Doxycycline for the treatment of nodding syndrome: a randomised, placebo-controlled, phase 2 trial

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Summary

Background Nodding syndrome is a poorly understood neurological disorder that predominantly occurs in Africa. We hypothesised that nodding syndrome is a neuroinflammatory disorder, induced by antibodies to *Onchocerca volvulus* or its *Wolbachia* symbiont, cross-reacting with host neuronal proteins (HNPs), and that doxycycline can be used as treatment.

Methods In this randomised, double-blind, placebo-controlled, phase 2 trial, we recruited participants from districts affected by nodding syndrome in northern Uganda. We included children and adolescents aged 8–18 years with nodding syndrome, as defined by WHO consensus criteria. Participants were randomly assigned (1:1) to receive either 100 mg doxycycline daily or placebo for 6 weeks via a computer-generated schedule stratified by skin microscopy results, and all parties were masked to group assignment. Diagnoses of *O volvulus* and antibodies to HNPs were made using luciferase immunoprecipitation system assays and immunohistochemistry. The primary outcome was change in the proportion with antibodies to HNPs, assessed at 24 months. All participants were included in safety analyses, and surviving participants (those with samples at 24 months) were included in primary analyses. Secondary outcomes were: change in concentrations of antibodies to HNPs at 24 months compared with baseline; proportion of participants testing positive for antibodies to *O volvulus*-specific proteins and concentrations of Ov16 or OVOC3261 antibodies at 24 months compared with baseline; change in seizure burden, proportion achieving seizure freedom, and the proportions with interictal epileptiform discharges on the diagnostic EEG; overall quality of life; disease severity at 24 months; and incidence of all-cause adverse events, serious adverse events, and seizure-related mortality by 24 months. This trial is registered with ClinicalTrials.gov, NCT02850913.

Findings Between Sept 1, 2016, and Aug 31, 2018, 329 children and adolescents were screened, of whom 240 were included in the study. 140 (58%) participants were boys and 100 (42%) were girls. 120 (50%) participants were allocated to receive doxycycline and 120 (50%) to receive placebo. At recruitment, the median duration of symptoms was 9 years (IQR 6–10); 232 (97%) participants had *O volvulus*-specific antibodies and 157 (65%) had autoantibodies to HNPs. The most common plasma autoantibodies were to human protein deglycase DJ-1 (85 [35%] participants) and leiomodin-1 (77 [32%] participants) and, in cerebrospinal fluid (CSF), to human DJ-1 (27 [11%] participants) and leiomodin-1 (14 [6%] participants). On immunohistochemistry, 46 (19%) participants had CSF autoantibodies to HNPs, including leiomodin-1 (26 [11%]), γ-aminobutyric acid B receptors (two [<1%]), CASPR2 (one [<1%]), or unknown targets (28 [12%]). At 24 months, 161 (72%) of 225 participants had antibodies to HNPs compared with 157 (65%) of 240 at baseline. 6 weeks of doxycycline did not affect the concentration of autoantibodies to HNPs, seizure control, disease severity, or quality of life at the 24-month follow-up but substantially decreased Ov16 antibody concentrations; the median plasma signal-to-noise Ov16 ratio was 16·4 (95% CI 6·4–38·4), compared with 27·9 (8·2–65·8; p=0·033) for placebo. 14 (6%) participants died and, other than one traffic death, all deaths were seizure-related. Acute seizure-related hospitalisations (rate ratio [RR] 0·43 [95% CI 0·20–0·94], p=0·028) and deaths (RR 0·46 [0·24–0·89], p=0·028) were significantly lower in the doxycycline group. At 24 months, 96 (84%) of 114 participants who received doxycycline tested positive for antibodies to Ov16, compared with 97 (87%) of 111 on placebo (p=0·50), and 74 (65%) participants on doxycycline tested positive for antibodies to OVOC3261, compared with 57 (51%) on placebo (p=0·039). Doxycycline was safe; there was no difference in the incidence of grade 3–5 adverse events across the two groups.

Interpretation Nodding syndrome is strongly associated with *O volvulus* and the pathogenesis is probably mediated through an *O volvulus* induced autoantibody response to multiple proteins. Although it did not reverse disease symptoms, doxycycline or another prophylactic antibiotic could be considered as adjunct therapy to antiseizure medication, as it might reduce fatal complications from acute seizures and status epilepticus induced by febrile infections.

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For the Luo translation of the abstract see Online for appendix 1

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Research in context

Evidence before this study

We searched PubMed from database inception to July 23, 2023, combining the search terms "nodding syndrome", "treatment", and "randomized trials", for specific treatment studies with no language restriction. Other than symptomatic treatments with antiseizure medicines, psychological treatment, lorazepam for catatonia, and ivermectin as adjunct treatment, no randomised trials have been reported for the treatment of nodding syndrome.

Added value of this study

Nodding syndrome is a complex neurological disorder predominantly occurring in Africa. It has been associated with infection by *Onchocerca volvulus*, but the pathogenesis is poorly understood, with contradictory findings in clinical, immunological, and post-mortem studies. This is the first specific treatment trial in people with nodding syndrome. Our findings further support the association of nodding syndrome with *O volvulus* and the hypothesised *O volvulus*-induced autoantibody-mediated pathogenesis. Although doxycycline did not reverse disease symptoms, the study suggests that it or other prophylactic antibiotics can be considered as adjunct therapy to antiseizure medications, to prevent the febrile illnesses that precipitated many of the severe seizures in these children.

Implications of all the available evidence

Eradication of endemic *O volvulus* infections with community-directed ivermectin is well underway and, concurrently, the nodding syndrome epidemic in Uganda has ended. However, individuals with nodding syndrome continue to live with the disease. In these individuals, treatment with doxycycline might help reduce severe morbidity and the number of potentially fatal complications. Similar considerations could be made for people with other epilepsies with uncontrolled seizures. Further studies are needed to better understand the pathogenesis of the disease.

