PWE with onchocerciasis-associated epilepsy with ≥2 seizures/month were randomly allocated to receive over a one year period, ivermectin once or thrice (group 1), while other onchocerciasis-infected PWE (OIPWE) were randomized to ivermectin twice or thrice (group 2). All participants also received anti-epileptic drugs (AED). Study outcomes included seizure freedom during the last four months (primary endpoint), decrease in microfilarial density, and occurrence of adverse events. A multiple logistic regression model was used to evaluate the primary outcome.

Results

Of the 197 OIPWE enrolled, 100 were randomized to receive ivermectin thrice, 52 twice, and 45 once. In an intent-to-treat combined analysis of data from group 1 and 2, the probability to become seizure-free for OIPWE treated with ivermectin twice per year was significantly higher than in those treated once (OR: 5.087, 95% CI: 1.378-19.749; p=0.018) and individuals who received ivermectin twice had a 4.471 (95% CI: 0.944-6.769, p=0.075) times higher odds of seizure freedom than those received ivermectin once per year. Absence of microfilariae during the last 4 months was associated with a higher probability of seizure freedom (p=0.027).

Conclusions

Increasing the number of ivermectin treatments per year was found to suppress both microfilarial density and seizure frequency in OIPWE, suggesting that *O. volvulus* infection plays an etiological role in causing seizures.

Registration: www.clinicaltrials.gov; NCT03852303

Introduction

An association between onchocerciasis (river blindness) and epilepsy was reported as early as 1938 [1]. This association was later documented in many cross-sectional studies [2-4] but the causal relationship between onchocerciasis and epilepsy remains controversial. A recent cohort study performed in an onchocerciasis-endemic region in Cameroon strongly suggested that infection with *Onchocerca volvulus* is able to cause epilepsy depending on the microfilarial (mf) density [5].

To investigate the role of *O. volvulus* in triggering and aggravating seizures, we evaluated the effect of ivermectin on the frequency of seizures in onchocerciasis-infected persons with epilepsy (OIPWE). Demonstrating such an effect would provide additional support that infection with *O. volvulus* is able to cause epilepsy. Conducting a clinical trial in a remote onchocerciasis-endemic area in Africa is logistically difficult and costly to organize. Therefore, we initially performed a four months proof-of-concept trial to investigate the effect of ivermectin on the frequency of seizures in OIPWE treated with phenobarbital. The trial was performed in the Logo health zone, an onchocerciasis-endemic area with a high epilepsy prevalence (4.6%) in the Ituri province in the Democratic Republic of Congo (DRC), where ivermectin had never been distributed previously [6]. Between October and November 2017, a community-based treatment of epilepsy was initiated and 387 persons with confirmed epilepsy (PWE) were enrolled in the program. Ninety-four of them who met the criteria for onchocerciasis-associated epilepsy (OAE) [7], and who experienced at least two seizures per month by the time of assessment, were enrolled in the proof-of-concept trial. The protocol of this trial was previously published [8]. In

March 2019 all participants had been followed for 4 months. However, given the small sample size the results of the trial were difficult to interpret (paper submitted for publication). During the trial, we realized that the planned sample size (110 individuals) could not be reached as the inclusion criteria were very strict. Moreover, trial participants requested to be followed up beyond four months, and the PWE with *O. volvulus* infection who had initially been excluded from the trial also wanted to participate. Therefore, before completion of the proof-of-concept trial, we decided to re-randomize the initial 94 participants (group 1) into two arms: one group would receive ivermectin once a year whereas individuals in the other one would be treated thrice a year. Furthermore, we randomized the remaining 103 OIPWE who had initially been excluded from the trial (group 2) to ivermectin treatment twice or thrice a year.

Ivermectin is very effective in killing the *O. volvulus* mf; however, it only temporarily represses mf production by the adult worm, which survives ivermectin treatment and resumes mf production at a slow rate after approximately 3-6 months [9]. Therefore, if ivermectin has an effect on the frequency of seizures in OIPWE, we expect that in a trial comparing annual ivermectin treatment with two or more doses per year, no difference in seizure frequency would be observed during the first few months. However, fewer seizures would be expected several months later particularly among persons who received more frequent doses of ivermectin. In this paper, we present the seizure outcomes of a single versus multiple dose ivermectin regimen in PWE after 12 months of follow-up.

Material and Methods

Study design and participants

Before starting the recruitment of study participants, village chiefs, nurses, and community health workers (CHW) of five onchocerciasis-endemic villages (Draju, Kanga, Wala, Ulyeko and Thedeja) within the Logo health zone were informed about the purpose and specificities of the study. Study

procedures, treatment regimens, potential risks and benefits were explained to interested participants and their parents/guardians. Consenting PWE were then assessed for eligibility to be enrolled into the study. Alur, the local language, was used for all communication with participants and parents/guardians.

To be enrolled, participants had to meet the 2014 International League Against Epilepsy (ILAE) definition of epilepsy: having experienced at least two seizures, unprovoked and without fever, with a minimal time difference of 24 hours between the two events [10] and either present mf detected during skin testing and/or onchocerciasis antibodies detected using an Ov16 rapid diagnostic test.

Two skin snips were obtained from each participant (one skin snip from each iliac crest), using a Holth corneoscleral punch. After incubation for 24 hours in isotonic saline, mf were counted using an inverted microscope and the arithmetic mean of the mf in the two skin snips was calculated. The Ov16 rapid test (SD BIOLINE Onchocerciasis IgG4 rapid test, Abbott Standard Diagnostics, Inc. Yongin, Republic of Korea) was used to test for the presence of onchocerciasis IgG4 antibodies.

