Evaluating post-treatment *Loa loa* microfilarial densities to classify serious adverse events caused by ivermectin: a retrospective analysis

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Summary

Background The elimination of onchocerciasis requires increasing ivermectin treatment coverage in communities hypoendemic for onchocerciasis. In areas where loiasis is co-endemic, this approach is complicated by the risk of serious adverse events following treatment with ivermectin in individuals with a high *Loa loa* microfilarial density (MFD). We aimed to evaluate the extent to which the pre-treatment MFD can be inferred from post-treatment MFDs.

Methods For this retrospective analysis, we used data from seven clinical or community trials (six were used for the main analysis and one for the secondary analysis) conducted in Cameroon, in which MFDs were measured both before and after (within 14 days) receiving a single dose of ivermectin (150–200 µg/kg bodyweight). The primary objective was to establish the receiver operating characteristic curves and the corresponding area under the curve statistics of MFD measured after treatment to classify pre-treatment MFD (MFD_{D0}) according to common risk thresholds of serious adverse events. We assessed the performance of post-treatment MFD to accurately classify MFD_{D0} according to commonly used thresholds using bootstrap procedures.

Findings 281 individuals with MFD measurements available before and 3–10 days after ivermectin treatment were enrolled. Our results show that an MFD of more than 3500 *L loa* microfilariae per mL of blood (mf per mL) 3 or 4 days after treatment indicates a 68·6% chance (positive predictive value) of an MFD_{D0} of more than 20000 mf per mL. An MFD of more than 3500 mf per mL at day 5–10 corresponds to a 72·2% chance of having an MFD_{D0} of more than 20000 mf per mL. Conversely, an MFD of less than 2500 microfilariae per mL at day 3–4 or day 5–10 corresponds to a probability of 92·3% or 92·8% (negative predictive value) of having MFD_{D0} of less than 20000 mf per mL. An MFD less than 1500 mf per mL on days 3–4 after treatment was associated with a 78·3% probability of having an MFD_{D0} less than 8000 mf per mL; this probability increased to 89·6% on days 5–10 after treatment.

Interpretation The MFD threshold of 1000 mf per mL within 1 month of treatment, which is commonly used to attribute the occurrence of a serious adverse event to ivermectin, should be revised. In this study, we present tables that can help to assess this attributability as part of mass or individual treatments.

Funding None.

Introduction

Mass drug administration (MDA)—ie, distribution of treatment without determining diagnosis—of ivermectin (Mectizan) has been or is at the core of the onchocerciasis elimination programmes conducted in Africa by the African Programme for Onchocerciasis Control (APOC) between 1995 and 2015, and the Expanded Special Project for Elimination of Neglected Tropical Diseases since 2016. APOC's initial objective was the elimination of onchocerciasis as a public health problem rather than complete elimination (ie, interruption of transmission). Thus, community-directed treatment with ivermectin was restricted to mesoendemic or hyperendemic areas, collectively defined as those where the prevalence of onchocercal nodules in adults exceeded 20%. The goal of elimination of onchocerciasis in 12 countries by 2030 was proposed by WHO following the demonstration that ivermectin MDA can interrupt *Onchocerca volvulus* transmission.[1,2] Reaching this target might require treatment in hypoendemic areas,[3,4] where an estimated 17 million people will live in 2025.[5]

Serious adverse events following ivermectin treatment have been reported since 1991,[6–8] and are closely associated with *Loa loa* microfilarial density (MFD) in the blood of individuals.[8] Elimination efforts are hampered in areas where onchocerciasis is hypoendemic and loiasis (*L loa* filariasis) is co-endemic, because the risk of serious adverse events in these settings can outweigh the benefits of community-directed treatment with ivermectin. In such areas, alternative treatment strategies have to be applied, such as test-and-not-treat, which relies on a mobile smartphone-based point-of-care diagnostic tool

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Research in context

Evidence before this study

During a meeting organised by the Mectizan Donation Program in 1995, experts proposed to define a probable case of *Loa loa* encephalopathy following ivermectin treatment as: an encephalopathy occurring fewer than 7 days after treatment in a previously healthy individual whose *L loa* microfilarial density (MFD) was higher than 10000 *L loa* microfilariae per mL of blood (mf per mL) before treatment, higher than 1000 mf per mL within 1 month after treatment, or higher than 2700 mf per mL within 6 months after treatment. However, these thresholds were not strongly supported by data, particularly for post-treatment microfilarial densities, which are often the only available data. Although post-treatment microfilarial densities are key for assigning a serious adverse event to ivermectin treatment, accurate thresholds for reliable classification have not been determined.

Added value of this study

Our results indicate that, for programmatic surveillance purposes, a threshold of 2500 mf per mL 3–10 days after ivermectin treatment is the best predictor of a baseline MFD higher than 8000 or 10000 mf per mL. This threshold provides reliable estimates of the overall number of attributable cases and facilitates monitoring of serious adverse event incidence. If a patient presents with symptoms consistent with *L loa* encephalopathy, the detailed tables provided in this Article can be used to assess the likelihood that the event is or is not related to ivermectin intake, thus informing the care of the patient.

