Effect of a Single Standard Dose (150–200 μg/kg) of Ivermectin on Loa loa Microfilaremia: Systematic Review and Meta-analysis

Sébastien D. Pion, Jules Brice Tchatchueng-Mbougua, Cédric B. Chesnais, Joseph Kamgno, Jacques Gardon, Jean-Philippe Chippaux, Stephane Ranque, Jean-Christophe Ernould, André Garcia, Michel Boussinesq

Open Forum Infectious Diseases

MAJOR ARTICLE

Effect of a Single Standard Dose (150–200 µg/kg) of Ivermectin on *Loa loa* Microfilaremia: Systematic Review and Meta-analysis

Sébastien D. Pion,[1] Jules Brice Tchatchueng-Mbougua,[2] Cédric B. Chesnais,[1] Joseph Kamgno,[3,4] Jacques Gardon,[5] Jean-Philippe Chippaux,[6] Stéphane Ranque,[7] Jean-Christophe Ernould,[6] André Garcia,[6] and Michel Boussinesq[1]

[1]IRD UMI 233-INSERM U1175- University of Montpellier, Montpellier, France; [2]Service d'Épidémiologie et de Santé Publique, Centre Pasteur du Cameroun, Yaoundé, Cameroon; [3]Centre for Research on Filariasis and other Tropical Diseases, Yaoundé, Cameroon; [4]Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon; [5]IRD UMR 050, HydroSciences Montpellier, CNRS, University of Montpellier, Montpellier, France; [6]IRD MERIT, Université Paris 5, Sorbonne Paris Cité, Paris, France; [7]Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, IHU-Méditerranée Infection, Marseille, France

Background. In central Africa, millions of individuals infected with *Loa loa* have received the anthelminthic drug ivermectin (IVM) as part of mass drug administration (MDA) campaigns targeting onchocerciasis control or elimination. Nonetheless, the parasitological surveys that are occasionally conducted to evaluate the impact of IVM treatments on *Onchocerca volvulus* do not include an assessment of the extra benefits of those MDA campaigns on *L. loa*.

Methods. We conducted a systematic review of trials on the effect of a single standard (150–200 µg/kg) dose of IVM on *L. loa* microfilarial density (MFD). The dynamics of MFD over 365 days of treatment were described using multilevel regression and latent class modeling.

Results. IVM brings about a rapid, dramatic, and sustained decrease, with reduction rates of 60%, 75%, 85%, and 90% on day 1 (D1), D2, D7, and D365, respectively. At D365, no participants (0/238) with an initial MFD of <20 000 microfilariae (mf)/mL were at risk of postivermectin severe adverse events, and only 1/57 individuals with an initial MFD of ≥20 000 mf/mL presented with an MFD above this value. The main predictor of post-treatment MFD was the pretreatment value, but this post-treatment value varied little between D8 and D365 regardless of the pretreatment level.

Conclusions. A single dose of IVM is very effective at substantially reducing *L. loa* MFD for at least a year, irrespective of the initial level of parasitemia. Individuals treated with IVM are probably not any more at risk of severe adverse events when retreated 1 year later.

Keywords. embryostatic; filariasis; ivermectin; loiasis; meta-analysis; microfilaricidal.

The broad-spectrum anthelmintic drug ivermectin (Mectizan) has been the mainstay of programs against onchocerciasis for the last 25 years. Ivermectin is also used, in combination with albendazole, against lymphatic filariasis (LF) in areas where onchocerciasis is also endemic. In 2017, 368 million treatments were approved by the Mectizan Expert Committee and donated by Merck & Co., Inc., for mass treatment of onchocerciasis and/or LF [1]. In large parts of central Africa, onchocerciasis coexists with loiasis [2, 3], and individuals can be co-infected with *Loa loa* and *Onchocerca volvulus*. For example, 15% of residents were found to harbor both *L. loa* and *O. volvulus* microfilariae in a rural area of Central Cameroon [4], whereas less impressive figures have been reported in the rainforest communities of Southwestern Nigeria (2.7%) [5] and Cameroon (5.6%) [6]. Consequently, in areas under mass drug administration (MDA) of ivermectin, millions of individuals infected with *L. loa* have received the drug, with contrasting outcomes: from self-reported improvement of loiasis-related signs and symptoms to potentially fatal serious adverse events (SAEs). The reason for SAEs is that ivermectin rapidly kills the embryonic stages of *L. loa* or microfilariae (mf)—the so-called "microfilaricidal effect." When the *L. loa* microfilarial density (MFD) exceeds 30 000 mf/mL, the rapid paralysis and death of these mf leads to the blockage of blood capillaries in various organs, including the brain, and to inflammatory processes and deposition of fibrin clots on the walls of the vessels [7, 8].

In addition to the microfilaricidal effect, the long-lasting reduction in *L. loa* MFD after treatment [9, 10] suggests that ivermectin, in a similar manner to that observed for *O. volvulus*, temporarily disrupts the production or release of mf by the female worms—a phenomenon referred to as an "embryostatic effect."

Received 12 September 2018; editorial decision 8 January 2019; accepted 11 January 2019.
Correspondence: Sébastien D. Pion, PhD, IRD, 911 Avenue Agropolis, 34394 Montpellier, France (sebastien.pion@ird.fr).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofz019

Outside the context of ivermectin treatment, it has been demonstrated that high *L. loa* MFD is associated with excess mortality in untreated individuals [11]. Therefore, in areas where loiasis is endemic, ivermectin MDA organized to eliminate onchocerciasis might have a supplementary beneficial effect by reducing the pathogenic processes associated with high *L. loa* MFD.

Here, we present the first systematic review and analysis of individual and aggregated data on the effect of a single standard (150–200 µg/kg) dose of ivermectin on *L. loa* MFD. The objectives of this analyses were (1) to gain insight into the impact of ivermectin MDA on the microfilarial reservoir of *L. loa* and (2) to assess whether an individual already treated with ivermectin may still be at risk of SAEs after a subsequent treatment.