Randomization and treatment regimens

Study participants were stratified into two groups; group 1 consisted of PWE who met the criteria for OAE [7] and experienced two or more seizures per month (these PWE were included in the four months proof-of-concept trial), and group 2 consisted of all other onchocerciasis-infected PWE (Figure 1). Separate randomization tables were developed for each of the two groups. Initially, the 94 PWE in group 1 were randomized to receive 150µg/kg ivermectin (Stromectol®) [11] plus anti-epileptic drugs (AED) (phenobarbital), or AED alone. Four months after the first dose, these 94 PWE were re-randomized to receive a total of either one dose or three doses 150µg/kg ivermectin (Stromectol®) plus AED. All the PWE in group 2 were randomized to receive 150µg/kg ivermectin (Stromectol®)

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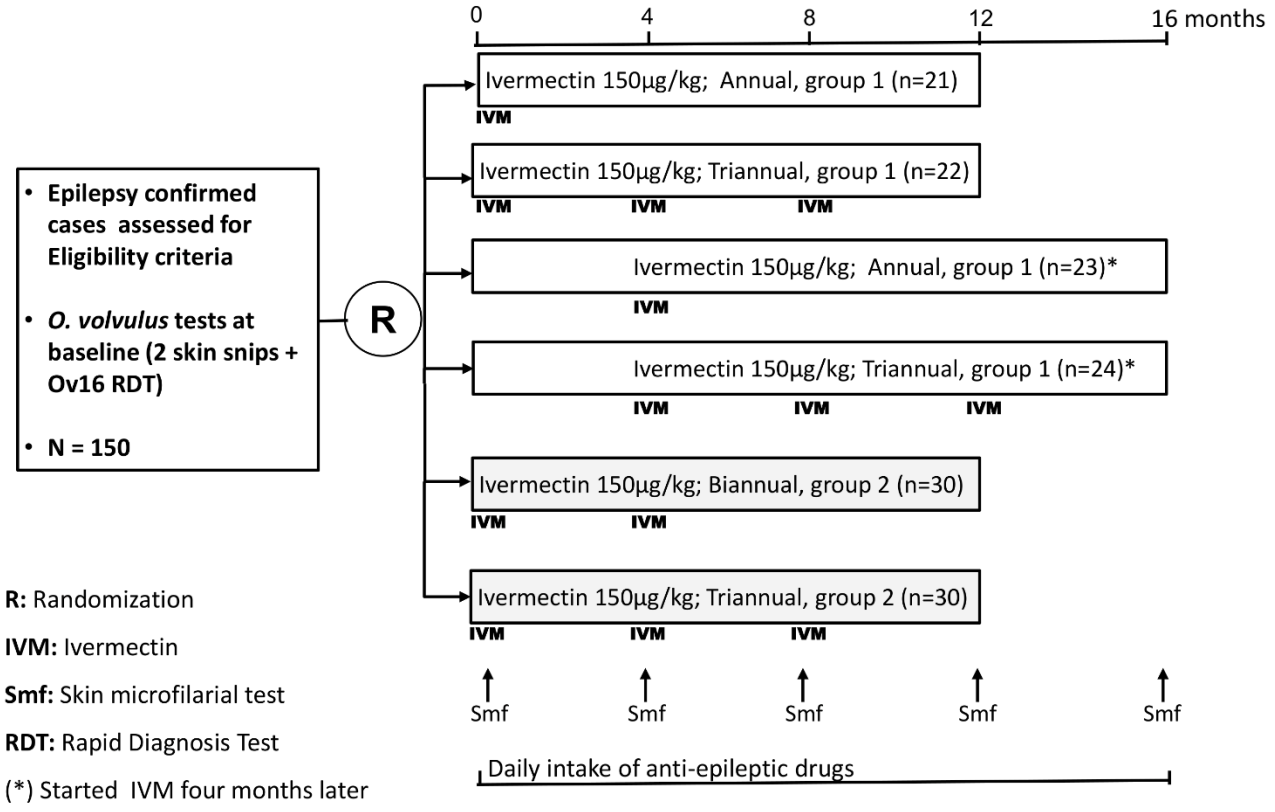
twice or thrice a year, plus AED during a one-year period. Overall, all study participants were followed for 12 months after the first dose of ivermectin.

Figure 1. Study participants recruitment and follow-up plan



Ivermectin (Stromectol®) was administered orally to the allocated study participants, under direct observation of an unblinded dispenser who was not involved in assessing the participants during follow-up. The minimal time interval between two consecutive doses of ivermectin was four months. All study staff involved in collecting and analyzing the data were kept blinded for treatment allocation until data lock.

The AED (phenobarbital) dose was based on the participants' weight: 5mg/kg for participants weighing <15kg; 3mg/kg for those weighing between 15–35kg, and 2mg/kg for participants with a weight above 35kg. Phenobarbital was taken orally once daily with possibilities to adjust the dose

based on seizure frequency and/or occurrence of side effects. AED were made available freely with support of the humanitarian organization Malteser International.

Baseline and follow-up procedures

All study procedures were done according to standard operating procedures developed by the study team. At baseline, information was collected on seizure semiology, seizure frequency, epilepsy risk factors, relevant medical history, previous AED and ivermectin use. Women of childbearing age (14–49 years) were tested for HCG (human chorionic gonadotropin) in urine to exclude pregnancy, and pre-conception counseling was given following national guidelines.

Trained community health workers (CHW) did monthly home visits, to monitor treatment adherence by counting AED pills, to identify potential side effects and to complete a seizure diary. Moreover, participants were seen monthly at the health center by project nurses and medical doctors during a one-year period. During visits, a physical and neurological examination was performed as well as an assessment of the frequency of seizures, adverse events, and adherence to AED treatment. Sub-optimal adherence was defined as ≥3 days/month without AED. Cognitive function was evaluated by determining whether the participant was well oriented in time and space, whether he/she could remember his/her name, was coherent in speech, and was obedient to orders. Medical doctors also reviewed the seizure diary and notes of the CHW. Based on their clinical assessment and information collected by CHW, medical doctors decided whether the initial dose of phenobarbital had to be continued or needed to be changed. If a participant was unable to reach the health center, a home visit was performed by a medical doctor or nurse trained in epilepsy management. Skin snip testing was repeated by the same laboratory technician every four months following the procedure described above. Skin snips were obtained before the administration of ivermectin. During each visit, female participants of childbearing age were questioned regarding menstrual aberrations and other signs of

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pregnancy. If pregnancy was suspected, a rapid pregnancy test was done and ivermectin treatment was interrupted upon confirmation of the gravid state but not the AED treatment. All pregnant women were followed up until delivery.

Primary outcome

The primary outcome was seizure freedom during the last four months of the 12-month trial. Medical doctors compared the number of seizures reported in the diaries completed by the CHW with the number of seizures reported by the participants during each visit, and a consensual seizure frequency was reached between the doctor, the CHW, and the PWE/guardians.