Introduction

Nodding syndrome is a complex neurological disorder of unknown aetiology that affects approximately 10 000 children in Africa. First reported in Tanzania in the 1960s,[1] subsequent cases of nodding syndrome were reported in South Sudan,[2] Uganda,[3,4] Democratic Republic of the Congo,[5] Cameroon, and Central African Republic.[6] The syndrome is characterised by episodes of repetitive head nodding (the pathognomonic feature). Symptoms develop in previously normally developing children aged 3–18 years, and head nodding often occurs in association with feeding, a breeze, or cold weather. With time, this syndrome is progressively complicated by the development of multiple types of seizures, malnutrition, and cognitive and physical decline.[3,7]

Despite multiple studies on environmental, metabolic, infectious, toxic, and genetic factors, the aetiology of nodding syndrome remains undetermined.[8] However, an epidemiologic association has been documented between nodding syndrome and infection with the filarial worm *Onchocerca volvulus*, the causative agent of onchocerciasis (river blindness).[9] The same parasite is associated with Nakalanga syndrome—another disorder characterised by short stature or growth retardation, physical deformities, endocrine dysfunction, mental impairment, and epilepsy—and there is growing evidence to suggest a causative association between infection with *O volvulus* and epileptic disorders.[10,11]

O volvulus is primarily endemic in Africa, with additional foci in Latin America and Asia. Although ocular and skin pathology are seen throughout the world, nodding syndrome has been confined to only a few areas of Africa. In one study, *O volvulus* microfilariae were observed in the cerebrospinal fluid (CSF) of people with nodding syndrome and high microfilarial loads.[10] However, in post-mortem investigations, *O volvulus* parasites were not detected in brain tissue of people with nodding syndrome.[7,11,12] Therefore, if the parasite is involved in the development of nodding syndrome, alternative mechanisms other than direct parenchymal injury are likely.

Antibodies against neuronal proteins such as leucine-rich glioma-inactivated protein 1 and contactin-associated protein 2 (CASPR2; complexed with the voltage-gated potassium channels), and the neurotransmitter receptors for N-methyl-D-aspartate, α-amino-3-hydrox y-5-methyl-4-isoxazolepropionic acid, γ-amino-butyric acid $(GABA)_A$, $GABA_B$, and glycine, are recognised causes of acquired epileptic disorders. Affected individuals have seizures, psychiatric features, and progressive encephalopathy. Previous unspecified infections might play a role in the development of some of these acquired epileptic disorders, most likely by stimulating the immune system and allowing pathogenic antibodies to gain access to the brain.[13] In a pilot study to investigate whether this role is the case in nodding syndrome, Johnson and colleagues[14] showed autoantibodies to the neuronal protein leiomodin-1 and to protein deglycase or human DJ-1 in both the sera and CSF of people with nodding syndrome. Antibodies against leiomodin-1 were neurotoxic, and antibodies against leiomodin-1 purified from individuals with nodding syndrome were cross-reactive with *O volvulus* antigens. The study provided initial evidence that nodding syndrome is an autoimmune epileptic disorder caused by molecular mimicry with *O volvulus* antigens.

Treatments are targeted towards symptoms, such as antiseizure medicines, psychological treatments,[15] lorazepam for catatonia,[16] and ivermectin as part of mass drug administrations to kill the microfilariae of *O volvulus*,[17] but no randomised trials have been

reported for the direct treatment of nodding syndrome. Although ivermectin readily kills microfilariae, there is no effective treatment for the adult Onchocerca worms, which can reside in subcutaneous nodules for up to 15 years. However, antibiotic depletion of the *O volvulus* symbiont *Wolbachia* results in sterilisation and premature death of the adult Onchocerca, leading to marked reduction in dermal microfilarial density.[18] We hypothesised that nodding syndrome is a neuroinflammatory disorder, induced by antibodies to *O volvulus* or its symbiont *Wolbachia* that cross-react with host neuronal proteins (HNPs), and that doxycycline—a known macrofilaricide—could be used to kill *Wolbachia* and thus the *O volvulus* parasite, thereby ameliorating nodding syndrome. We examined the effects of treating nodding syndrome with oral doses of 100 mg doxycycline daily for 6 weeks compared with placebo on the presence and concentrations of antibodies to neuronal proteins at 24 months, seizure control, and markers of disease severity.

Methods

Study design and participants

This was a randomised, double-blind, parallel-group, placebo-controlled, phase 2 trial of oral doxycycline versus placebo for the treatment of nodding syndrome.[19] The study was conducted in districts of northern Uganda affected by nodding syndrome, and the recruitment, study procedures, and follow-up were based at Kitgum General Hospital, Kitgum, Uganda (appendix 2 p 1). The trial setup was described in a previous study.[20]

Participants were children and adolescents aged 8–18 years with nodding syndrome as defined by WHO consensus criteria, ie, those who have experienced head nodding on two or more occasions, observed by a health worker or documented on electroencephalogram (EEG), with symptom onset between the ages of 3 and 18 years, plus any one of: symptoms being triggered by food or cold weather; presence of other seizures or neurological abnormalities and cognitive decline; or clustering in space or time in the local population.[21] Sex data were reported as boys or girls. Participants were identified through nodding syndrome outpatient clinics. Girls with a positive urinary human chorionic gonadotropin hormone and those with known hypersensitivity to tetracycline or high likelihood of non-compliance to the study schedule were excluded. All participants were provided with oral sodium valproate (15–30 mg/kg per day titrated against seizure control) as the standard antiseizure medication.

Ethical approval was provided by the Makerere University School of Medicine Research and Ethics Committee and the University of Oxford Tropical Medicine Research Ethics Committee. All parents or primary caregivers provided written consent, and participants provided assent except if severely cognitively impaired. This trial is registered at ClinicalTrials.gov, NCT02850913.

Randomisation and masking

Participants were randomly assigned (1:1) to receive either daily doxycycline or placebo using a computer-generated schedule stratified by skin microscopy results (positive or negative) into one of four groups: A, B, C, and D. All parties were masked. The intervention drug, doxycycline capsules (Azudox) and identical placebo (containing starch), were from the same source (Kampala Pharmaceutical Industries, Kampala, Uganda). Each Azudox capsule contained doxycycline hyclate equivalent to 100 mg of doxycycline base.