METHODS

Search Strategy

We searched the PubMed and ISI Web of knowledge databases using the terms "(loiasis OR loase OR Loa loa) AND (ivermectin$) AND (trial OR therapeuti$)" for articles published in English or French. We also checked the references of all studies identified by the above methods.

Data Extraction

Two authors (S.D.P., M.B.) independently assessed eligibility and study quality and extracted data (Figure 1). Eligible trials had to present data on (1) individuals with *L. loa* infection, defined by the presence of mf in the blood (clinical trials), or (2) a population living in an endemic community eligible for treatment regardless of *L. loa* microfilarial status (community trials). Only trials during which ivermectin was given as a single standard dose (150–200 µg/kg of body weight) were included. Outcome measures were *L. loa* microfilaremia prevalence and MFD. Basic parasitological and demographic information from each eligible study was entered into a purpose-built database. The extracted data included demographics of participants (age range and sex ratio) and methods used to select the subjects and for parasitological examinations. In addition, the authors of the included studies were contacted and asked to share the raw data of these trials.

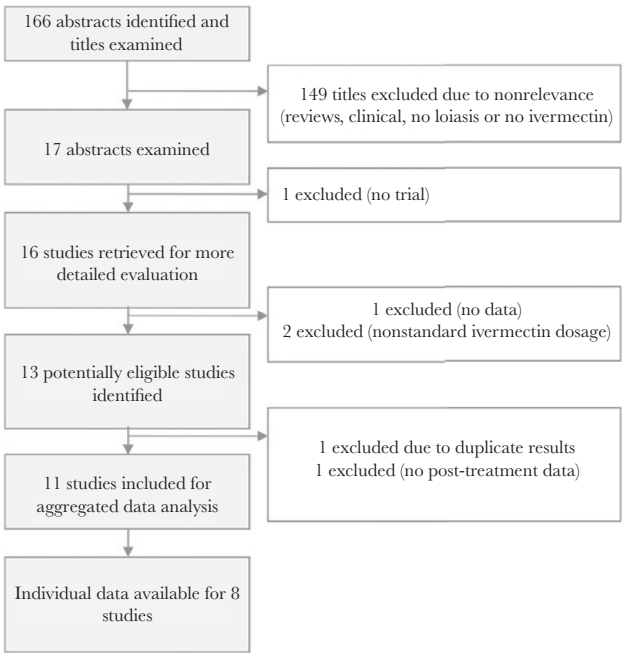

Figure 1. Flowchart summarizing the results of the literature search of a standard dose of ivermectin on *Loa loa* microfilarial density.

Data Analysis

Average Trend Analysis

The effect of a single dose of ivermectin was illustrated by the change (expressed as a percentage) in the pooled geometric mean (GM) of MFD from baseline (pretreatment) values. The pooled GM of this ratio at time t was calculated as a weighted GM in which the sample size of each study was used as a proxy measure for the inverse of the variance (Appendix, Equation 1).

Analysis of Individual Factors Associated With the Effect of Ivermectin on L. loa *MFD*

Using individual participant data, we assessed whether the individuals' age, gender, and pretreatment *L. loa* MFD were associated with the dynamics of post-treatment *L. loa* MFD (dependent variable). The pretreatment *L. loa* MFD was coded in 4 different categories, according to the 4 quartiles: 0–166 (n = 216), 167–3139 (n = 208), 3140–16 179 (n = 212), and 16 180–198 660 mf/mL (n = 211). To account for the nature of the data (repeated measures on sets of individuals from different trials), we built a 3-level regression model: A random intercept was set at the study level, and a random intercept was nested in the study-level random effect to account for repeated measures on individuals. Intraclass correlations were calculated to quantify the variance attributable to interstudy and interindividual heterogeneity. This analysis was performed using the *xtmixed* procedure in Stata software [12]. This analysis was conducted separately for the 7-day period after treatment (when ivermectin exerts its microfilaricidal effect) and the period from day 8 (D8) to D365. The same random-effects regression model was used for both periods. In addition to this main analysis, we performed latent class modeling to test for the existence of different subtypes of responders, defined as the possible "latent classes" (Appendix).

RESULTS

Study Characteristics

Eleven published studies [9, 10, 13–21] provided usable results for the purpose of this analysis (Table 1). The studies were conducted in Cameroon, Gabon, and the Republic of Congo. The

Table 1. Ivermectin Trials Providing Results on *Loa loa* Microfilaremia Included in the Analysis

Reference	Country	Sex, Age Range, y	Inclusion Criteria for IVM Treatment	Blood Sample/Processing	IVM Dose, µg/kg	No. of Participants[a]	Initial MFD (WGM) [range], mf/mL
[13]	Republic of Congo	M+F, NA	MFD >0 mf/mL	2 × 20 µL/leucoconcentration if no mf in blood smears	200	28	5850 [2500–46 000]
[14]	Cameroon	M+F, >5	MFD >0 mf/mL	30 µL	200	80	1914 [33–139 086]
[15]	Cameroon	M+F, 19–60	MFD >30 mf/mL	30 µL	200	112	15 725 [633–164 550]
[10]	Gabon	M+F, 15–76	MFD >0 mf/mL	10 µL + 4 mL if no mf in blood smear	200	71	39 [0.25–35 700]
[9]	Cameroon	M+F, ≥15	Random sampling in 7 microfilaremia strata	50 µL	150	533	503.8 [0–198 660]
[16]	Cameroon	M+F, 18–70	5 ≤ MFD ≤ 150 mf/mL	50 µL	150	13	535 [80–2240]
[17]	Cameroon	M+F, ≥15–70	100 ≤ MFD ≤ 15 000 mf/mL	50 µL	150	31	3261 [420–16 220]
[18]	Gabon	M+F, 7–78	Symptoms of loiasis	4 mL/leucoconcentration if no mf found in 10-µL aliquot	200	84	129.3 [NA]
[19]	Cameroon-Gabon	M+F, NA	MFD >0 mf/mL	NA	200	7	1222 [300–11 150]
[20]	Cameroon	M+F, 2–81	Not documented	30 µL	200	448	8813 [67–101 690]
[21]	Gabon	M, 18–70	Moderate loiasis	3–5 mL/leucoconcentration	150	5 (group 1)	290 [NA]
		M, 18–70	MFD >0 mf/mL & infected with *O. volvulus*		200	5 (group 2)	292 [NA]
		M+F, 13–72			200	17 (group 3)	222 [NA]

Abbreviations: IVM, ivermectin; MFD, microfilarial density; WGM, Williams geometric mean.