Secondary outcomes

Skin mf density during the last four months of the trial, adverse events at any point during the trial were considered as secondary outcomes. Adverse events were assessed through clinical examination, by questioning participants and guardians, and by reviewing the reports of CHW. We used the Common Terminology Criteria for Adverse Events (CTCAE), version v5.0 for the classification of adverse events and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use criteria to determine whether adverse events were serious [12].

Sample size calculation

Assuming that 60% of OIPWE in group 1 receiving ivermectin thrice a year and 30% receiving ivermectin once per year will no longer have seizures during the last four months of the trial (9 to 12 months after receiving the first dose of ivermectin), implying an odds ratio (OR) of 3.5, 84 (2 × 42) participants will be needed to detect a significant difference in odds between the treatment regimens with a power of 80% and a two-sided 5% significance level. Assuming that 65% of OIPWE in group 2 (most of them having less seizures compared to individuals in group 1) receiving ivermectin thrice

a year will no longer have seizures during the last four months of the trial, compared to 40% of those receiving ivermectin twice per year (OR = 2.5), 123 (2 × 62) participants will be required to detect a significant difference with a power of 80% and a two-sided 5% significance level. Assuming the rate of lost to follow-up or early withdrawal to be 10%, a minimum of 229 participants would be required (i.e., 94 in group 1, and 135 in group 2).

Statistical Analysis

All randomized participants who received at least one dose of ivermectin were included in the primary analysis according to their assigned treatment arms (intent-to-treat analysis). Categorical variables were reported as counts and percentages, while continuous variables were described using medians and interquartile ranges (IQRs). Baseline characteristics of the two groups (group 1 and 2) were compared using a Fisher's exact test for categorical variables and a median test for continuous variables. Multiple logistic regression models were used to evaluate the effect of different ivermectin regimens on the probability of being seizure-free during the last four months of the study. The relationship between seizure freedom during the last four months and the presence of mf in skin snips during the last four months was evaluated using multiple logistic regression. Stratified analyses by group were performed and model fit was compared with a combined model fitted to all data using the Akaike Information Criterion (AIC).[12-13] An additional analysis based on the actual number of ivermectin treatments received (as-treated) was also performed. All PWE who died, were lost to follow-up, or discontinued their participation before the 12th month were considered as not being seizure free. In the presence of separation, Firth's modified score equation approach was used to estimate the model parameters [13]. The 95% confidence intervals (CIs) for all estimates were constructed. All analyses were performed using the statistical software programs SAS 9.4 (SAS Institute Inc.) and R version 3.6.1, relying on a two-sided 5% significance level.

Ethical considerations

The study was approved by the Ethics committee of the School of Public Health of the University of Kinshasa in the DRC (ESP/CE/063/2017) and the one of the University of Antwerp, Belgium (17/32/369). All participants gave their consent or assent (with parental consent) through signature or thumbprint in the presence of a literate witness. PWE who refused to participate were still examined and given AED. In specific cases, a special consent was obtained for photos.

Trial registration

The clinical trial was registered at www.clinicaltrials.gov (NCT03052998)

Results

Description of the study population

Between October 1st 2017, and March 16th 2018, 387 PWE were screened. One hundred ninety-seven PWE (50.9%) with evidence of *O. volvulus* infection were included in the trial. The median age of these OIPWE was 23 years (IQR: 17–30). Ninety-four of them fulfilled the criteria for OAE with ≥2 seizures/months (group 1), of which 45 (47.9%) were randomly allocated to receive ivermectin once and 49 (52.1%) to receive ivermectin thrice. Of the remaining 103 OIPWE (group 2), 52 (50.5%) were randomly allocated to receive ivermectin twice and 51 (49.5%) to receive ivermectin thrice (Figure 2). Overall, 100 participants were assigned to receive ivermectin thrice, 52 twice, and 45 once.

Figure 2. Trial profile of screened, randomized, treated, and analyzed patients by study group

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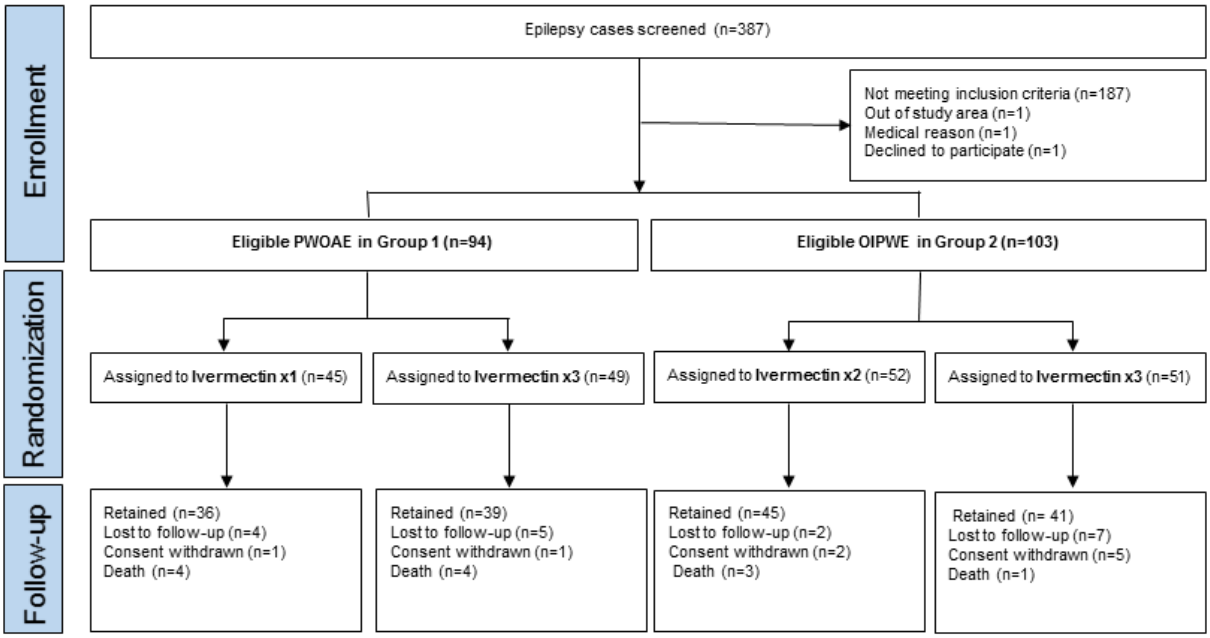

Participants from group 1 were significantly younger, with a higher prevalence of burn scars and cognitive impairment compared to those in group 2 (Table 1).