Procedures

Consenting participants, recruited from across a wide geographical area with a very poor road network, were admitted to hospital for 1–2 weeks to rationalise antiseizure medication doses and allow accurate collection of baseline tests. The clinical assessment included a review of participants' medical history, description of progressive development of symptoms, burden and types of seizures, and neurological examination. The Modified Rankin Scale was used to describe gross motor abilities and the Quality of Life in Childhood Epilepsy questionnaire to determine quality of life.[22] All participants had a 30-min diagnostic EEG recording and those with ongoing seizures had doses of sodium valproate optimised to 20–30 mg/kg per day for seizure control.

The intervention drugs were packaged in capped plastic bottles each with 50 capsules (42 plus eight extra capsules). The first dose was administered in hospital under direct observation. Subsequent doses were continued at home, and participants were visited in their homes at 2, 4, and 6 weeks for adherence and safety monitoring. In between visits, parents and carers received weekly telephone reminders to administer the assigned intervention. Each participant was observed for at least 30 min after taking the medication and, should they vomit during this time, the full dose was readministered. The eight additional capsules in each pack were to cater for such eventualities.

See Online for appendix 2

On discharge, participants were accompanied by a home visitor who documented the home location using a GPS instrument. During the next home visits, the home visitor documented any adverse event, noted seizures in a seizure diary, conducted a pill count, and counselled on adherence. Those with less than 80% adherence at weeks 2 and 4 were visited again the following week for additional counselling.

Participants were re-evaluated clinically at 6, 12, 18, and 24 months. At 24 months, participants were again hospitalised for a week during which all the pre-randomisation procedures and tests were repeated. The rationale for assessing outcomes at 24 months but not 6 weeks is that doxycycline does not act directly on *O volvulus* but indirectly by killing their symbiont, *Wolbachiae*. Without *Wolbachiae*, the adult worms slowly

degenerate and the life span is reduced from 10–15 years down to 9–24 months.[23] It is this long-term effect that the study outcomes are based on.

In between the scheduled visits, parents and carers were instructed to bring the participants to the study clinic for any suspected illness. Tetracycline was prohibited during the study period because doxycycline is its derivative. Doxycycline also decreases the effectiveness of penicillin; hence, during the 6-week period, alternative antibiotics were used if indicated.

All clinical data were directly recorded at the point of contact on handheld computers. These data were uploaded to protected databases before the end of day.

We drew 10 mL of blood for standard-of-care testing (full blood count, liver and renal function tests, and HIV), inflammatory markers, and antibody tests. We obtained skin snips for *O volvulus* microfilariae density testing on plain microscopy. Basic laboratory testing was conducted on site. All patients had lumbar puncture and 3–4 mL of CSF obtained for antibody testing. Infection by *O volvulus* was determined by luciferase immunoprecipitation system (LIPS) assay for anti-Ov16 or anti-OVOC3261.[24,25] We performed two types of test for antibodies against neuronal proteins.

Fusion proteins for Ov16 (accession number P31729), OVOC3261 (A0A2K6VY22), human leiomodin-1 (NP036266), and human DJ-1 (Q99497) were made by cloning the full-length gene coding for the proteins into a FLAG epitope-tagged mammalian *Renilla reniformis* luciferase-containing expression vector pREN2. Lysates containing the fusion proteins were prepared by transfecting 293F cells (Thermo Fisher Scientific, Waltham, MA, USA) as per the manufacturer's instructions. Briefly, 30 µg of plasmid was used to transfect 293F cells at a final concentration of 1 µg of plasmid for 1×10^6 cells in FreeStyle 293 expression medium (Thermo Fisher Scientific) and cultured for 48 h at 37°C, 8% CO_2, and shaking at 125 rpm. The cells were centrifuged, the pellet was lysed as described previously, and the lysate frozen until used.[24]

A standard LIPS antibody assay was used for the evaluation of IgG responses to each of the antigens using 1 million light units for each sample with plasma at a dilution of 1:100 or with 20 µL of undiluted CSF. The output was measured as relative light units using a Berthold LB 960 Centro microplate luminometer and coelenterazine substrate mixture (Promega, Madison, WI, USA). Data are reported as signal-to-noise ratios by dividing the light units of participant samples by light units of control wells.

Separately, we conducted cell-based studies for a more in-depth study of CSF antibodies.[26] CSFs were tested for binding to fixed rat brain sections and to live cell-based assays for binding to CASPR2, $GABA_A$, and $GABA_B$ receptors (based on preliminary findings). For intracellular leiomodin-1, antibody testing was performed after fixing and permeabilising the cells.

Over the study period, all participants continued to receive the twice-yearly mass drug administration of ivermectin (as part of the semi-annual mass drug administration for *O volvulus* elimination).

Parents were provided a diary to record all seizures and document any adverse events. All sick events and sick visits to health units were also documented. In addition, parents were asked to call the clinic telephone number in the event of any illness considered serious. In this case, the study clinical team either visited the child or evacuated them to hospital.