[a]With at least 1 post-treatment measure.

dates on which post-treatment *L. loa* MFD were measured (and the number of subjects examined at each time point) were D1 (24 hours after treatment; 73 subjects), D2 (72), D3 (103), D4 (69), D5 (13), D6 (13), D7 (140), D8 (8), D15 (44), D20 (60), D22 (33), D30 (31), D50 (53), D60 (31), D90 (86), D180 (216), and 365 (256) (Figure 2). Individual data were available for 8 [9, 10, 14–17, 19, 20] of the 11 included studies, representing a pool of 847 individuals, of whom 757 had *L. loa* mf at baseline (Figure 2).

General Trend in *L. loa* MFD After Ivermectin Treatment

Seventy-five point four percent (571/757) of the initially microfilaremic subjects remained microfilaremic after treatment. Nonetheless, when compared with the pretreatment values, *L. loa* MFD decreased sharply within the week after treatment, with reduction rates of about 60%, 75%, and 85% on D1, D2, and D7, respectively (Figure 3). The minimum MFD, corresponding to about 90% reduction of pretreatment value, was reached 3 weeks after treatment, with the MFD remaining substantially decreased until D365. At this latter date, the MFDs of individuals from quartiles 1, 2, 3, and 4 were reduced, on average, by 65.3% (95% confidence interval [CI], 31.9%–98.7%), 72.5% (95% CI, 52.5%–92.6%), 81.2% (95% CI, 76.4%–85.9%), and 83.3% (95% CI, 79.9%–86.7%), respectively.

Complete disappearance of *L. loa* microfilaremia was not observed before D4 (0/124 on D1, 0/122 on D2, 0/153 on D3, and 2/118 on D4). The proportion of individuals with undetectable *L. loa* microfilaremia subsequently fluctuated between 20% and 75% from D15 to D365 in the lowest *L. loa* density group (<167 mf/mL) but remained very low in the higher *L. loa* density group throughout the follow-up period.

At D0, 666 individuals presented strictly less than 20 000 mf/mL, a threshold that has been used for implementing safe treatment with ivermectin using a "test and treat" approach in an area where onchocerciasis is co-endemic with loiasis [22]. No individuals presented an *L. loa* MFD above this value at any time after treatment (Figure 2A). A group of 199 microfilaremic individuals with MFD <20 000 mf/mL at D0 were re-examined 1 year after treatment; their MFD ranged from 0 to 13 160 mf/mL after a median decrease (interquartile range [IQR]) of 95.1% (75%–100.0%) (Figure 2A).

At D0, 181 individuals presented at least 20 000 mf/mL. One year after treatment, 57 of those individuals were re-examined; their MFD ranged from 0 to 34 540 mf/mL following a median decrease (IQR) of 89.6% (75.2%–94.2%). Thirty-five (61.4%) and 21 (36.8%) had an MFD below 8000 mf/mL and between 8000 and 20 000 mf/mL, respectively. Only 1 individual (1.3%) still presented an MFD above 20 000 mf/mL despite a significant decrease from 74 140 to 34 540 mf/mL (Figure 2B).

Ninety individuals were amicrofilaremic at baseline, among whom 39 were reexamined at D180 and 51 others at D365. At each of these time points, only 1 subject was found microfilaremic (1/39 and 1/51), both of them with an MFD of 20 mf/mL.

Effect of Individual Factors on the Dynamics of *L. loa* MFD

The regression model using the data collected from D1 to D7 indicated that, on average, the level of MFD after ivermectin is positively correlated with the pretreatment level (quartile