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1 **Table 1**. Baseline characteristics of the participants in the different treatment arms

	Group 1			Group 2			P-value*
	Ivermectin x1	Ivermectin x3	Total	Ivermectin x2	Ivermectin x3	Total	
	n=45	n=49	n=94	n=52	n=51	n=103	
Median age in years (IQR)	24 (18-30)	22 (16-28)	20 (16-27)	24 (17-35)	28 (21-35)	26 (18-35)	0.001
Males, n (%)	29 (64)	29 (59)	58 (62)	21 (40)	22 (43)	43 (42)	0.006
Height in cm, median (IQR)	150 (144-159)	152 (146-158)	152 (144-158)	155 (150-163)	154 (146-160)	155 (147-162)	0.072
Weight in kg, median IQR)	42 (34-51)	47 (38-50)	45 (35-51)	49 (44-54)	45 (40-51)	48 (41-53)	0.112
Ivermectin use in the past, n (%)	0 (0)	0 (0)	0	2 (4)	1 (2)	3 (3)	0.247
Seizure characteristics:							
History of generalized seizures, n (%)	43 (95)	46 (95)	89 (95)	52 (100)	49 (96)	101 (98)	0.261
History of absence seizures, n (%)	26 (58)	29 (47)	55 (59)	14 (27)	18 (35)	32 (31)	0.002
History of nodding seizures, n (%)	5 (11)	2 (3)	7 (8)	1 (2)	1 (2)	2 (2)	0.089
Seizure frequency per month, median (IQR)	2 (1-4)	3 (2-7)	3 (2-6)	1 (1-4)	2 (1-3)	2 (1-3)	<0.001
Epilepsy duration in years, median (IQR)	10 (6-15)	13 (7-17)	12 (16-27)	6 (3-14)	10 (2-17)	7 (3-16)	0.093
Physical examination:							
Altered general state, n (%)	23 (51)	18 (30)	41 (44)	6 (11)	12 (24)	18 (17)	0.001
Itching, n (%)	22 (49)	19 (40)	41 (44)	18 (35)	21 (41)	39 (38)	0.468
Palpable nodules, n (%)	3 (6)	7 (10)	10 (11)	5 (10)	3 (6)	8 (8)	0.621
Burn scars, n (%)	17 (38)	19 (30)	36 (38)	10 (19)	11 (22)	21 (20)	0.007

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Leopard skin, n (%)	1 (2)	1 (2)	1 (1)	2 (4)	1 (2)	3 (3)	0.734
Lizard skin, n (%)	4 (8)	4 (7)	8 (9)	1 (7)	1 (2)	2 (2)	0.234
Cognitive impairment, n (%)	21 (47)	22 (33)	43 (46)	10 (19)	11 (22)	21 (20)	0.002
Microfilarial density per skin snip, median (IQR)	11 (0-53)	28 (3-48)	17 (1-72)	6 (0-78)	6 (1-37)	6 (0-56)	0.062
Skin snip positivity, n (%)	33 (73)	40 (79)	73 (78)	39 (56)	29 (57)	68 (66)	0.078
Ov16 positivity	32 (71)	35 (71)	67 (71)	42 (81)	35 (63)	77 (75)	0.338

Group 1: OIPWE meeting the OAE criteria with ≥2 seizures/month at baseline, Group 2: OIPWE not meeting the OAE criteria or < 2 seizures/month at baseline;

**Fisher exact test for counts and median test for continuous variables comparing baseline characteristics of group 1 and 2 participants; IQR: Interquartile range.*

2

A total of 177 (90%) participants attended the one-year follow-up visit; nine (4.5%) were lost to follow-up, and 12 (6.1%) had died (Table 2). Although the median mf density at month 12 was similar in both study groups, a greater proportion of participants in group 1 still experienced seizures and itching by the end of the study period compared to individuals in group 2.

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7 **Table 2**. Characteristics of the participants at month 12 in the different treatment arms

	Group 1			Group 2			P-value*
	Ivermectin x1	Ivermectin x3	Total	Ivermectin x2	Ivermectin x3	Total	
Status of participants at 12 months:							
Intent-to-treat set, n (%)	45 (23)	49 (51)	94 (100)	52 (26)	51 (26)	103 (100)	
As-treated set, n (%)	41 (91)	39 (79.6)	80 (85)	49 (94.2)	37 (72.5)	86 (83.5)	
Attended last follow-up visit, n (%)	36 (91)	39 (79.6)	75 (79.7)	45 (86.5)	41 (80.4)	86 (83.5)	0.139
Lost to follow up, n (%)	4 (8.8)	5 (10.2)	9 (9.6)	2 (3.8)	7 (13.7)	9 (8.7)	0.838
Consent withdrew, n (%)	1 (2.2)	1 (2)	2 (4.1)	2 (3.8)	5 (10)	7 (6.8)	0.117
Death, n (%)	4 (8.9)	4 (8.2)	8 (8.5)	3 (5.8)	1 (1.9)	4 (3.9)	0.235
Pregnant, n (%)	1 (2)	0 (0)	1 (1)	2 (3.8)	5 (10)	7 (6.8)	0.035
Seizure-free during last four months, n (%)	12 (27)	21 (43)	33 (35)	31 (59)	21 (41)	52 (50)	0.034
Seizure frequency during last four months, median (IQR)	2 (0-5)	1 (0-3)	2 (0-5)	0 (0-2)	0 (0-2)	0 (0-2)	0.016
Optimal adherence to AED during last four months, n (%)	28/45 (62)	29/49 (59)	57 (61)	31/51 (57)	36/52 (77)	67 (65)	0.562
Increased AED dose more than 30mg	21/45 (47)	18/49 (37)	39/94 (41)	14/52 (27)	12/51 (23)	26 (24)	0.022
Switch to a different AED, n (%)	2/45 (4)	4/49 (8)	6 (6)	0/52 (0)	3/51 (6)	3 (3)	0.308
Physical examination:							
Altered general state, n (%)	5 (14)	7 (14)	12 (17)	5 (10)	8 (17)	13 (14)	0.582
Itching, n (%)	3 (8)	5 (5)	8 (9)	0 (0)	0 (0)	0	0.003
Burn scars, n (%)	1 (3)	2 (5)	3 (3)	0 (0)	2 (4)	2 (2)	0.669