Implications of all the available evidence

Reaching the WHO target of eliminating onchocerciasis will probably require treatment in hypoendemic areas, including where loiasis is co-endemic. Such expansion of treatment programmes requires adequate surveillance and monitoring of potential adverse effects. Our study provides the basis for programmatic surveillance, in the form of tables that can assess the attributability of adverse reactions to ivermectin, using MFDs measured 3–4 days, 5–10 days, and up to 180 days after treatment with ivermectin.

(LoaScope) to estimate pre-treatment *L loa* MFD (MFD_{D0}).[9–11] Although the contribution of test-and-not-treat to elimination efforts might make the strategy cost-effective in the long term,[12] the immediate costs in many settings could be prohibitive.[2] Currently, the test-and-not-treat strategy has not been endorsed by WHO.

In 1997, the first large-scale study[8] on post-ivermectin serious adverse events classified adverse events as (1) mild with no functional impairment, (2) marked with functional impairment lasting fewer than 7 days, and (3) serious with impairment that lasts more than 7 days, with or without neurological involvement. Symptoms usually develop 2–3 days after treatment and can progress into coma and death.[13] Specific surveillance measures, including visits to the communities by trained staff, must accompany community-directed treatment with ivermectin in onchocerciasis–loiasis co-endemic areas to ensure the early identification and management of patients who develop serious adverse events. The treatment of serious adverse events is not standardised and consists mainly of supportive care after having eliminated other causes of encephalopathy. 65 (63%) of 103 cases of encephalopathy reported from 1989 to 2001 during ivermectin MDA in Cameroon, Central African Republic, and southern Sudan were linked to loiasis.[14] However, MFD_{D0} is generally not measured during MDA. Indeed, only eight cases of post-ivermectin *L loa*-related serious adverse events with documented MFD_{D0} have been published.[6–8,15] In these eight cases, the MFD_{D0} ranged from 50520 *L loa* microfilariae per mL of blood (mf per mL) to 217000 mf per mL.

Ivermectin is a potent microfilaricidal drug against *L loa*, rapidly lowering MFDs to a low steady state within 1 week of treatment.[16,17] Probable *L loa* encephalopathy has been defined as an encephalopathy occurring within 7 days of ivermectin treatment in a previously healthy individual whose *L loa* MFD was recorded as higher than 10000 mf per mL before treatment, or higher than 1000 mf per mL 1 month after treatment, or more than 2700 mf per mL 6 months after treatment.[18] However, these thresholds were established in the early years of ivermectin MDA and are not strongly supported by data. Given the probable expansion of ivermectin treatment into onchocerciasis hypoendemic areas where loiasis is co-endemic, and the possible occurrence of *L loa*-related serious adverse events in these ivermectin-naive areas if the drug is given using an MDA strategy, it is crucial to establish a robust classification of probable serious adverse event cases. Therefore, our aim was to assess the usefulness of post-treatment MFDs in predicting MFD_{D0} above the thresholds known to be associated with serious adverse events.

Methods

Selection of trials

For this retrospective analysis, we used data from six clinical or community trials conducted in Cameroon.[6,19–23] We searched the PubMed and Web of Science databases using the terms "(loiasis OR loase OR Loa loa) AND (ivermectin$) AND (trial OR therapeuti$)" for papers published in English or French from database inception until Jan 1, 2018. The reference lists of identified studies were also screened by SDP and MB. Eligible studies were clinical or community trials of ivermectin treatment (given as a single standard dose [150–200 µg/kg bodyweight]) that collected data on the MFD of individuals with *L loa* infection before and after treatment with

ivermectin. After reviewing 166 abstracts, 11 original studies were identified from Cameroon, Gabon, and Republic of the Congo, and seven trials conducted in Cameroon met the inclusion criteria and had raw data available that could contribute to the present analysis, including timing of post-treatment measurement of microfilarial densities. Four additional studies[24–27] were found to have MFD data obtained before and after treatment, but we had no access to the individual data. No new clinical trials meeting those criteria were identified between Jan 1, 2018, and Nov 2, 2022, in a repeated search of the two databases. Details on the trials used in this study can be found in the appendix (pp 1–2).

All trials had been conducted in compliance with the regulations in force, and the present retrospective observational study did not require additional ethical clearance.

Parasitological examination

As part of the studies, calibrated (30 µL or 50 µL) thick blood smears prepared between 1000 h and 1600 h were examined to measure the *L loa* MFD.

Outcomes

The primary objective was to establish the receiver operating characteristic (ROC) curves and the corresponding area under the curve (AUC) statistics of MFD measured after ivermectin treatment to classify MFD_{D0} according to common risk thresholds of serious adverse events. Secondary objectives were to establish the positive predictive values (PPVs) and negative predictive values (NPVs) at those same thresholds, and to assess the feasibility of using late MFDs (measured after more than 14 days) for the same purpose.