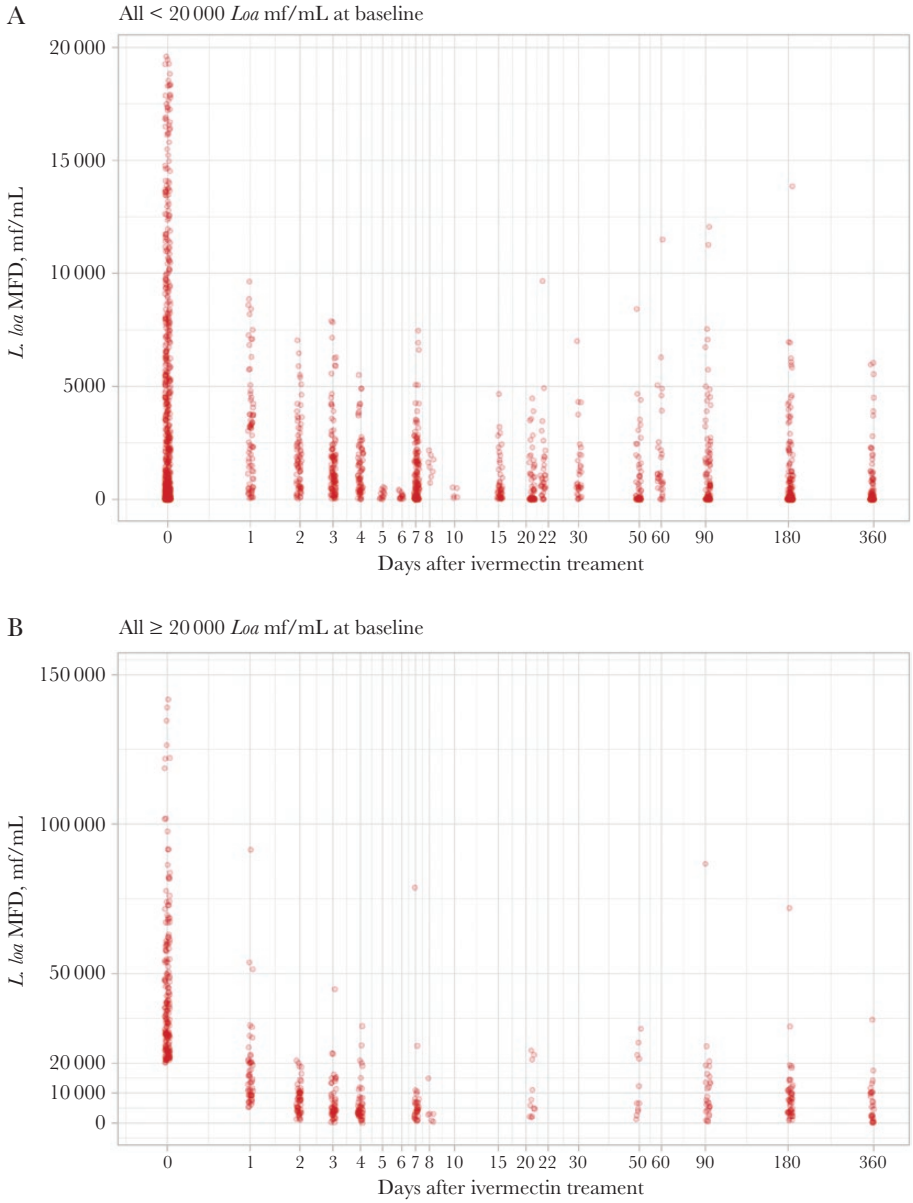

Figure 2. *Loa loa* microfilarial density before (time = 0) and after ivermectin treatment in individuals with initial microfilaremia (A) below 20 000 mf/mL and (B) above 20 000 mf/mL. Abbreviation: MFD, microfilarial density.

effect all significant in Table 2, Figure 4); however, the dynamics of MFD over this period of time did not depend on the pretreatment level (P = .313, .178, and .521 for the second, third, and fourth quartiles, respectively) (Table 2), and the decrease rates were similar in the 4 quartiles. The MFD reduction rate increased minimally with age (P = .012) and did not differ between sexes.

Intraclass correlations indicate that only 1.4% of the total variance in *L. loa* MFD is explained by an interstudy effect and that 71.6% is explained by interindividual variations. Of note, when the quartile of pretreatment *L. loa* MFD was not included in the model, the fraction of variance explained by interindividual variation was barely affected (72.2%), indicating that the variability around the post-treatment value is not correlated with the pretreatment value.

When applied to the following period (D8 to D360), the regression model indicated that *L. loa* MFDs remain stable in patients belonging to 3 of the pretreatment MFD categories. Only individuals belonging to the second quartile (167–3139 mf/mL) showed a slight but significant (P = .004) decrease of approximately 1.5% when compared with the reference group (Table 3, Figure 4). The part of the total variance explained by interstudy variation was negligible, whereas that explained by interindividual variations was 74.4%. When the quartile of

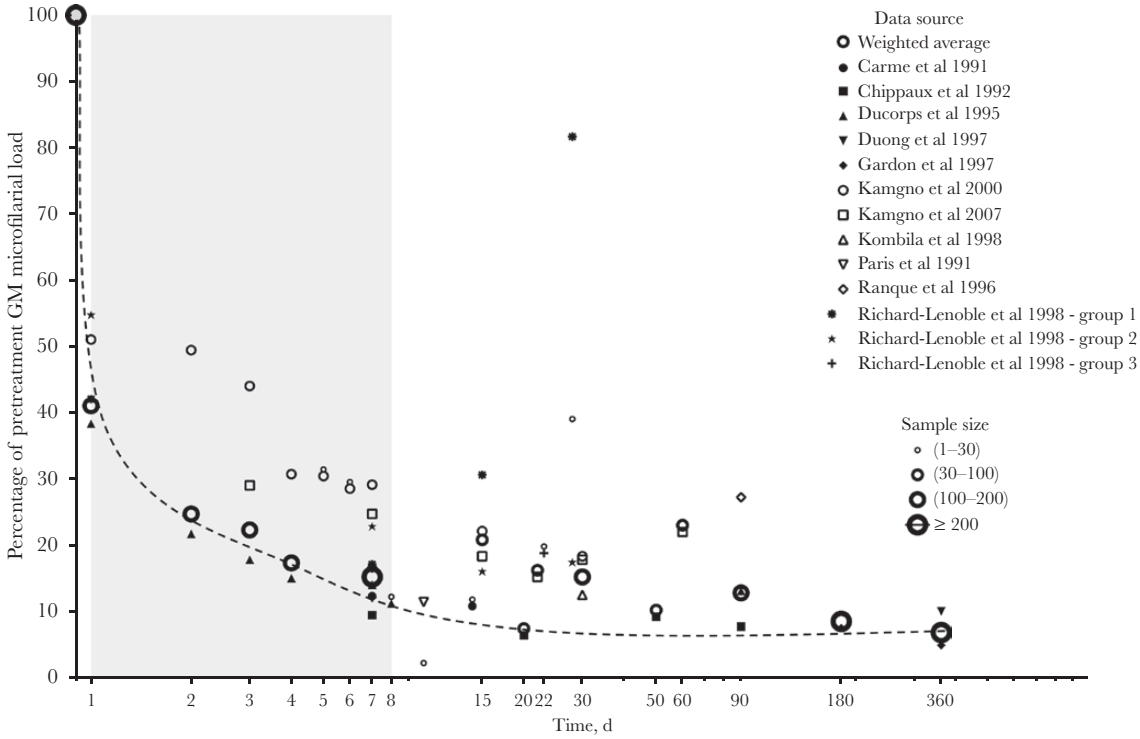

Figure 3. Weighed geometric mean of *Loa loa* microfilarial density after ivermectin treatment. The dashed line is a hand drawing representing the average trend in microfilarial density.

pretreatment *L. loa* MFD was not included in the model, the fraction of variance explained by interindividual variation increased to 80.4%, indicating again a weak association between the variability around the post-treatment MFD and the level of initial MFD.