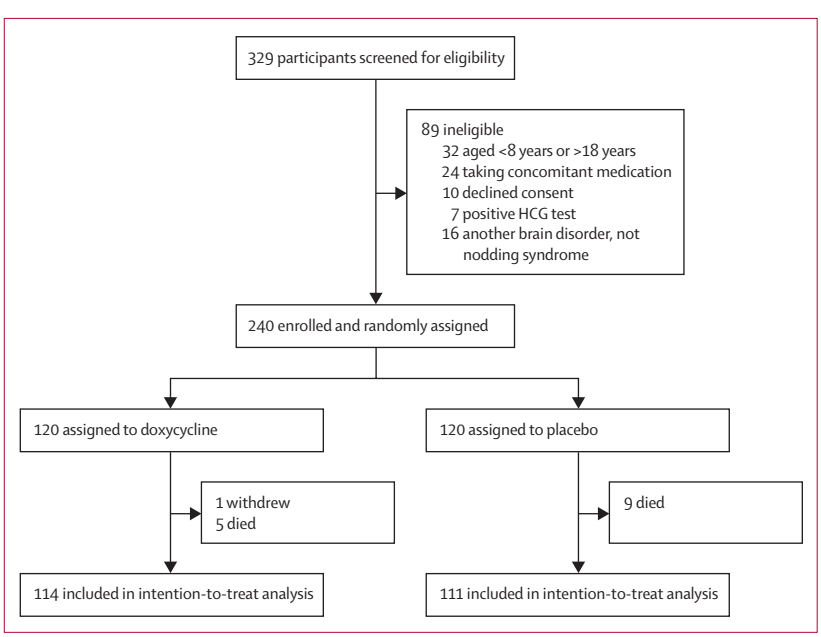

Figure 1: **Trial profile**

Outcomes

The primary outcome was change in the proportion of participants testing positive for any antibodies to HNPs at 24 months. Secondary outcomes were: change in concentrations of antibodies to HNPs at 24 months compared with baseline; proportion of participants testing positive for antibodies to *O volvulus*-specific proteins and concentrations of Ov16 or OVOC3261 antibodies at 24 months compared with baseline; mean change in BMI Z scores; change in overall quality of life; seizure burden, proportion achieving seizure freedom, and the proportions with interictal epileptiform discharges on the diagnostic EEG; disease severity at 24 months; and incidence of all-cause adverse events, serious adverse events, and seizure-related mortality by 24 months.

Statistical analysis

We hypothesised that, among people with new-onset nodding syndrome (symptoms beginning within the past 12 months), 6 weeks of treatment with doxycycline would cause a 50% reduction in the proportion of individuals with antibodies to neuronal proteins and the

concentrations of these antibodies at 24 months. However, the intervention might be less efficacious in individuals with established symptoms (a lower reduction of 40%). Bi-annual mass ivermectin administration was started in 2012 and, at the study start, there were no incident cases of nodding syndrome. Thus, to show a 40% reduction at 80% power and 5% level of significance while allowing for 10% losses to follow-up, we needed to recruit 240 participants with established symptoms.

Analysis was by intention to treat. For the antibody LIPS assays, a signal-to-noise ratio greater than 2·0 was considered positive. No efficacy analysis was conducted at 6 weeks for the reason detailed above. We compared the proportions of participants testing positive for any antibody to an HNP at baseline with values at 24 months. We then determined and compared differences in the proportions and changes in concentrations of specific antibodies between the two timepoints. Similar comparisons were made for antibodies to Ov16 or OVOC3261 antigens, safety, the proportion achieving seizure freedom, proportions with interictal discharges on EEG, and disease severity at 24 months. For the changes in proportions and concentrations, only participants with assessments at 24 months were considered. The χ^2 test was used to compare proportions, Student's *t* test for means of normally distributed data, and the Mann–Whitney *U* test for unpaired or Wilcoxon signed rank test for paired non-normally distributed data. Unless otherwise stated, medians were used as a measure of central tendency.

Role of the funding source

The funder of the study had no role in the study design, collection, management, analysis and interpretation of data, or writing and submission of the report.

Results

Between Sept 1, 2016, and Aug 31, 2018, 329 children and adolescents with nodding syndrome were screened for eligibility; 89 were excluded and 240 eligible participants were enrolled and randomly assigned (1:1) to receive either doxycycline or placebo (figure 1). Of these, 140 (58%) were boys and 100 (42%) girls. The median age was 16 years (IQR 15–17) and the median duration of symptoms was 9 years (6–10). 138 (58%) participants had severe disease (table 1). 234 (98%) participants had taken one or more doses of oral ivermectin from mass drug administration programmes, and the mean number of doses taken was 5·3 (SD 3·5). There were no differences between the two groups in the number of ivermectin doses received.

The two allocation groups had similar baseline demographic and clinical characteristics, including duration of symptoms, age, sex, seizure burden, number with interictal epileptiform discharges on the diagnostic EEG, and disease severity (table 1), but different nutritional status (wasting). Two participants (one in each group) had an HIV infection. The median dose of the antiseizure medication sodium valproate was 21·7 mg/kg per day (IQR 18·8–26·2). The average daily dose of doxycycline was 2·5 mg/kg.

232 (97%) of 240 participants tested positive for antibodies to Ov16 or OVOC3261 in plasma at baseline; of these, 228 (95%) tested positive for anti-Ov16 IgG and 192 (80%) tested positive for anti-OVOC3261 IgG. The

	Doxycycline (n=120)	Placebo (n=120)
Age, years	15·4 (2·5)	15·7 (2·4)
Sex		
Male	68 (57%)	72 (60%)
Female	52 (43%)	48 (40%)
Duration of symptoms, years	8·4 (2·8)	8·2 (2·9)
Number of seizures in the previous month	1·5 (0·0–4·0)	2·0 (0·0–4·0)
Any epileptiform discharges on electroencephalogram	80 (67%)	79 (66%)
Focal interictal discharges	76 (63%)	72 (60%)
Generalised discharges	9 (8%)	15 (13%)
Nutritional status		
BMI	15·6 (14·2–17·2)	16·9 (15·2–18·1)
Wasting (BMI Z score < –2)	66 (55%)	42 (35%)
Disease severity		
Mild	28 (23%)	21 (18%)
Moderate	23 (19%)	30 (25%)
Severe	69 (58%)	69 (58%)
Overall quality-of-life score on the Quality of Life in Childhood Epilepsy survey	50·4 (10·4)	49·5 (11·4)
Infection by *Onchocerca volvulus*	115 (96%)	117 (98%)
Plasma Ov16 seropositive	113 (94%)	115 (96%)
Plasma OVOC3261 seropositive	97 (81%)	95 (79%)
Microfilariae on skin snip	10 (8%)	11 (9%)
Number of doses of ivermectin taken	5·1 (3·7)	5·5 (3·3)
Any antibody to neuron proteins in plasma, by LIPS	59 (49%)	63 (53%)
IgG antibodies to human DJ-1	43 (36%)	42 (35%)
IgG antibodies to leiomodin-1	37 (31%)	40 (33%)
Any antibody to neuron proteins in CSF, by LIPS	20 (17%)	19 (16%)
CSF DJ-1	13 (11%)	14 (12%)
CSF leiomodin-1	8 (7%)	6 (5%)
Any neuronal antibody to neuronal proteins on cell-based assays in CSF	23 (19%)	23 (19%)
Neuropilar staining	12 (10%)	16 (13%)
Leiomodin-1	15 (13%)	11 (9%)
$GABA_A$ receptors	0	0
$GABA_B$ receptors	1 (<1%)	1 (<1%)
CASPR2	0	1 (<1%)

Data are mean (SD), median (IQR), or n (%). Ov16 and OVOC3261 are *Onchocerca volvulus*-specific antigens with over 99% diagnostic specificity for infection by *O volvulus*. LIPS=luciferase immunoprecipitation system assay. CSF=cerebrospinal fluid. GABA=γ-aminobutyric acid. CASPR2=contactin-associated protein-like 2.