Statistical analysis

Individual MFDs are expressed in mf per mL. The following MFD_{D0} were considered as MFD thresholds in the analysis: (1) 8000 mf per mL, corresponding to a 10-fold increase in the risk of a marked adverse event[8] and used in therapeutic guidance;[28] (2) 10000 mf per mL, which is the threshold proposed by experts at a meeting organised by the Mectizan Donation Program in 1995 and included in the 2003 classification of *L loa*-related adverse events;[18] (3) 20000 mf per mL, which is the threshold used by the LoaScope point-of-care device, which measures MFD within 3 min of blood sampling;[10] (4) 30000 mf per mL, corresponding to a 1000-fold increase in the risk of a marked adverse event,[8] the threshold above which ivermectin is contraindicated in therapeutic guidance;[28] (5) 50000 mf per mL, corresponding to the lowest MFD_{D0} among the eight cases of *L loa*-related serious adverse events for which the MFD_{D0} had been measured.

Considering that clinical manifestations of concern usually occur at day 3–4, and thus that the investigation of potential serious adverse events cannot start earlier, the first timepoint was set at day 3–4 after treatment ($MFD_{D3–4}$).

	Study participants (n=281)
Age*, years	
Median (IQR)	43 (32–54)
≤15	12 (4%)
16–30	47 (17%)
31–45	94 (34%)
46–60	88 (32%)
>60	35 (13%)
Sex*	
Female	124 (45%)
Male	152 (55%)
MFD_{D0}, mf per mL	
Median (IQR)	7640 (2266–22 064)
Mean (SD)	16 707 (24 606)
MFD_{D0} category, mf per mL	
1–2000	67 (24%)
2001–8000	82 (29%)
8001–10 000	19 (7%)
10 001–20 000	36 (13%)
20 001–30 000	29 (10%)
30 001–50 000	26 (9%)
>50 000	22 (8%)
Cumulated numbers of individuals with MFD_{D0} below or above thresholds, mf per mL	
8000	Below: 149 (53%); above: 132 (47%)
10 000	Below: 168 (60%); above: 113 (40%)
20 000	Below: 204 (73%); above: 77 (27%)
30 000	Below: 233 (83%); above: 48 (17%)
50 000	Below: 259 (92%); above: 22 (8%)
Study reference	
Paris et al (1991)[23]	5 (2%)
Chippaux et al (1992)[19]	76 (27%)
Ducorps et al (1995)[6]	112 (40%)
Ranque et al (1996)[20]	42 (15%)
Kamgno et al (2000)[21]	13 (5%)
Kamgno et al (2007)[22]	33 (12%)

Data are n (%), unless otherwise stated. MFD_{D0}=*Loa loa* microfilarial density before ivermectin treatment. mf per mL=*L loa* microfilariae per mL of blood.
*Five missing values from one study (Paris et al).[23]

Table 1: **Baseline characteristics of the participants in the trials**

We used the arithmetic mean of the values for individuals who had their MFD measured on both day 3 and day 4. Given the significant decrease in MFD between day 3–4 and day 7, a second timepoint was set around day 7 (from day 5 to 10 [$MFD_{D5–10}$]). $MFD_{D5–10}$ could be the value of a single MFD measured between day 5 and day 10 or the average of several measurements made between day 5 and day 10, if available. Analyses on data collected at days 5–10 were performed because, in remote locations, serious adverse events might be investigated during this interval of time.

The capacity of $MFD_{D3–4}$ or $MFD_{D5–10}$ (measured as a continuous variable in microfilariae per mL) to discriminate between MFD_{D0} that were lower or higher