Two latent classes were identified from latent trajectory modeling. Latent class 1 comprised 50.8% of all included individuals, and their median pretreatment *L. loa* MFD (IQR) was 480 (100–2240) mf/mL. Latent class 2 comprised 49.2% of individuals, and their median pretreatment *L. loa* MFD (IQR) was 17 780 (8676–35 582) mf/mL. During the first week after treatment, the mean trends of *L. loa* MFD for both latent classes were similar. After the first week, the trends were different between the 2 latent classes: MFD tended to increase over time

Table 2. Multilevel Regression of *Loa loa* MFD Over the First Week of Treatment

D0-D7	Coef.	95% CI Lower Limit	95% CI Upper Limit	*P*
Time, d	–0.2370	–0.3924	–0.0815	.003
Quartile of pretreatment density (ref: 0–166 mf/mL)				
167–3139 mf/mL	3.2746	2.0505	4.4986	.0001
3140–16 179 mf/mL	5.3553	4.1415	6.5691	.0001
16 180–198 660 mf/mL	7.0997	5.8848	8.3146	.0001
Pretreatment density × time				
167–3139 mf/mL × time	0.0847	–0.0800	0.2493	.313
3140–16 179 mf/mL × time	0.1133	–0.0514	0.2780	.178
16 180–198 660 mf/mL × time	–0.0541	–0.2193	0.1111	.521
Sex (ref: females)	–0.1569	–0.4227	–0.1089	.0247
Age	–0.0113	–0.0201	–0.0025	.012
Constant	3.3148	2.0809	4.5487	.0001
Random effect (SD)	Estimate	95% CI Lower Limit	95% CI Upper Limit	
Study	0.1403	0.0291	0.6752	
Individual	1.0105	0.9040	1.1297	
Residual	0.6213	0.5797	0.6659	

Abbreviations: CI, confidence interval; MFD, microfilarial density.

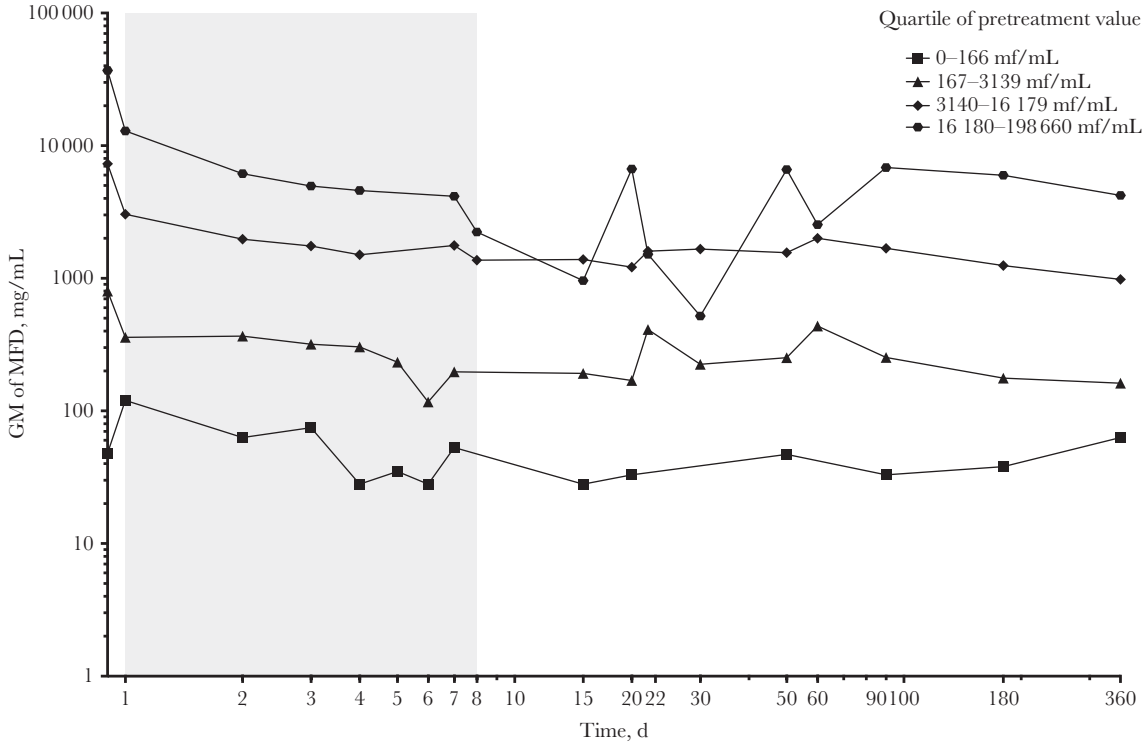

Figure 4. Geometric mean of *Loa loa* microfilarial density after ivermectin treatment in the 4 different quartiles of pretreatment density. Abbreviations: GM, geometric mean; MFD, microfilarial density.

for latent class 2, whereas the MFD for latent class 1 remained stable and low over this period (Figure 5). The MFD reduction rate was positively and significantly associated with age in latent class 1 but was not associated with age in latent class 2 (Table 4). Pretreatment MFD was the only individual factor significantly associated with the probability of belonging to one or the other latent class; individuals from the first and second quartiles of pretreatment *L. loa* MFD were more likely to belong to latent class 1 than individuals from the upper quartiles (Table 5).

DISCUSSION

The first trials of ivermectin on *L. loa* were conducted after demonstration of the high effectiveness and safety of ivermectin

Table 3. Multilevel Regression of *Loa loa* MFD Over the Period D8–D365 After Treatment

D8-D360	Coef.	95% CI Lower Limit	95% CI Upper Limit	*P*
Time, d	–0.0003	–0.0026	0.0020	.809
Quartile of pretreatment density (ref: 0–166 mf/mL)				
167–3139 mf/mL	3.8833	3.1102	4.6563	.0001
3140–16 179 mf/mL	6.7864	6.0258	7.5470	.0001
16 180–198 660 mf/mL	8.3234	7.4741	9.1726	.0001
Pretreatment density × time				
167–3139 mf/mL × time	–0.0044	–0.0074	–0.0014	.004
3140–16 179 mf/mL × time	–0.0020	–0.0050	0.0011	.216
16 180–198 660 mf/mL × time	–0.0019	–0.0051	0.0013	.251
Sex (ref: females)	0.0363	–0.2337	0.3063	.792
Age	0.0024	–0.0057	0.0104	.565
Constant	0.4417	–0.2698	1.1533	.224
Random effect (SD)				
Study	0	0	0.3757	
Individual	1.5937	1.4803	1.7159	
Residual	0.9353	0.8520	1.0267	

Abbreviations: CI, confidence interval; MFD, microfilarial density.