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Skin snip positivity, n (%)	16 (44)	7 (15)	23 (31)	13 (32)	5 (11)	18 (19)	0.065
Microfilarial density per skin snip, median (IQR)	0 (0-3)	0 (0-0)	0 (0-2)	0 (0-1)	0 (0-0)	0 (0-1)	0.126

Group 1: OIPWE meeting the OAE criteria with ≥2 seizures/month at baseline, Group 2: OIPWE not meeting the OAE criteria or < 2 seizures/month at baseline;

**Fisher exact test for counts and median test for continuous variables comparing the characteristics of group 1 and 2 participants at month 12;*

IQR: Interquartile range; AED: Anti-epileptic drug.

8

Of the100 participants randomized to ivermectin thrice, 76 (76%) were treated as randomized and were followed for 12 months. Twenty four (24%) did not receive all three doses of ivermectin; five of them died, five were pregnant, 10 missed a dose because not visiting the health center and five withdrew consent. Of the 52 participants who were randomized to receive ivermectin twice, 49 (94.2%) were treated as randomized and 45 were followed for 12 months; three died, two were lost to follow up and two withdrew consent. Of the 45 participants who were randomized to receive ivermectin once, 41 (80%) were treated as randomized and 36 were followed for 12 months; four died, four were lost to follow up and one withdrew consent.

In both groups, the mean skin mf density decreased drastically between month 0 and month 4, and remained low at month 8 through month 12 (Figure 3). In group 1, the mf density at month 12 was significantly lower (p=0.032) in participants who received ivermectin thrice compared to those who received ivermectin once per year (Figure 3A), in contrast to individuals in group 2 in which there was no difference in mf density between the thrice and twice a year treatment arms (Figure 3B).

Figure 3. Average skin microfilarial density (with error bars) at baseline, month 4, 8 and 12, Figure 3A. Group 1 participants (ivermectin thrice vs ivermectin once per year), Figure 3B. Group 2 participants (ivermectin thrice vs ivermectin twice per year)

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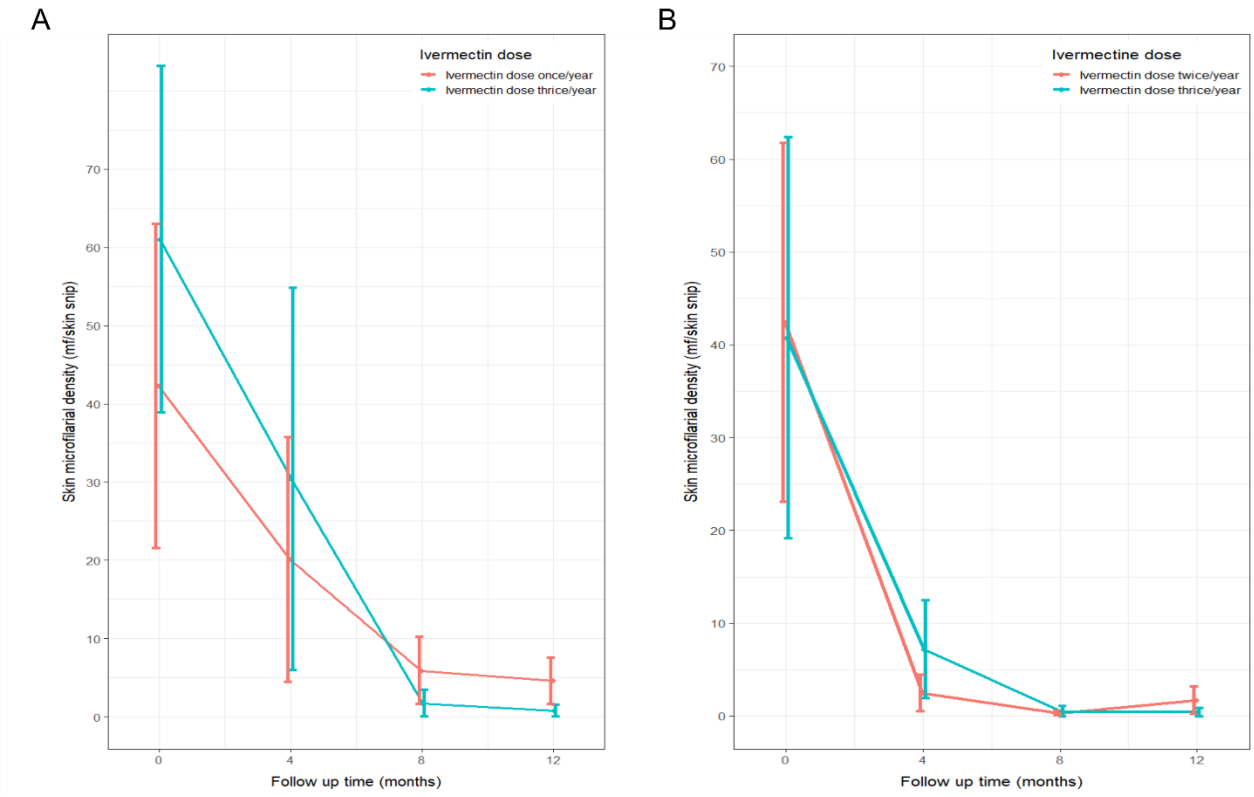

Group 1 included a higher number of PWE for which the AED dose was increased above 30mg during the trial (Table 2). Moreover, the AED dose was increased above 30mg in a slightly higher number of persons on an ivermectin once regimen (in 21 out of 45 persons, 46.6%) compared to an ivermectin twice regimen (in 14 out of 52 persons, 26.9%) and an ivermectin thrice regimen (in 30 out of 100 persons, 30%). The proportion of individuals with an increase of AED dose >30mg in persons on an ivermectin once regimen was significantly higher compared to the proportion of AED increases in persons on an ivermectin thrice regimen (unadjusted OR: 2.142, 1.034-4.457; p=0.039); no such difference was observed in group 2 between individuals on ivermectin twice and ivermectin thrice regimens (p=0.786).