Table 1: **Baseline characteristics of participants**

proportions of participants testing positive were similar in the two groups (figure 2). The concentrations of Ov16 IgG, expressed as signal-to-noise ratios, were also similar in the two groups (median 28·0 [IQR 9·4–52·6] *vs* 22·7 [8·5–39·5], p=0·070), as were the concentrations of OVOC3261 IgG (median 7·6 [3·2–19·2] *vs* 7·3 [2·5–20·2], p=0·63).

At baseline, 157 (65%) of 240 participants had at least one autoantibody to an HNP in plasma or CSF on LIPS or immunohistochemistry. On LIPS testing, at baseline, 122 (51%) participants had at least one autoantibody to an HNP in plasma alone and 140 (58%) in plasma and CSF (table 1). Commonly detected antibodies in plasma were to human DJ-1 in 85 (35%) participants and to human leiomodin-1 in 77 (32%) participants. In CSF, 14 (6%) participants had antibodies to leiomodin-1 and 27 (11%) to DJ-1 Ig. There were no differences between the two groups in either the plasma or CSF concentrations or the proportion of samples with antibodies to either protein (table 1, appendix 2 p 2).

The circulating leiomodin-1 antibodies (76 [33%] of 232 Ov16 or OVOC3261 positive *vs* one [13%] of eight Ov16 or OVOC3261 negative, p=0·044) but not CSF leioimodin-1 antibodies (25 [11%] of 232 Ov16 or OVOC3261 positive *vs* one [13%] of eight Ov16 or OVOC3261 negative, p=1·0) were associated with infection by *O volvulus*.

From cell-based studies and immunohistochemistry testing, the CSF of 46 (19%) of 240 participants had evidence of autoantibodies to HNPs; 26 (11%) participants had anti-leiomodin-1 antibodies in CSF and 28 (12%) had neuropilar staining on fixed tissue with various patterns. Of the 28 participants with neuropil-positive CSF, eight (29%) had leiomodin-1 antibodies, two (7%) had $GABA_B$ receptor antibodies (one also with leiomodin-1 antibodies), one (4%) had CASPR2 antibodies, and 18 (64%) were positive only for leiomodin-1 antibodies. No participant had N-methyl-D-aspartate receptor antibodies. There were no differences between the two baseline groups (table 1).

Adherence, determined by pill count, was more than 95% and almost all participants completed the full 6-week course of treatment.

Treatment with doxycycline did not affect the autoantibodies to HNPs. At 24 months, 161 (72%) of 225 participants had antibodies to HNPs compared with 157 (65%) of 240 at baseline, and there were no differences between treatment groups. Similarly, no differences were observed in the proportions and concentrations of the individual antibodies to human leiomodin-1, human DJ-1,