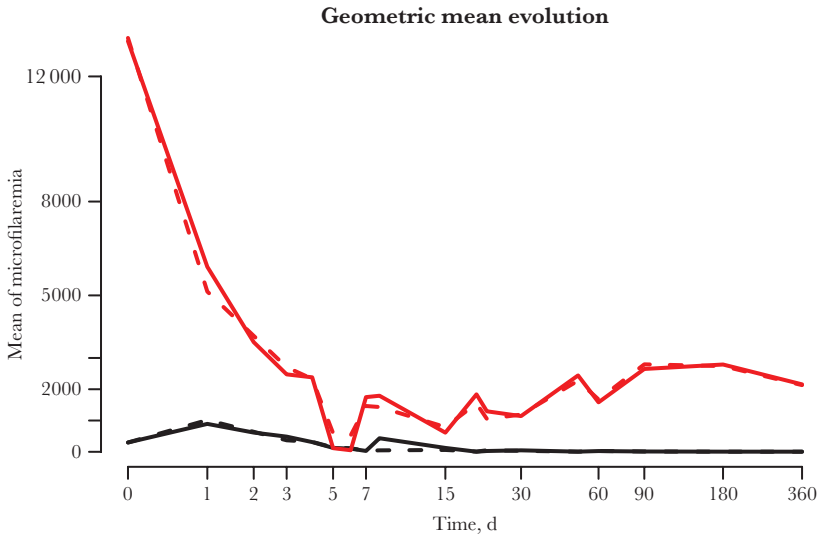

Figure 5. Predicted and observed geometric mean of *Loa loa* microfilaremia. Predicted geometric means were obtained using a latent trajectory model with 2 latent classes. Solid lines: observed geometric mean of *L. loa* microfilarial density. Dashed lines: predicted geometric mean of *L. loa* microfilarial density.

against onchocerciasis. All trials conducted so far have been unanimous in proving the high microfilaricidal efficacy of a single standard dose of ivermectin on *L. loa*. However, although the very first trials [14, 19, 21] concluded the total acceptability and safety of ivermectin for treatment of loiasis, subsequent trials warned about [13]—or confirmed [15]—the possible occurrence of SAEs in individuals with very high *L. loa* microfilaremia. In 1997, the risk threshold for postivermectin *Loa*-related SAEs was estimated to be 30 000 mf/mL [23]. The database gathered to conduct the analyses presented in this paper is therefore unique as it includes a large number of individuals with a known pretreatment *L. loa* MFD as part of a trial or individual treatment.

Table 4. Characteristics Associated With *Loa loa* MFD Evolution After Ivermectin Treatment in Each Latent Class

Microfilaremia at Initiation	Coefficient	95% CI	*P*
Class 1			
Age	–0.04	(–0.05 to –0.03)	<.001
Time	1.81	(0.29 to 0.33)	.02
Time^2	–1.16	(–2.23 to 0.09)	.03
Time^3	3.50	(–0.72 to 7.74)	.1
Time × age	–0.06	(–0.09 to 0.02)	.003
Time^2 × age	–0.04	(–0.07 to 0.01)	.002
Time^3 × age	–0.16	(–0.26 to 0.05)	<.001
Class 2			
Age	0.001	(–0.01 to 0.01)	.76
Time	–1.84	(–2.62 to 1.06)	<.001
Time^2	–1.9	(–2.53 to 1.28)	<.001
Time^3	1.10	(–1.34 to 3.55)	.38
Time × age	0.03	(0.01 to 0.05)	.004
Time^2 × age	–0.01	(–0.03 to 0)	.06
Time^3 × age	–0.04	(–0.1 to 0.02)	.27

Abbreviations: CI, confidence interval; MFD, microfilarial density.

Ivermectin starts acting within hours of intake and, in some individuals, total clearance is observed within 4–5 days. This rapid disappearance of a proportion of microfilariae suggests a direct effect of the drug on *L. loa* mf. Although the mode of action of ivermectin on *L. loa* has not been specifically addressed, this drug is known to act on helminths by altering neuromuscular functions, resulting in paralysis of the parasite [24]. Clinical description of postivermectin SAEs indicates that some embolization processes occur at the cerebral level, pointing out that mf exposed to ivermectin are indeed paralyzed and accumulate in microcirculation rather than being rapidly cleared from the bloodstream. Besides this, it has been demonstrated that ivermectin treatment induces a passage of the mf from the blood into the urine and the cerebrospinal fluid [8, 15], which could lead to the occurrence of adverse events.

Analysis of aggregated data indicate that, on average, MFD is reduced by 90% a few weeks after treatment and that this decrease is sustained for at least a year. Analysis of individual data shows some heterogeneity in the dynamics of MFDs after ivermectin treatment. It is important to note that interstudy heterogeneity was found to be minimal compared with interindividual heterogeneity, suggesting little if any study-related bias in the data, and that we could pool the data together to conduct multivariate analyses.

Multivariate regression indicated that the MFD reduction rate during the first week after treatment was very homogeneous; average MFD was weakly (positively) correlated with pretreatment value, but the evolution between D1 and D8 was similar and showed little variation across the different quartiles

Table 5. Risk Factor Associated With Each Latent Class (Reference Class 2)

Risk Factor	OR	95% CI	*P*
Microfilaremia at initiation			
0–167	6124	(699 to 53 630)	<.001
168–3139	1236	(138 to 11 121)	<.001
3140–16 179	1.8	(0.55 to 5.9)	.33
16 180–198 660	1		
Male	0.7	(0.25 to 2.17)	.58
Age	1	(0.95 to 1.03)	.62

Abbreviations: CI, confidence interval; OR, odds ratio.

of initial MFD. In other words, all MFDs were drastically and very rapidly reduced regardless of their pretreatment value.