Intent-to-treat analysis

The intent-to-treat population included all 197 randomized participants. Of these, 12 did not take the total number of ivermectin doses assigned, nine were switched to another AED, and in 65 individuals

the AED dose had been increased by more than 30 mg during the 12-month trial because seizures were not controlled. Additionally, 73 PWE did not adhere optimally to AED. On an intent-to-treat basis, 43% of participants were seizure-free during the last four months of the follow-up period.

Primary outcome: **probability of seizure freedom during the last four months**

Group 1 (OIPWE meeting the OAE criteria with ≥2 seizures/month at baseline)

In group 1, the probability of being seizure-free during the last four months of the one-year follow-up period was not significantly different for those who received ivermectin thrice than for those who received ivermectin once per year (p=0.111) (Table 3).

Table 3. Multiple logistic regression exploring the association between ivermectin once and thrice a year and seizure freedom during last four months (group 1).

Variables	OR	95% CI		P-value
Ivermectin dose once/year vs thrice/year	2.310	0.865	6.592	0.111
Female vs male	1.418	0.521	3.939	0.505
Age	1.070	0.981	1.175	0.156
Weight (in kg)	1.020	0.968	1.079	0.476
Number of seizures at baseline	0.667	0.301	1.341	0.291
Duration of epilepsy (years)	0.604	0.263	1.355	0.236
Microfilarial density at baseline	0.779	0.591	1.009	0.073
Optimal vs sub-optimal AED adherence during last four months	3.358	1.206	10.181	0.029
>30mg of AED increase (baseline-month 12) vs no increase	0.278	0.087	0.797	0.026

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug

Group 2 (OIPWE not meeting the OAE criteria or experiencing < 2 seizures/month)

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In group 2 the probability of being seizure-free during the last four months for PWE treated with ivermectin thrice was not significantly different than for those treated twice annually (p=0.107) (Table 4).

Table 4. Multiple logistic regression exploring the association between ivermectin thrice and twice a year and seizure freedom during last four months (group 2).

Variables	OR	95% CI		P-value
Ivermectin dose thrice/year vs twice/year	0.477	0.196	1.125	0.107
Female vs male	0.871	0.347	2.131	0.772
Age	0.988	0.946	1.030	0.583
Weight (in kg)	0.990	0.941	1.041	0.713
Number of seizures at baseline	0.823	0.458	1.462	0.527
Duration of epilepsy (years)	1.105	0.688	1.812	0.693
Microfilarial density at baseline	0.739	0.573	0.934	0.018
Optimal vs sub-optimal AED adherence during last four months	2.792	1.141	7.281	0.034
>30mg of AED increase (baseline-month 12) vs no increase	0.212	0.067	0.595	0.006

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug

Group 1 and 2 combined

Adjusting for study group membership (group 1 and group 2), the intake of ivermectin twice or thrice per year resulted in higher odds of seizure freedom during the last four months of the trial as compared to an annual ivermectin regimen. After adjusting for other covariates, the odds of seizure freedom when receiving ivermectin thrice was 5.087 (95% CI: 1.378-19.749) times higher compared to the odds in individuals who received ivermectin once a year (p=0.018). The odds of seizure freedom for individuals who received ivermectin twice was 4.471 (95% CI: 0.944-6.769, p=0.075) times the odds of those who received ivermectin once per year (Table 5). The combined model presented in Table 5 outperforms a model allowing for differential effects of the covariates for both study groups (as is the case for the separate analyses) based on a smaller AIC-value (183.03 versus 193.79).

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Table 5. Multiple logistic regression exploring the association between taking ivermectin thrice, twice or once a year with seizure freedom during the last four months of the trial adjusted, for study group membership (combined analysis for group 1 and group 2).

Variables	OR	95% CI		P-value
Ivermectin dose thrice/year vs once/year	2.471	0.944	6.769	0.075
Ivermectin dose twice/year vs once/year	5.087	1.378	19.749	0.018
Female vs male	1.152	0.593	2.235	0.681
Age	0.999	0.964	1.036	0.967
Weight (in kg)	1.004	0.969	1.039	0.838
Number of seizures at baseline	0.731	0.460	1.133	0.180
Duration of epilepsy (years)	1.039	0.711	1.538	0.848
Microfilarial density at baseline	0.752	0.627	0.892	0.002
Optimal vs sub-optimal AED adherence during last four months	3.274	1.673	6.635	0.001
>30mg of AED increase (baseline-month 12) vs no increase	0.253	0.116	0.525	<0.001
Study group 1 vs group 2	1.989	0.749	5.466	0.182

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug

The probability of being seizure free was positively associated with the absence of mf during the last four months (month 8 and month 12) OR 2.618 (95% CI: 1.136-6.289) (p=0.027) (Table 6).

Table 6. Multivariable analysis investigating the effect of absence of mf during the last four months on seizure freedom during the same period.

Variables	OR	95% CI		P-value
Absence vs presence of mf during month 8 and month 12 visits	2.618	1.136	6.289	0.027
Female vs male	1.089	0.539	2.187	0.811
Age (years)	0.992	0.949	1.038	0.711
Weight (in kg)	1.013	0.977	1.051	0.477

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Number of seizures at baseline	0.719	0.432	1.175	0.192
Duration of epilepsy (years)	1.001	0.952	1.053	0.960
Optimal vs sub-optimal AED adherence during last four months	1.885	0.909	3.988	0.092
>30mg of AED increase (from baseline to month 12) vs no increase	0.193	0.088	0.407	<0.001
Study group 1 vs group 2	0.861	0.391	1.912	0.711

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug; Mf: microfilariae

As-treated analysis

Group 1

In group 1**,** of the 49 randomized to receive ivermectin thrice, 39 were treated as randomized (Table 2). Of the 45 randomized to receive ivermectin once, 41 were treated as randomized, four were treated once and two were treated twice (Table 2). The participants who received ivermectin thrice a year were 3.318 (1.161-10.500; p=0.035) times more likely to be seizure free than those who received ivermectin once (Table 7).