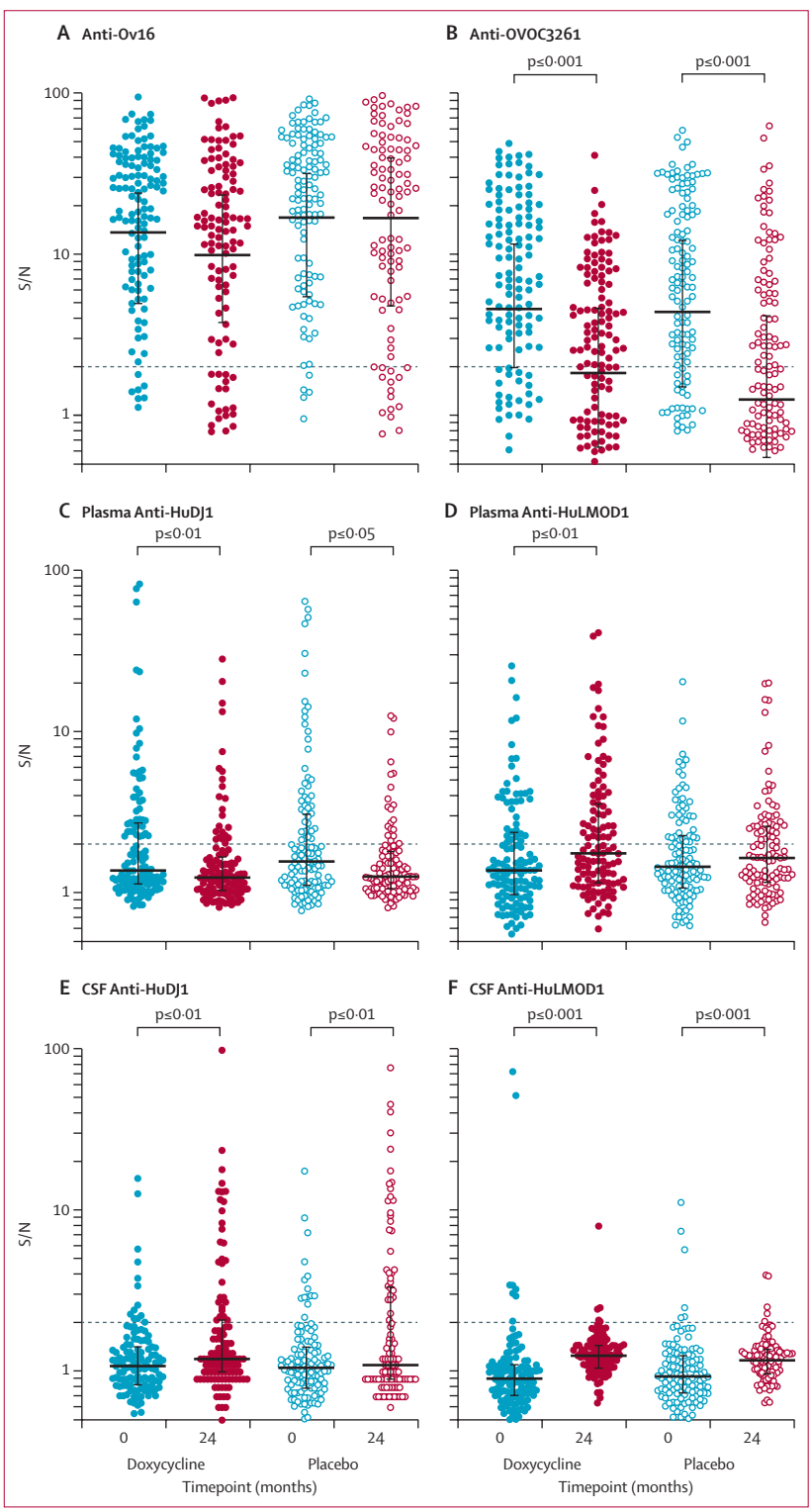

Figure 2: **Antibodies associated to *Onchocerca volvulus* and to the host neuronal proteins measured by LIPS between baseline and 24 months**
Differences between treatment groups at baseline and 24 months for (A) anti-Ov16, (B) anti-OVOC3261, (C) anti-DJ-1, and (D) anti-leiomodin-1 IgG antibodies in plasma on LIPS assays. CSF levels of (E) anti-DJ-1 and (F) anti-leiomodin-1 IgG antibody concentrations at baseline on LIPS assays. Filled circles represent participants in the doxycycline group and open circles represent participants in the placebo group. Blue circles represent antibody levels at baseline and red circles represent levels at 24 months. CSF=cerebrospinal fluid. LIPS=luciferase immunoprecipitation system. S/N=signal-to-noise ratio.

CASPR2, and $GABA_B$ receptors between the two groups at 24 months in either plasma or CSF.

6 weeks of treatment with doxycycline significantly reduced the plasma concentrations of Ov16 antibodies at 24 months; the median signal-to-noise ratio in participants who received doxycycline was 16·4 (95% CI 6·4–38·4) compared with 27·9 (8·2–65·8, p=0·033) in those who received placebo (table 2). There was, however, no notable difference in the proportions testing positive. A similar reduction was not seen in OVOC3261 concentrations (table 2, figure 2).

The average BMI for age Z score in children treated with doxycycline improved by 0·47 (from –2·14 to –1·67) *vs* 0·29 (from –1·59 to –1·30) in the placebo group (p=0·0010). The intervention was also associated with better resolution of wasting. Although there were significant improvements in quality of life scores in both groups over the study period (overall Quality of Life in Childhood Epilepsy scores in the doxycycline group improved from 50·4 [SD 10·4] at baseline to 54·4 [11·8] at 24 months, p=0·0058, and those in the placebo group improved from 49·5 [11·8] at baseline to 53·8 [12·5] at 24 months, p=0·0082), there were no differences in Quality of Life in Childhood Epilepsy scores at 24 months or the proportions of participants with seizure freedom or interictal epileptiform discharges on the diagnostic EEG or motor disability at 24 months between the two groups (table 2, appendix p 3). Eight (7%) participants treated with doxycycline were hospitalised with acute exacerbation of seizures or status epilepticus over the 24 months, compared with 20 (17%) who received placebo (RR 0·43 [95% CI 0·20–0·94], p=0·028). All eight events in the doxycycline group occurred 6 weeks after completing the intervention. Participants with more severe disease had smaller improvements in seizure control and nutritional status.

Doxycycline was well tolerated: only six (3%) participants reported vomiting or any other gastrointestinal symptoms. The intervention was safe; the incidence rate of grade 3 adverse events among participants who received doxycycline was 0·69 per person-year of observation (PYO) compared with 0·64 per PYO among those who received placebo. The most common reasons for adverse events were respiratory tract infections. The incidence of grade 4 and 5 adverse events in participants who received doxycycline was 0·13 per PYO compared with 0·16 per PYO in those who received placebo (incidence rate ratio 0·68 [95% CI 0·41–1·11], p=0·10; table 3). Although there were no significant differences in rates of adverse events overall, severe febrile illnesses (acute respiratory tract infections, malaria, and suspected sepsis) were less common in those who received doxycycline (RR 0·40 [0·18–0·87], p=0·016) and often presented with acute exacerbation or clusters of seizures, status epilepticus, and seizure-related injuries (soft tissue injuries, fractures, and severe burns).

	Doxycycline (n=114)	Placebo (n=111)	p value
Infection by *Onchocerca volvulus*			
Infection by *O volvulus* (Ov16 or OVOC3621 antibody S/N >2·0)	99 (87%)	98 (88%)	0·74
Microfilariae in skin snip (n=217)	9 (8%)	11 (10%)	0·60
Plasma anti-Ov16 IgG, S/N	16·4 (6·4–38·4)	27·9 (8·2–65·8)	0·033
Plasma anti-OVOC3261 IgG, S/N	3·0 (1·1–7·6)	2·1 (0·9–6·9)	0·46
Any antibody to HNP in plasma or CSF on LIPS or immunohistochemistry testing at 24 months	80 (67%)	81 (68%)	0·85
Any antibody to HNP in plasma on LPS testing	59 (52%)	51 (46%)	0·38
IgG antibodies to human DJ-1	23 (20%)	20 (18%)	0·68
IgG antibodies to leiomodin-1	49 (43%)	41 (37%)	0·36
Any antibody to HNP in CSF on LPS testing	33 (29%)	39 (35%)	0·32
CSF leiomodin	6 (5%)	5 (5%)	0·79
CSF DJ-1	30 (26%)	34 (31%)	0·47
Any antibody to HNP in CSF by cell-based assays	12 (11%)	8 (7%)	0·52
Leiomodin-1, (n=224)	10 (9%)	8 (7%)	0·84
$GABA_AR$ (n=224)	0	0	1·0
$GABA_BR$ (n=224)	2 (2%)	0	0·50
CASPR2 (n=224)	0	0	1·0
Seizures			
Attained seizure freedom	52 (46%)	46 (41%)	0·55
Number of convulsions in the last month	1·0 (0·0 to 4·0)	1·0 (0·0 to 5·0)	0·54
Quality of Life in Childhood Epilepsy score at 24 months	53·8 (12·5)	..	0·65
Hospitalised for acute exacerbation of seizures or status epilepticus	8 (7%)	20 (18%)	0·028
Interictal epileptiform discharges on the 24 month diagnostic electroencephalogram	72 (63%)	70 (63%)	0·99
Nutritional status			
BMI for age Z scores	–1·67 (–2·46 to 0·72)	–1·30 (–2·07 to 0·62)	0·080
Normal	66 (58%)	81 (73%)	0·058
Wasted	33 (29%)	20 (18%)	..
Severe wasting	15 (13%)	10 (9%)	..
Disease severity	..	..	0·32
Mild	28 (25%)	20 (18%)	..
Moderate	22 (19%)	29 (26%)	..
Severe	64 (56%)	62 (56%)	..
All-cause mortality	5 (4%)	9 (8%)	0·42
Adverse events (n=240)	2·38 (1·49)	2·28 (1·36)	0·56
Severe adverse events (n=240)	1·23 (0·68)	1·28 (0·65)	0·56