See **Online** for appendix

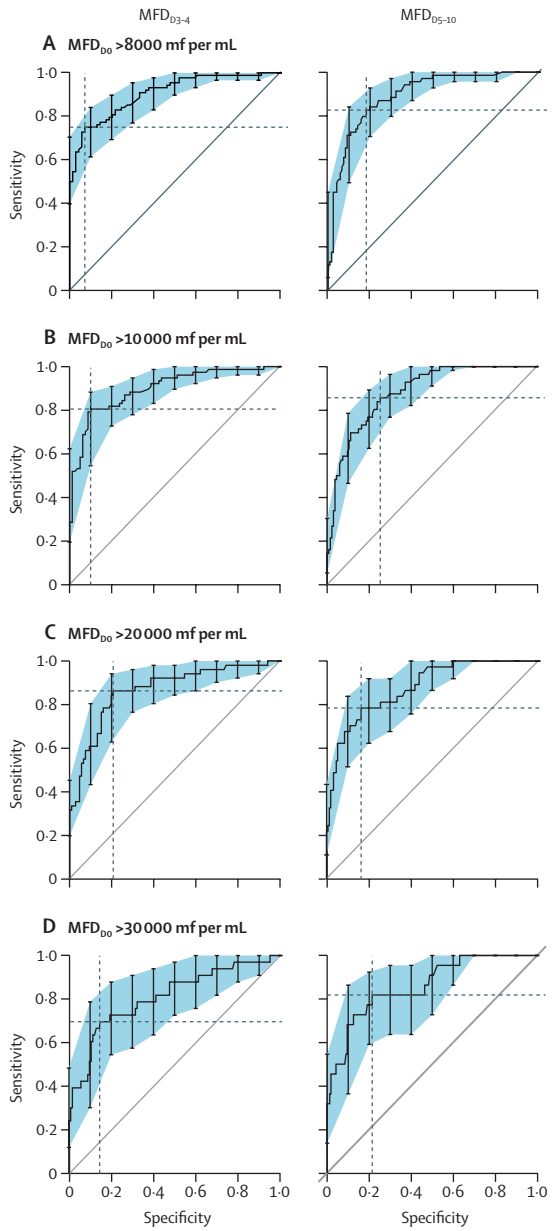

***Figure*: ROC curves for determining *Loa loa* MFD$_{D0}$ from *L loa* MFD$_{D3-4}$ or *L loa* MFD$_{D5-10}$**

(A) ROC curves for determining *L loa* MFD$_{D0}$ higher than 8000 mf per mL. (B) ROC curves for determining *L loa* MFD$_{D0}$ higher than 10000 mf per mL. (C) ROC curves for determining *L loa* MFD$_{D0}$ higher than 20000 mf per mL. (D) ROC curves for determining *L loa* MFD$_{D0}$ higher than 30000 mf per mL. The curves are determined from *L loa* MFD$_{D3-4}$ (first column) or *L loa* MFD$_{D5-10}$ (second column). The intersection of the dotted lines represents the best sensitivity–specificity pairing according to Youden's method. ROC=receiver operating characteristic. mf per mL=*L loa* microfilariae per mL of blood. MFD=*Loa loa* microfilariae density. MFD$_{D0}$=MFD before ivermectin treatment. MFD$_{D3-4}$=MFD at day 3–4 after treatment. MFD$_{D5-10}$=MFD at day 5–10 after treatment.

than each of the prespecified thresholds was evaluated by analysis of ROC curves and the corresponding AUC statistics. The theoretical best cutoff for each pairing of pre-treatment threshold and post-treatment measurement timepoint was determined using Youden's index. Intrinsic (ie, sensitivity, specificity, and accuracy) and extrinsic (ie, PPV and NPV) attributes of possible cutoffs were estimated by bootstrapping with 1000 resampling iterations.

After the initial drop in MFD in the first week, post-treatment levels remain fairly stable during the next 6 months.[17] Therefore, we also assessed the statistical accuracy of MFDs measured between 14 and 180 days after treatment to classify MFD$_{D0}$ at the 8000 mf per mL, 10000 mf per mL, 20000 mf per mL, and 30000 mf per mL thresholds using the cutoffs determined using the methodology described above. We used the first MFD measurement available between 14 and 180 days (MFD$_{D14–180}$) of the 167 individuals already included in the first part of the study (ie, who had MFD$_{D3–4}$ or MFD$_{D5–10}$ available), but also of 271 other individuals who had MFD$_{D0}$ and MFD$_{D14–180}$ measured but no MFD data between day 3 and 10.[16] As the Mectizan Donation Program classification also mentions MFD values measured 1 month and 6 months after treatment, additional analyses were done using data collected after day 10.

All analyses were performed using R, version 4.1.0, and Stata, version 17.0.

Role of the funding source

There was no funding source for this study.

Results

281 individuals with MFD measurements available before and 3–10 days after ivermectin treatment were enrolled in the six studies included in this analysis (table 1).[6,19–23] Median MFD$_{D0}$ was high, at 7640 mf per mL (IQR 2266–22064]). The distribution was right-skewed, with 48 (17%) participants having an MFD$_{D0}$ higher than 30000 mf per mL. 157 participants had MFD$_{D3–4}$ measurements available: 39 with MFD measured on day 3, four on day 4, and 114 on day 3 and day 4. 216 MFD$_{D5–10}$ measurements were available for 190 participants, including 13 individuals sampled on day 5, 13 sampled on day 6, 171 sampled on day 7, 14 sampled on day 8, and five sampled on day 10.

Using MFD$_{D3–4}$ measurements, AUC estimates for predicting MFD$_{D0}$ were 0·903 at the threshold value of 8000 mf per mL, 0·897 at the threshold value of 10000 mf per mL, 0·866 at the threshold value of 20000 mf per mL, 0·809 at the threshold value of 30000 mf per mL, and 0·757 at the threshold value of 50000 mf per mL. When MFD estimates were taken at a later time (MFD$_{D5–10}$), the corresponding AUC estimates were 0·892 for 8000 mf per mL, 0·881 for 10000 mf per mL, 0·878 for 20000 mf per mL, 0·860 for 30000 mf per mL, and 0·888 for 50000 mf per mL. The corresponding 95% CIs are presented in the appendix (p 3). Corresponding ROC curves for thresholds from 8000 to 30000 mf per mL are presented in the figure.

The best cutoffs determined from Youden's index are shown in table 2. For the 10 000 mf per mL threshold proposed to define probable *L loa*-related serious adverse events,[18] the optimal cutoff was 2245 mf per mL when MFD was measured at day 3–4 and 1483 mf per mL when measured at day 5–10.