Table 7. Multiple logistic regression exploring the association between ivermectin thrice and once a year on seizure freedom during last four months (as-treated analysis, group 1)

Variables	OR	95% CI		P-value
Ivermectin dose thrice/year vs once/year	3.318	1.161	10.500	0.035
Female vs male	1.526	0.521	4.671	0.458
Age	1.061	0.967	1.177	0.248
Weight (in kg)	1.011	0.952	1.074	0.732
Number of seizures at baseline	0.815	0.347	1.755	0.627
Duration of epilepsy (years)	0.681	0.291	1.553	0.380
Microfilarial density at baseline	0.746	0.542	0.999	0.065
Optimal vs sub-optimal AED adherence during last four months	1.897	0.635	6.001	0.278
>30mg of AED increase (baseline-month 12) vs no increase	0.201	0.063	0.581	0.006

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug

Group 2

In group 2, of the 51 who were randomized to receive ivermectin thrice, 37 were treated as randomized (Table 2), seven were treated twice and 5 were treated once. Of the 52 participants who were randomized to receive ivermectin twice, 49 were treated as randomized (Table 2). The probability of seizure freedom of the participants who received ivermectin thrice and those who received ivermetin twice was similar (p=0.183) (Table 8).

Table 8. Multiple logistic regression exploring the association between ivermectin thrice and twice a year on seizure freedom during last four months (as-treated analysis, group 2)

Variables	OR	95% CI		P-value
Ivermectin dose thrice/year vs twice/year	0.484	0.168	1.348	0.183
Female vs male	0.851	0.306	2.279	0.758
Age	0.988	0.943	1.034	0.630
Weight (in kg)	0.980	0.924	1.037	0.508
Number of seizures at baseline	0.775	0.388	1.521	0.480
Duration of epilepsy (years)	1.009	0.592	1.756	0.975
Microfilarial density at baseline	0.657	0.484	0.859	0.005
Optimal vs sub-optimal AED adherence during last four months	2.107	0.767	6.075	0.170
>30mg of AED increase (baseline-month 12) vs no increase	0.147	0.041	0.451	0.002

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug

Group 1 and 2 combined

In total, 76 participants received ivermectin thrice a year, 58 ivermectin twice and 50 ivermectin once. The participants who received ivermectin thrice and those who received ivermectin twice were respectively 4.795 (95% CI: 1.790-14.089) and 10.033 (95% CI: 2.670-42.496) times more likely to be seizure free than those who received ivermectin once (Table 9).

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Table 9. Multiple logistic regression exploring the association between ivermectin once, twice and thrice a year on seizure freedom during last four months adjusted for study group (as-treated analysis, group 1 and 2 combined)

Variables	OR	95% CI		P-value
Ivermectin dose thrice vs once/year	4.795	1.790	14.089	0.003
Ivermectin dose twice vs once/year	10.033	2.670	42.496	0.001
Female vs male	1.135	0.548	2.348	0.738
Age (years)	0.999	0.960	1.038	0.947
Weight (in kg)	0.993	0.954	1.033	0.731
Number of seizures at baseline	0.771	0.453	1.279	0.333
Duration of epilepsy (years)	1.031	0.686	1.574	0.886
Microfilarial density at baseline	0.691	0.561	0.837	0.000
Optimal vs sub-optimal AED adherence during last four months	1.966	0.935	4.238	0.085
>30mg of AED increase (from baseline to month 12) vs no increase	0.171	0.074	0.372	<0.001
Study group 1 vs group 2	3.141	1.097	9.668	0.043

OR: Odds ratio; CI: Confidence interval; AED: Antiepileptic drug

Trial adverse events

In total, 169 adverse events were reported among the 197 randomized participants; 133 (79%) of them were considered to be possibly related to ivermectin or the AED (Table 10).

Table 10. Adverse events reported during the 12 months of follow-up

	Ivermectin x 3	Ivermectin x 2	Ivermectin x 1	0verall
Number of participants	100	52	45	197
AE reported	84	44	41	169
Average number of AE per person	0.84	0.85	0.91	0.86
AE possibly related to the treatment, n (%)	64 (76)	37 (84)	32 (78)	133 (79)
AE severity				

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Minimal, n (%)	31 (37)	14 (32)	11 (27)	56 (33)
Moderate, n (%)	34 (41)	21 (48)	20 (49)	75 (44)
Severe, n (%)	17 (20)	6 (14)	10 (24)	33 (20)
Life-threatening, n (%)	1 (1)	2 (5)	0	3 (2)
Fatal outcome, n (%)	5 (6)	3 (7)	4 (10)	12 (7)
Any SAE, n (%)	13 (16)	3 (7)	4 (10)	20 (12)
SAE possibly related to the treatment, n (%)	9 (11)	1 (2)	2 (5)	12 (7)

AE: adverse event; SAE: serious adverse events

One serious adverse event, toxic epidermal necrosis (TEN), was related to the intake of phenobarbital. The affected person was a 42 years old male weighing 46 kg and being randomized in the ivermectin thrice arm. He received a first dose of ivermectin at the start of the study and was treated with 100 mg of phenobarbital daily, indicated for generalized tonico-clonic seizures (one seizure per month at the start of the study).

Two weeks after starting phenobarbital he experienced itching. A maculo-papular skin rash was noted at the first month visit, predominantly on the limbs. Ten days later, he was hospitalized at the Logo hospital because he was severely ill, presenting with fever, injected conjunctiva, and swollen lips with oral ulcerations. On day 6 of hospitalization, he developed bullous lesions over his entire body except his feet (Figure 4). A diagnosis of toxic epidermal necrolysis (TEN) was made; phenobarbital was stopped and treatment was switched to sodium valproate. He was treated in the intensive care unit with corticosteroids and antibiotics for two weeks. Thirty days after admission, his skin lesions had healed completely.

Figure 4. Evolution of the toxic epidermal necrosis, from hospital admission up to discharge

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Five participants developed burns; four in the ivermectin thrice arm and one in the ivermectin once arm. In one of them, the burn was classified as a life-threatening serious adverse event requiring hospitalization.