Data are mean (SD), median (IQR), n (%), or p value. Ov16 and OVOC3261 are *O volvulus*-specific antigens with over 99% diagnostic specificity for infection by *O volvulus*. S/N=signal-to-noise ratio. HNP=host neuronal protein. CSF=cerebrospinal fluid. LIPS=luciferase immunoprecipitation system assay. LPS=lipopolysaccharide. GABA=γ-aminobutyric acid. CASPR2=contactin-associated protein-like 2.

Table 2: **Study outcomes at 24 months**

14 (6%) participants died during the 24-month follow-up (table 2). Other than one death from traumatic brain injury in a road traffic accident, all were from seizure-related events such as status epilepticus, severe burns, drowning, or sudden unexpected death in epilepsy (appendix p 4). The number of such seizure-related deaths was significantly lower in participants who received doxycycline (4 [3%] of 120) compared with those

	Doxycycline (N=120; of 30 events)	Placebo (N=120; of 44 events)
Abscesses	1 (1%)	0
Acute gastritis	0	1 (1%)
Acute malnutrition	1 (1%)	0
Acute psychosis	2 (2%)	1 (1%)
Severe burns	3 (3%)	2 (2%)
Contracture secondary to cellulitis	1 (1%)	0
Severe dehydration	1 (1%)	0
Deaths	5 (4%)	9 (8%)
Fractures	4 (3%)	0
Severe gastritis	0	1 (1%)
Herpes zoster	1 (1%)	0
Hypoglycaemia	0	1 (1%)
Open head injury	0	1 (1%)
Pulmonary tuberculosis	1 (1%)	0
Septicaemia	0	2 (2%)
Severe malaria	0	3 (3%)
Soft tissue injury	2 (2%)	2 (2%)
Status epilepticus or acute exacerbation of seizures	8 (7%)	20 (17%)
Typhoid fever	0	1 (1%)

Data are n (%). Person-years of observation were 232·0 in the doxycycline group, with an incidence rate of 0·13 per person-year, and 230·7 in the placebo group, with an incidence rate of 0·19 per person-year.

Table 3: **Serious adverse events by treatment group**

who received placebo (9 [8%] of 120; RR 0·46 [0·24–0·89], p=0·028).

Discussion

We hypothesised that nodding syndrome is a neuroinflammatory disorder, induced by antibodies to *O volvulus* or its *Wolbachia* symbiont cross-reacting with HNPs, and that doxycycline, which kills *O volvulus* through depletion of *Wolbachia,* could be used as treatment for nodding syndrome. In a phase 2 trial of children and adolescents with longstanding nodding syndrome who were treated with a 6-week course of either 100 mg oral doxycycline daily or placebo and assessed at 24 months, we found that—at baseline—nearly all had evidence of exposure to *O volvulus* and two-thirds had at least one autoantibody against an HNP in plasma or CSF. After 24 months, treatment with doxycycline had decreased plasma Ov16 antibody concentrations by more than 40% relative to those in control group participants, with no significant effect on OVOC3261 antibody concentrations. Doxycycline also significantly decreased acute seizure-related hospitalisations and deaths. However, doxycycline neither decreased the proportion of participants testing positive for the HNP antibodies nor the concentrations of such antibodies. It also did not reverse the symptoms of disease.

There is increasing evidence to support an aetiological role for *O volvulus* in the causation of nodding syndrome. Before the current study, the most recent evidence was the disappearance of incident cases of nodding syndrome in northern Uganda with the implementation of twice-yearly mass drug administration of ivermectin to eliminate *O volvulus*. Since 2015, no incident cases of nodding syndrome have been reported. Conversely, there are several observations that do not support *O volvulus* as the cause of nodding syndrome. First, why are only children affected, when *O volvulus* infects both children and adults? Second, why is nodding syndrome only found in a few areas in eastern and central Africa and not universally in all *O volvulus* endemic areas? Third, how does a parasite resident in the skin and not readily found in the brain cause a brain disorder?[12] Fourth, why have leiomodin-1 antibodies not been detected in blood or CSF in all participants?[27] The situation is further complicated by one post-mortem study suggesting that nodding syndrome is a tauopathy,[28] but a subsequent series disputed this.[29] Instead, in support of the neuroinflammation hypothesis, there was marked cerebral and cerebellar atrophy with loss of Purkinje cells and hyperplasia of the Bergmann glia, in addition to gliosis and features of past ventriculitis or meningitis. Similar atrophy is seen on brain imaging. Furthermore, in addition to the initial findings by Johnson and colleagues,[14] we recently documented elevated complement C5a levels in CSF of adolescents with longstanding nodding syndrome, suggesting local intracranial complement activation.[30] A decrease in HNP antibody titres with doxycycline treatment, together with improvements in the clinical status of the participants, would have strongly supported our hypothesis.