After 1 week, the *Loa* MFD remained positively correlated with the pretreatment value and remained low up to 1 year after treatment. This dependence on the initial MFD value was confirmed by latent class modeling, which demonstrated that the data are consistent with the existence of 2 distinct individual profiles: (1) individuals with low initial MFDs and whose MFDs were sustainably reduced for at least 1 year and were frequently below the threshold of detectability and (2) individuals with higher initial MFDs, whose post-treatment MFDs were higher than in the first group and tended to increase very slightly over time. The main result from an epidemiological perspective is that even individuals in the highest MFD quartile still had their MFD reduced by about 85% 1 year after treatment.

As with observed effects on *O. volvulus*, the microfilaricidal effect of ivermectin on *L. loa* MFD is only partial. Indeed, total clearance of *L. loa* microfilaremia was nearly entirely observed in individuals with low *L. loa* microfilaremia. This indicates that a proportion of microfilariae would escape the effect of the drug. Whether it is due to a suboptimal exposure to the drug, related to parasite sensitivity to the drug, or related to another kind of mechanism should be investigated.

To date, there are no markers to estimate the number of *L. loa* adult worms harbored by a given individual, and the effects of ivermectin on the parasite rely mainly on the dynamics of *L. loa* MFD after treatment. This is the result of (1) the microfilaricidal effect of the drug (and marginally the natural death of mf) and (2) the production and release of new mf by fertilized adult female worms. From results obtained in an *L. loa*/baboon experimental model, it was estimated that a single female adult worm can produce between 12 000 and 39 000 mf per day [25]. The fact that MFD remains at a very low level for several months after ivermectin treatment could therefore potentially be the result of any of the following 4 processes acting separately or in synergy: (1) a temporary or permanent cessation of mf production (embryogenesis) by the female worms, (2) a long-lasting cessation of the release of mf (embryostatic effect), (3) an increased efficacy of the immunological response leading to an increased destruction of the released mf, and (4) a partial macrofilaricidal effect. Unfortunately, no attempt has been made to collect adult worms migrating under the conjunctiva in patients who have received ivermectin recently. The examination of such worms might help to assess whether the embryostatic effect of ivermectin seen in *O. volvulus* also exists for *L. loa*. Whether the frequency of "eyeworm" episodes decreases after ivermectin treatment has also never been investigated. Given these uncertainties, mathematical modeling similar to that conducted for *O. volvulus* [26] might be useful to assess the respective role of each of the possible effects of ivermectin on *L. loa*.

Based on the observed MFD dynamics after a first ivermectin treatment, one could expect that community-directed treatment with ivermectin (CDTI) could have a significant and rapid impact on the transmission of *L. loa*. One year after a first ivermectin treatment, the MFD of most individuals should be reduced to such low levels that an additional treatment with ivermectin might result in mf clearance. A study conducted in Cameroon whose objective was to compare entomological indicators measured in 2000 and 2012 in an area benefitting from annual CDTI showed that the infection rates of *Chrysops* had decreased significantly but that the infective rates and mean number of *L. loa* infective larvae per infective fly did not differ significantly between the 2 investigation periods [27]. We assume that this result, which was unexpected given the promising observations made in another area treated with ivermectin every 3 months [28], might be due to a relatively low therapeutic coverage (see figures reported by the National Programme for Onchocerciasis Control in [27]) or to a high proportion of individuals who never take the drug because of fear of SAEs. The latter parameter was not assessed during the aforementioned study (in Kokodo [27]). However, in a health district (Yabassi) located some 200 km west of Kokodo and where the endemicity levels for loiasis were lower, the proportion of individuals who had never taken ivermectin during the last 5 MDAs was 15.4% (95% CI, 11.5%–20.5%) [29]. The proportion of systematic noncompliers might have been higher in Kokodo than in Yabassi.

This fear of SAEs could be overcome using a test-and-treat strategy during which the MFD of every participant is assessed before treatment. Such a strategy typically leads to the exclusion of less than 3% of the population from ivermectin treatment [22]. Given the phenomenon of increased *Chrysops* mortality after ingesting a high number of mf [30, 31], the possibility that those few individuals constitute a group of super-spreaders enabling widespread maintainance of *L. loa* transmission in the community should be assessed.

In individuals with *L. loa* microfilaremia, ivermectin is highly efficacious: a single oral dose is able to drastically and rapidly reduce the MFD, leading to reductions that are sustained for at least a year. In onchocerciasis-hypoendemic areas where CDTI cannot be implemented but where a test-and-treat strategy can be applied, our results indicate that individuals treated with ivermectin do not need to be retested for *L. loa* 1 year later, during

the next test-and-treat campaign. Testing only those who did not receive ivermectin 1 year before would have a huge impact on lowering the costs of the campaign, which could reach very low levels after several years. In onchocerciasis-meso-hyperendemic areas, CDTI has been in place for many years, but one of the main challenges is to convince the systematic noncompliers (who can represent up to 20% of the total population) to take ivermectin treatment. The data presented here might help program managers to develop specific messages directed toward this subpopulation. By highlighting that (1) ivermectin is very effective against the parasite inducing the SAEs, (2) that the drug received for many years by the population has reduced the transmission of this parasite and thus the infection intensity and the risk of SAEs in the whole population, and (3) that a single test just before ivermectin treatment would ensure that, if they are below a given threshold, they would not need to be retested every year, managers would have strong arguments that could convince at least a proportion of people systematically refusing treatment.

Presently, control of loiasis is not considered a priority, even if evidence is emerging that this disease is a significant public health problem [11]. Our results suggest that ivermectin distribution, should a good coverage be achieved, might have a significant impact on reducing *L. loa* transmission and help decrease the burden related to loiasis. More information is needed to evaluate to what extent this is true. Measuring *L. loa* infection levels systematically during impact assessment surveys for onchocerciasis and LF programs would provide data useful to refining the parameters of the *L. loa* transmission models that are currently being developed.

Acknowledgments

Financial support. As a literature review and meta-analysis, this study did not receive any funding.