The 1000 mf per mL threshold, proposed by Twum-Danso,[18] exhibited a low accuracy, of around 72%, at both day 3–4 and day 5–10 (table 3) to predict an MFD_{D0} of more than 10000 mf per mL. In particular, although sensitivity (94·9% at day 3–4 and 89·5% at day 5–10) and NPV (90·9% at day 3–4 and 93·7% at day 5–10) were high at these two timepoints, specificity (50·6% at day 3–4 and 72·0% at day 5–10) and PPV (64·8% at day 3–4 and 51·2% at day 5–10) were low (table 3).

In relation to the thresholds discussed at the Mectizan Donation Program meeting in 1995, to predict an MFD_{D0} of more than 8000 mf per mL (T_{8000}) or 10000 mf per mL (T_{10000}), the accuracy of classification was highest for the 2500 mf per mL cutoff, reaching 80·9% (for T_{8000}) and 84·1% (for T_{10000}) at day 3–4 (table 3). At day 5–10, accuracy was maximal at 2000 mf per mL for T_{8000} (83·2%) and T_{10000} (80·5%), although for this latter threshold, the cutoff of 2500 mf per mL was slightly better (82·6%). Retaining a cutoff of 2500 mf per mL for T_{10000} corresponds to an NPV of 80·2% and a PPV of 89·5% at day 3–4, or to an NPV of 86·3% and PPV of 72·7% at day 5–10 (table 3).

Sensitivity and NPV can be optimised by lowering the cutoff closer to the best threshold by Youden's index method. Choosing a 1500 mf per mL cutoff results in NPV and PPV values of 85·3% and 75·3% at day 3–4 and 92·6% and 58·5% at day 5–10. A 2000 mf per mL cutoff would provide NPV and PPV values of 81·2% and 80·5% at day 3–4 and 87·6% and 65·5% at day 5–10.

For predicting an MFD_{D0} of more than 20000 mf per mL (T_{20000}), classification performance plateaued for MFD_{D0} threshold values higher than 20000 mf per mL. The fewer number of observations available results in asymptotic parameter estimates being closely related to the prevalence of cases. However, at T_{20000}, classification was still performing quite well and showed a maximum accuracy of 81·5% for the cutoff of 3000 mf per mL at day 3–4 and 87·9% for the cutoff of 3500 mf per mL at day 5–10 (table 3).

The cutoff of 3000 mf per mL corresponded to an NPV of 88·0% and a PPV of 69·4% at day 3–4 and an NPV of 92·3% and a PPV of 65·8% at day 5–10 (table 3). The cutoff of 3500 mf per mL corresponded to an NPV of 84·8% and a PPV of 68·6% at day 3–4, and an NPV of 91·2% and a PPV of 72·2% at day 5–10. Lowering the cutoff closer to 2000 mf per mL would then provide NPV and PPV values of 91·1% and 56·7% at day 3–4 and 93·9% and 47·3% at day 5–10. A cutoff of 2500 mf per mL would yield NPV and PPV values of 92·3% and 66·2% at day 3–4 and 92·8% and 52·6% at day 5–10.

The NPV, PPV, sensitivity, and specificity of MFD estimates at the two post-treatment timepoints for MFD_{D0} cutoffs ranging from 500 to 5000 mf per mL according to the prespecified thresholds are reported in the appendix (pp 4–6).

	MFD day 3–4 after treatment			MFD day 5–10 after treatment		
	Cutoff*	Sensitivity	Specificity	Cutoff*	Sensitivity	Specificity
8000	2230	75·0%	92·8%	1563	82·6%	81·8%
10 000	2245	80·5%	90·0%	1483	85·7%	74·6%
20 000	2478	86·3%	79·2%	2283	78·4%	83·6%
30 000	4091	69·7%	85·5%	2283	81·8%	78·6%
50 000	4166	64·3%	79·7%	3100	84·6%	85·3%

Data are mf per mL, unless otherwise stated. MFD=*Loa loa* microfilarial density. MFD_{D0}=MFD before ivermectin treatment. mf per mL=*L loa* microfilariae per mL of blood. *Cutoffs are determined using Youden's index.

Table 2: **MFD_{D0} thresholds and best cutoffs**

Participants followed up after 2 weeks did not differ in terms of age, sex ratio, or initial MFD category (1–2000 mf per mL: 34 [20%] of 167; 2001–8000 mf per mL: 57 [34%]; 8001–10000 mf per mL: 11 [7%]; 10001–20000 mf per mL: 16 [10%]; 20001–30000 mf per mL: seven [4%]; 30001–50000 mf per mL: 12 [7%]; >50000 mf per mL: ten [6%]). Applying the previous cutoffs to MFDs measured 14–180 days after ivermectin treatment led to good discrimination performance, with an accuracy of more than 80% in most cases (table 4). Although global performance indicators were elevated for the 30 000 microfilariae per mL threshold, PPV and sensitivity were low, due to the aggregation of values below the threshold. Therefore, caution should be used when considering this threshold.