Despite the long duration for which participants had nodding syndrome in the current study, more than two-thirds still had strong systemic autoantibody responses to several HNPs. A smaller number had similar antibodies in their CSF. Specifically, these were antibodies to proteins with close amino acid sequence similarity to *O volvulus* proteins,[14] supporting the view that nodding syndrome is an *O volvulus* autoantibody-induced disease. Despite this finding, and although treatment with doxycycline caused a 40% reduction in Ov16 antibody concentrations, no significant changes were observed in concentrations of autoantibodies to the neuronal proteins at 24 months. Moreover, there was no significant change in the number of participants with seizure freedom at 24 months. However, doxycycline reduced the proportion with possible bacterial and malarial infections, as well as acute exacerbations of seizures requiring hospitalisation and episodes of status epilepticus. These findings could explain, in part, why improvement in nutritional status was improved in children receiving doxycycline. We hypothesise that the immune-mediated pathogenic processes might have continued despite parasite death and, therefore, a longer follow-up could show better outcomes; or, and most

probably, extensive brain injury had already occurred that could not be reversed by the intervention and so seizures persisted. In support of the latter hypothesis, most participants who were hospitalised with status epilepticus or who died with seizure-related events had more severe disease.

The most commonly identified autoantibodies were against DJ-1 and leioimodin-1. Others were against $GABA_A$ and $GABA_B$ receptors and CASPR2, associated with seizures in individuals with autoimmune encephalitis. Non-specific neuropilar staining patterns were also seen. Human DJ-1 is a protein encoded by the PARK7 parkinsonism gene. Its functions include the regulation of transcription, antioxidative stress reaction, mitochondrial regulation, and as a chaperone and protease. Although the role of DJ-1 in nodding syndrome remains unknown, its potential involvement in mitochondrial complex I activity could underlie some of the features of nodding syndrome, such as the flat facies and catatonia.[16] On the other hand, leiomodin-1 is expressed in all regions of the brain by both neurons and glia, and especially on membranes of newly formed neurons. Such membrane expression might mediate leiomodin-1 antibody toxicity in nodding syndrome pathogenesis.[31] However, only some participants had these autoantibodies and only small changes in HNPs were observed following the intervention, possibly due to the duration for which participants had the disease before enrolment into the study. A trial in incident cases would be more informative. We did not examine antibodies to the HNPs in community controls as our aim was to compare levels in the two groups. In addition, it would be difficult to justify obtaining CSF from unaffected children.

To the best of our knowledge, this is the first drug treatment trial to target a proposed aetiological agent for nodding syndrome. Earlier studies examined whether ivermectin can be used as adjuvant therapy for seizure control in *O volvulus*-associated epilepsy; there was only borderline benefit with both single and multiple doses.[17] The observation that participants who received doxycycline had fewer hospitalisations for febrile illnesses and acute seizures might have wider application for people with uncontrolled epilepsy. In tropical Africa, where children experience up to six febrile illnesses annually, including malaria and respiratory tract infections, antimicrobial prophylaxis could prevent fever-induced acute seizures and reduce hospitalisations and deaths in people with uncontrolled epilepsy in particular. Trials are needed to establish this theory. The ideal treatment should be one that prevents both malaria and bacterial respiratory tract infections, eg, cotrimoxazole, as is the case in HIV/AIDS. On the other hand, the decrease in Ov16 titres might also have lowered *O volvulus*-associated inflammatory and epileptogenic activity or doxycycline might have directly inhibited inflammation.

In conclusion, nodding syndrome is strongly associated with infection by *O volvulus*. The pathogenesis can be mediated through an *O volvulus*-induced autoantibody response to multiple neuronal proteins. 6 weeks of treatment with doxycycline in individuals with advanced disease decreases Ov16 antibody concentrations and is associated with reductions in acute seizure-related hospitalisations and deaths, probably through the prevention of acute febrile illnesses, but doxycycline does not reverse the disease process. Whether failure to reverse the disease is because of the existing and extensive brain injury or whether another cofactor is involved remains unclear. Eradication of endemic *O volvulus* infections with community-directed ivermectin and use of entomological approaches to control blackfly populations is well underway and, with it, the nodding syndrome epidemic in Uganda has ended. However, affected individuals continue to live with the disease. In these individuals, treatment with doxycycline might help reduce severe morbidity and the number of potentially fatal complications. Similar considerations could be made for other epilepsies with uncontrolled seizures in similar settings, and a specific trial focused on this is recommended. Further studies are also needed to better understand the pathogenesis of the disease.

Contributors

RI, KM, and CRN conceived the study and together with AV, AE, PN, and MT designed the study and obtained funding. RO, RA, PA, BO, CA, MPG, JK, ADM, TBN, and AN obtained the data, including participant assessment and laboratory testing. PA and RA conducted project administration. RI, AN, RO, and PN curated the data and conducted data analysis. RI and RO wrote the original draft, and all authors critically reviewed the manuscript. AN, RO, and RI accessed and verified the data. All authors had access to all the data in the study and had final responsibility for the decision to submit for publication.

Equitable partnership declaration

The authors of this paper have submitted an equitable partnership declaration (appendix 3). This statement allows researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This statement is part of *The Lancet Global Health*'s broader goal to decolonise global health.

See Online for appendix 3

Declaration of interests

AV reports a patent held with the University of Oxford for leucine-rich glioma-inactivated protein 1 and CASPR2 antibody assays, for which she receives a proportion of royalties. TBN reports a provisional patent issued on OVOC3261. MT reports being an inventor (with Liverpool School of Tropical Medicine) on patents and patent applications US20170368088 and EP3242662A1-A4, which cover treatment of filarial diseases. All other authors declare no competing interests.

Data sharing

Data collected for the study will be made available upon request after publication. Data will include individual de-identified participant data and the data dictionary. Requests can be addressed to the corresponding author. Requests will be examined by a committee of relevant people involved in the study. The scientific aspects of the proposal and the ethical and legal implications of data sharing will be considered. Data will be shared after approval of the proposal and after signing of a data sharing agreement by all parties involved.

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