Six participants died in the ivermectin thrice arm and five in the ivermectin once arm for reasons not directly related to study procedures or drugs. More specifically, four participants died during seizures (two of them by drowning in the river and two during seizures while sleeping), seven died of co-morbidities, i.e., two died in the hospital due to infection, malnutrition and anemia while the remaining five individuals died in the village because of septicemia (1), suspected pulmonary tuberculosis (1), with severely altered consciousness (2), or with Nakalanga features dying from malnutrition (1).

Eight women got pregnant during the study period. One of them, 32 years old, randomized in the ivermectin twice arm and taking daily 100 mg of phenobarbital since November 2017, delivered a girl with a separation of the upper lip not extended to the base of the nose and not involving the palate in June 2019. The birth weight of the newborn was 3.5 kg and there were no other visible malformations. The Venereal Disease Research Laboratory test for syphilis of the mother was negative. In February 2018, this woman had received four tablets of praziquantel and during pregnancy she received two doses of sulfadoxine-pyrimethamine and 5 mg folic acid daily for three months. Since AED initiation in November 2017, she has been seizure-free.

Discussion

In the intent-to-treat analysis of all OIPWE participants enrolled in the study (group 1 and 2 combined), those receiving multiple doses of ivermectin were more likely to be seizure-free during the last four months of the trial, compared to OIPWE who only received ivermectin once. Analyzing the results of the two study groups separately, an advantage of the thrice ivermectin regimen over the once or twice ivermectin regimen could not be confirmed. However, this may be because of the small sample size and because a large percentage of persons randomized to receive ivermectin thrice did not receive all doses, respectively 20.4% in group 1 and 27.5% in group 2. The advantage of the multiple dose regimens was shown in the combined as-treated analysis of the two groups but also in analyzing the group 1 data only.

Most likely the advantage of the multiple dose ivermectin is explained by the lower mf density by the end of the study period in participants on a multiple dose ivermectin regimen, confirming the previously documented positive association between seizure frequency and mf density [14]. Our findings strongly suggest that *O. volvulus* mf may induce seizures and that the beneficial effect of ivermectin was not caused by a direct anti-seizure mechanism. This was expected, as ivermectin given at therapeutic doses does not cross the blood-brain barrier and therefore cannot elicit a direct anti-epileptic effect in the central nervous system [15]. A small study conducted in a non-onchocerciasis endemic area reported that treatment with ivermectin decreased the frequency of seizures in persons with refractory epilepsy [16]. However this may have been a placebo effect as no controls were enrolled.

Our study illustrates that epilepsy in Africa is associated with high mortality. Over a period of one year, 12 (6.1%) of the 197 OIPWE enrolled in the trial died, despite the availability of free AED. This number could be even higher as nine participants were lost to follow-up. A high mortality due to

epilepsy, between 200 and 300 per 100.000 person years was documented in onchocerciasis-endemic villages in Maridi, South Sudan [17]. Also, in a cohort study in an onchocerciasis-endemic area in Cameroon, 37 (28%) of 158 PWE died over a 10-year period [18]. In the latter study, the overall relative risk of dying in PWE over a 10-year period compared to controls was 6.2 (95% CI: 2.7–14.1) [18]. In our study, five OIPWE died because their parents refused to take them to the clinic and preferred traditional treatment instead of AED. To avoid such deaths, the inclusion of traditional healers in epilepsy treatment programs should be considered [19].

In our study, a number of adverse events were observed, most of which were minor and transitory. The frequency of drug-related adverse events was similar across the different treatment arms. Nonetheless, one OIPWE developed TEN induced by phenobarbital. TEN has previously been observed with several AED including phenobarbital [20-22]. Our study illustrates the importance of counseling PWE, caretakers, CHW and local health personnel about early identification of adverse events caused by AED to ensure prompt discontinuation of potentially harmful drugs. One mother who did not receive ivermectin during pregnancy but who was treated with phenobarbital gave birth to a child with a cleft lip. Phenobarbital, as all first line AED available in sub-Saharan Africa, has been associated with congenital abnormalities [23]. Therefore more advocacy is needed to make safer second line AED [24] available at an affordable price.

This study is not void of limitations. The fact that PWE were not blinded to the number of ivermectin treatments they received could have influenced the observed outcomes. The required sample size to detect the hypothesized difference in probability of seizure freedom among individuals with administration of ivermectin twice or thrice per year was not reached. Moreover, up to seven participants were switched to another AED and in 64 the AED dose had been increased > 30mg during the trial because seizures were not controlled. An increase of the AED dose >30mg was associated

with a more severe form of epilepsy (as AED increase was more frequent in PWE from group 1 than in group 2) and of seizures uncontrolled by ivermectin. Indeed, increase in AED dose was associated with a lower probability of seizure freedom, possibly because the neurological damage in these PWE was severe, rendering the seizures difficult to manage even with high AED doses. Finally, PWE who became pregnant during the study were not given further doses of ivermectin because according to international guidelines ivermectin should not be given to pregnant women [11].

In conclusion, this study, in the combined analysis of group 1 and 2, shows the advantage to treat OIPWE with ivermectin at least bi-annually in addition to AED. Moreover, it lends support to the accumulating evidence that infection with *O. volvulus* can trigger seizures. Increasing the frequency of community-directed treatments with ivermectin in areas with ongoing onchocerciasis transmission and high epilepsy prevalence will not only decrease the incidence of OAE, but also improve the quality of life of OIPWE while accelerating the elimination of onchocerciasis.

Supplementary material

S1: Consort 2010checklist

S2: Clinical trial protocol

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Author contributions

Conceptualization, MM and RC; Methodology, MM and RC; Software, SN and AD; Validation, MM, AH and RC; Formal Analysis, AD, SH and SA; Investigation, MM, JNSF, DM, RC, RL, SN, FN, GA, DW; Resources, RC; Data Curation, SN and AD; Writing – Original Draft Preparation, MM and RC; Writing – Review & Editing, MM, JNSF, DM, RC and AH; Visualization, SH, AD, AH; Supervision, RC; Project Administration, RC, MM, FN; Funding Acquisition, RC,

Declaration of interest

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