Discussion

Assessing causal links between treatment and adverse events is a primary challenge when monitoring serious adverse events occurring during community-directed treatment with ivermectin and for pharmacovigilance in general. Therefore, on a scale as large as that of an international elimination programme, it is important to have pragmatic and robust definitions to guide the investigation of attributability. When monitoring suspected *L loa*-related adverse events following ivermectin treatment, *L loa* MFDs are generally not known until several days after treatment and after the onset of symptoms. The issue is that ivermectin is a very potent microfilaricidal drug. A meta-analysis published in 2019 found that a single standard dose resulted in an 85% reduction in *L loa* MFD, on average, 1 week after treatment, with important interindividual variability.[17] To our knowledge, our study provides the first evaluation of threshold values proposed during a meeting on post-ivermectin serious adverse events organised in 1995 by the Mectizan Donation Program. These proposed thresholds have also been used in the classification by Twum-Danso in 2003.[18]

In this study, we accessed all published studies with pre-treatment and post-treatment measurements of *L loa* MFD, for which individual data were available. Our results

show that, although the 1000 mf per mL threshold is very sensitive, it is probably too low to be an accurate predictor. Indeed, the probability that an individual with an $MFD_{D3–4}$ measurement of more than 1000 mf per mL had an MFD_{D0} measurement of more than 10000 mf per mL is only 64·8%. This probability is further lowered to 51·2% if MFD is measured 5–10 days after treatment. Furthermore, the utility of the 10 000 mf per mL pre-treatment threshold might also be questioned, as the original publication acknowledged.[18] Raising the pre-treatment threshold is

	Day 3–4					Day 5–10				
	MFD_{D0} thresholds, mf per mL					MFD_{D0} thresholds, mf per mL				
	8000	10000	20000	30000	50000	8000	10000	20000	30000	50000
Accuracy										
500	71·3%	64·3%	47·8%	36·3%	25·5%	70·5%	64·7%	55·8%	47·9%	44·2%
1000	76·4%	72·0%	56·7%	45·9%	35·7%	76·8%	72·1%	64·2%	57·4%	54·2%
1500	80·3%	79·6%	70·7%	59·2%	49·7%	81·6%	77·9%	70·0%	64·2%	61·6%
2000	80·3%	80·9%	74·5%	63·1%	54·8%	83·2%*	80·5%	78·9%	75·3%	72·6%
2500	80·9%*	84·1%*	81·5%*	70·1%	61·8%	81·1%	82·6%*	82·1%	79·5%	77·9%
3000	77·1%	80·3%	81·5%*	73·9%	67·5%	78·4%	82·1%	86·8%	85·3%	84·7%
3500	73·9%	77·1%	79·6%	77·1%	70·1%	77·4%	82·1%	87·9%*	87·4%	86·8%
4000	70·7%	75·2%	80·3%	81·5%	75·8%	73·7%	78·4%	86·3%	86·8%	88·4%
4500	63·7%	69·4%	79·6%	82·2%	80·3%	72·6%	78·4%	87·4%	87·9%	89·5%
5000	62·4%	68·2%	79·6%	82·2%	81·5%	71·1%	76·8%	85·8%	89·5%	91·1%
PPV										
500	66·4%	58·1%	38·0%	24·6%	10·5%	55·4%	45·3%	30·1%	17·5%	10·7%
1000	72·6%	64·8%	42·3%	26·5%	11·3%	62·6%	51·2%	33·7%	19·4%	12·0%
1500	82·1%*	75·3%*	52·5%	32·5%	13·3%	70·7%*	58·5%*	37·6%	21·8%	13·0%
2000	87·1%*	80·5%*	56·7%	33·8%	12·9%	80·4%*	65·5%*	47·3%	29·3%	17·5%
2500	94·2%*	89·5%*	66·2%*	39·4%	15·2%	82·6%*	72·7%*	52·6%*	33·3%	20·8%
3000	96·6%	91·4%	69·4%*	42·9%	16·0%	86·8%	79·4%	65·8%*	42·2%	28·2%
3500	96·3%	90·5%	68·6%*	46·8%	17·5%	90·6%	84·6%	72·2%*	47·1%	30·3%
4000	100·0%	95·5%	73·9%	54·8%	21·2%	88·0%	80·0%	72·2%	43·5%	31·5%
4500	100·0%	97·0%	80·8%	58·3%	22·6%	87·0%	83·0%	78·6%	47·6%	34·4%
5000	100·0%	96·8%	83·3%	59·4%	24·1%	85·0%	80·0%	75·0%	55·0%	38·9%
NPV										
500	96·3%	96·3%	96·3%	96·3%	100·0%	95·8%	97·2%	98·6%	98·6%	100·0%
1000	86·4%	90·9%	93·3%	93·3%	97·8%	91·4%	93·7%	95·7%	96·8%	98·9%
1500	78·3%*	85·3%*	94·0%	94·0%	97·1%	89·6%*	92·6%*	94·4%	96·3%	98·2%
2000	74·0%*	81·2%*	91·1%	91·1%	95·1%	84·6%*	87·6%*	93·9%	96·9%	98·5%
2500	71·6%*	80·2%*	92·3%*	92·3%	95·7%	80·6%*	86·3%*	92·8%*	96·4%	98·6%
3000	66·3%	74·0%	88·0%*	91·0%	95·1%	76·1%	83·0%	92·3%*	96·1%	98·7%
3500	63·3%	70·4%	84·8%*	91·4%	95·4%	74·5%	81·8%	91·2%*	95·5%	98·1%
4000	60·0%	67·6%	82·8%	91·2%	95·7%	71·4%	78·2%	88·5%	93·3%	97·0%
4500	54·9%	62·5%	79·5%	87·9%	94·5%	70·7%	77·9%	88·7%	93·4%	97·1%
5000	54·1%	61·5%	79·1%	87·3%	94·6%	69·5%	76·6%	87·1%	93·5%	97·1%
Sensitivity										
500	98·9%	98·7%	98·1%	97·1%	100·0%	95·7%	96·5%	97·4%	95·7%	100·0%
1000	93·1%	94·9%	94·2%	91·0%	93·3%	88·6%	89·5%	89·2%	87·0%	92·9%
1500	83·1%*	87·1%*	92·2%	88·0%	86·7%	84·0%*	85·7%*	83·8%	82·1%	85·4%
2000	76·3%*	80·6%*	86·2%	78·8%	71·4%	71·0%*	71·2%*	78·1%	82·1%	85·4%
2500	70·5%*	76·5%*	86·2%*	78·8%	71·4%	60·6%*	66·0%*	73·0%	77·8%	85·4%
3000	61·1%	65·9%	75·9%*	72·4%	64·3%	47·5%	53·3%	67·6%	72·6%	85·4%
3500	55·4%	59·3%	68·3%*	72·4%	64·3%	41·5%	47·8%	62·4%	68·0%	76·9%
4000	47·6%	51·5%	60·7%	69·4%	64·3%	31·4%	35·3%	48·5%	50·0%	61·5%
4500	34·9%	38·5%	48·8%	54·2%	50·0%	28·6%	33·9%	48·5%	50·0%	61·5%
5000	32·6%	36·0%	46·8%	51·4%	50·0%	24·3%	28·3%	40·0%	50·0%	61·5%

(Table 3 continues on next page)

	Day 3–4					Day 5–10				
	MFD_{D0} thresholds, mf per mL					MFD_{D0} thresholds, mf per mL				
	8000	10000	20000	30000	50000	8000	10000	20000	30000	50000
(Continued from previous page)										
Specificity										
500	36·1%	31·3%	23·4%	20·2%	18·1%	71·3%	64·3%	47·8%	36·3%	25·5%
1000	55·1%	50·6%	38·7%	33·1%	30·1%	76·4%	72·0%	56·7%	45·9%	35·7%
1500	76·7%*	72·4%*	60·2%	51·6%	46·1%	80·3%*	79·6%*	70·7%	59·2%	49·7%
2000	85·7%*	81·3%*	68·8%	59·1%	53·2%	80·3%*	80·9%*	74·5%	63·1%	54·8%
2500	94·4%*	91·4%*	79·3%*	68·0%	61·1%	80·9%*	84·1%*	81·5%*	70·1%	61·8%
3000	97·2%	94·0%	84·1%*	74·6%	67·6%	77·1%	80·3%	81·5%*	73·9%	67·5%
3500	97·2%	94·0%	85·0%*	78·6%	71·0%	73·9%	77·1%	79·6%*	77·1%	70·1%
4000	100·0%	97·6%	89·8%	84·9%	77·1%	70·7%	75·2%	80·3%	81·5%	75·8%
4500	100·0%	98·8%	94·4%	89·8%	83·5%	63·7%	69·4%	79·6%	82·2%	80·3%
5000	100·0%	98·8%	95·5%	90·7%	84·9%	62·4%	68·2%	79·6%	82·2%	81·5%

Values indicate median bootstrapped values of accuracy, PPV, NPV, sensitivity, and specificity of pre-treatment MFD according to post-treatment MFD measured at day 3–4 or 5–10 after ivermectin treatment. MFD=*Loa loa* microfilariae density. MFD_{D0}=MFD before ivermectin treatment. mf per mL=*L loa* microfilariae per mL of blood. NPV=negative predictive value. PPV=positive predictive value. *Thresholds that might be considered for predicting pre-treatment levels on the basis of their combination of sensitivity and specificity for various thresholds (except for 30000 mf per mL and 50000 mf per mL, owing to larger uncertainty in estimates).

Table 3: **Accuracy, PPV, NPV, sensitivity, and specificity of post-treatment MFD for predicting pre-treatment MFD**

consistent with the observations of Twum-Danso,[18] who found that the average post-treatment (<1 month) MFD in 20 so-called probable serious adverse event cases was 4114 mf per mL. Such a high density suggests that pre-treatment MFD was probably closer to 30000 mf per mL, assuming an 85% relative reduction at day 7, as shown by Pion and colleagues,[17] or perhaps even as high as 100000 mf per mL or more, assuming a 96% reduction in pre-treatment levels after 1 month, as shown by Kombila and colleagues.[26] This finding is consistent with data obtained from the eight individuals with neurological serious adverse events for whom MFD_{D0} were known, most of which were greater than 100 000 mf per mL (range 50 520–217 000 mf per mL).[6–8,15] Our work provides a set of tables and charts estimating the probability that an observed post-treatment *L loa* MFD is associated with an MFD_{D0} exceeding commonly used thresholds predictive of *L loa*-related adverse events. These thresholds include those for the risk of a marked adverse event (8000 mf per mL) and the risk of a serious adverse event (associated with higher MFD_{D0} threshold values of 10 000–50 000 mf per mL). Inferring MFD_{D0} from post-treatment measurements can be helpful at the programmatic and individual levels, and might rely on different cutoffs depending on whether NPV is favoured over PPV.

In clinical practice, an encephalopathy occurring shortly after ivermectin treatment can be considered *L loa*-related in cases in which the post-treatment MFD is above a threshold of 3000 mf per mL (a threshold favouring specificity). An individual with a post-treatment MFD of more than 3500 mf per mL has a 69% risk of having more than 20 000 mf per mL before treatment. Post-treatment MFD assessment can also help rule out adverse event diagnoses when the diagnosis is not clear. For instance, an individual with an *L loa* MFD of fewer than 2500 mf per mL on day 3–4 has a 92% chance of having an MFD_{D0} of fewer than 20 000 mf per mL; and an individual with an MFD of fewer than 2000 mf per mL from day 5 onwards has an 85% chance of having an initial MFD_{D0} of fewer than 8000 mf per mL.

	Sensitivity	Specificity	PPV	NPV	Accuracy
8000 mf per mL MFD_{D0} threshold					
1500	83·0%	87·5%	74·7%	92·0%	86·0%
2000	80·0%	91·8%	81·2%	91·2%	79·0%
2500	71·1%	94·1%	84·2%	88·0%	87·0%
10000 mf per mL MFD_{D0} threshold					
1500	85·3%	84·2%	66·0%	94·1%	84·5%
2000	81·9%	88·2%	71·4%	93·1%	86·5%
2500	75·0%	91·6%	76·3%	91·1%	87·2%
20000 mf per mL MFD_{D0} threshold					
2500	85·5%	86·5%	57·0%	96·6%	86·3%
3000	84·2%	88·4%	60·4%	96·4%	87·7%
3500	79·0%	89·8%	61·9%	95·3%	87·9%
30000 mf per mL MFD_{D0} threshold					
3500	84·2%	87·1%	49·5%	97·4%	86·8%
4000	79·0%	89·8%	53·6%	96·6%	88·4%
4500	75·4%	91·3%	56·6%	96·1%	89·3%
5000	73·7%	93·7%	63·6%	96·0%	91·1%

PPV=positive predictive value. NPV=negative predictive value. mf per mL=*L loa* microfilariae per mL of blood. MFD=*Loa loa* microfilariae density. MFD_{D0}=MFD before ivermectin treatment.

Table 4: **Observed sensitivity, specificity, PPV, and NPV when using post-treatment MFD measured 14–180 days after ivermectin treatment at selected cutoffs to predict the pre-treatment MFD category according to various thresholds**

These NPV data are also important guides for clinicians to carefully consider and search for a differential diagnosis. For individual purposes, MFDs measured at day 3–4 tended to perform slightly better than those measured later, which is explained by the highly skewed and overdispersed distribution of initial loads, combined with post-treatment clustering due to the slightly faster reduction in *L loa* microfilaria counts in patients with the highest initial MFD. Therefore, in areas where loiasis is endemic, or for individuals returning from such areas, thick blood smears should be prepared immediately upon observing potential signs of adverse events following treatment. Although PPV and NPV data depend on the prevalence of each class of MFD, the overdispersion parameter of the distribution of MFD was not found to depend on the endemicity level,[29] and similar reasoning can be conducted using the sensitivity and specificity data provided herein. For programmatic purposes, thresholds with the greatest accuracy can be used to obtain reliable estimates of the number of attributable cases overall and monitor the incidence of these effects.

Analysis of the MFD data collected between 14 and 180 days after treatment confirms the generally good prediction accuracy and adds that thresholds might retain utility even for MFD measurements obtained much later during follow-up of programmes.

This study has some limitations. The study population was included as part of trials. However, the fact that some were community trials reduces the risk of selection bias. Most importantly, there were few serious neurological adverse events, which are to be attributed, with a risk of classification bias. Nevertheless, it is probable that there is a continuum between the different types of serious adverse events, limiting this issue. Finally, all data were collected in Cameroon, where most of the *L loa*-related post-ivermectin adverse events have been reported (with the Democratic Republic of the Congo), and certainly permits generalisation to central Africa.

The predictive performance was not improved by taking into account the age and sex of the participants (data not shown). Nevertheless, we cannot exclude that some other factors could help improve the classification performance. Efforts should be made by the international community of clinicians receiving imported cases of loiasis to gather a systematised and comprehensive set of clinical and biological information on their patients during treatment follow-up.

Contributors

CB, SDP, CBC, and MB conceived the study. MB, SDP, JK, JG, J-PC, SR, and AG were investigators of the trials from which we used the data. CB performed and interpreted the statistical analyses, and wrote the first version of the manuscript. CB, SDP, CBC, and MB accessed and verified all the data in this study. All authors critically reviewed the manuscript, had full access to all the data in the study, and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The datasets analysed during the current study are available from MB (michel.boussinesq@ird.fr) upon reasonable request and provided that the principal investigator of the original trial agrees.

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