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Mectizan Donation Programme. 2017 annual highlights. **2018**. https://mectizan.org/wp-content/uploads/2018/06/MDP_AH17_English.pdf. Accessed 11 March 2019.
2. Cano J, Basáñez M-G, O'Hanlon SJ, et al. Identifying co-endemic areas for major filarial infections in sub-Saharan Africa: seeking synergies and preventing severe adverse events during mass drug administration campaigns. Parasit Vectors **2018**; 11:70.
3. Zouré HG, Wanji S, Noma M, et al. The geographic distribution of *Loa loa* in Africa: results of large-scale implementation of the Rapid Assessment Procedure for Loiasis (RAPLOA). PLoS Negl Trop Dis **2011**; 5:e1210.
4. Pion SD, Clarke P, Filipe JA, et al. Co-infection with *Onchocerca volvulus* and *Loa loa* microfilariae in central Cameroon: are these two species interacting? Parasitology **2006**; 132:843–54.
5. Ojurongbe O, Akindele AA, Adeleke MA, et al. Co-endemicity of loiasis and onchocerciasis in rain forest communities in southwestern Nigeria. PLoS Negl Trop Dis **2015**; 9:e0003633.
6. Wanji S, Tendongfor N, Esum M, et al. Epidemiology of concomitant infections due to *Loa loa*, *Mansonella perstans*, and *Onchocerca volvulus* in rain forest villages of Cameroon. Med Microbiol Immunol **2003**; 192:15–21.
7. Fobi G, Gardon J, Santiago M, et al. Ocular findings after ivermectin treatment of patients with high *Loa loa* microfilaremia. Ophthalmic Epidemiol **2000**; 7:27–39.
8. Wanji S, Eyong EJ, Tendongfor N, et al. Ivermectin treatment of *Loa loa* hyper-microfilaraemic baboons (*Papio anubis*): assessment of microfilarial load reduction, haematological and biochemical parameters and histopathological changes following treatment. PLoS Negl Trop Dis **2017**; 11:e0005576.
9. Gardon J, Kamgno J, Folefack G, et al. Marked decrease in *Loa loa* microfilaraemia six and twelve months after a single dose of ivermectin. Trans R Soc Trop Med Hyg **1997**; 91:593–4.
10. Duong TH, Kombila M, Ferrer A, et al. Reduced *Loa loa* microfilaria count ten to twelve months after a single dose of ivermectin. Trans R Soc Trop Med Hyg **1997**; 91:592–3.
11. Chesnais CB, Takougang I, Paguélé M, et al. Excess mortality associated with loiasis: a retrospective population-based cohort study. Lancet Infect Dis **2017**; 17:108–16.
12. Graubard BI, Korn EL. Modelling the sampling design in the analysis of health surveys. Stat Methods Med Res **1996**; 5:263–81.
13. Carme B, Ebikili B, Mbitsi A, Copin N. Essai thérapeutique de l'ivermectine au cours de la loase à moyenne et forte microfilarémie. Ann Soc Belg Med Trop **1991**; 71:47–50.
14. Chippaux JP, Ernould JC, Gardon J, et al. Ivermectin treatment of loiasis. Trans R Soc Trop Med Hyg **1992**; 86:289.
15. Ducorps M, Gardon-Wendel N, Ranque S, et al. Effets secondaires du traitement de la loase hypermicrofilarémique par l'ivermectine. Bull Soc Path Ex **1995**; 88:105–12.
16. Kamgno J, Gardon J, Boussinesq M. Analysis of the prevention of post-ivermectin *Loa loa* encephalopathy by administration of initial low dose. Med Trop (Mars) **2000**; 60:275–7.
17. Kamgno J, Pion SD, Tejiokem MC, et al. Randomized, controlled, double-blind trial with ivermectin on *Loa loa* microfilaraemia: efficacy of a low dose (approximately 25 microg/kg) versus current standard dose (150 microg/kg). Trans R Soc Trop Med Hyg **2007**; 101:777–85.
18. Kombila M, Duong TH, Ferrer A, et al. Short- and long-term action of multiple doses of ivermectin on loiasis microfilaremia. Am J Trop Med Hyg **1998**; 58:458–60.
19. Paris L, Datry A, Durepaire R, et al. Intérêt de l'ivermectine dans le traitement initial de la loase. La Presse Médicale **1991**; 20:1393.
20. Ranque S, Garcia A, Boussinesq M, et al. Decreased prevalence and intensity of *Loa loa* infection in a community treated with ivermectin every three months for two years. Trans R Soc Trop Med Hyg **1996**; 90:429–30.
21. Richard-Lenoble D, Kombila M, Rupp EA, et al. Ivermectin in loiasis and concomitant *O. volvulus* and *M. perstans* infections. Am J Trop Med Hyg **1988**; 39:480–3.
22. Kamgno J, Pion SD, Chesnais CB, et al. A test-and-not-treat strategy for onchocerciasis in *Loa loa*-endemic areas. N Engl J Med **2017**; 377:2044–52.
23. Gardon J, Gardon-Wendel N, Demanga-Ngangue, et al. Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. Lancet **1997**; 350:18–22.
24. Bennett J, Dolin R, Blaser M. *Principles and Practise of Infectious Diseases*. Philadelphia, PA: Elsevier; **2018**.
25. Eberhard ML, Orihel TC. *Loa loa*: output of microfilariae in single pair infections. Trop Med Parasitol **1986**; 37:369–74.
26. Walker M, Pion SDS, Fang H, et al. Macrofilaricidal efficacy of repeated doses of ivermectin for the treatment of river blindness. Clin Infect Dis **2017**; 65:2026–34.
27. Kouam MK, Tchatchueng-Mbougua JB, Demanou M, et al. Impact of repeated ivermectin treatments against onchocerciasis on the transmission of loiasis: an entomologic evaluation in central Cameroon. Parasit Vectors **2013**; 6:283.
28. Chippaux JP, Bouchité B, Boussinesq M, et al. Impact of repeated large scale ivermectin treatments on the transmission of *Loa loa*. Trans R Soc Trop Med Hyg **1998**; 92:454–8.
29. Kamga GR, Dissak-Delon FN, Nana-Djeunga HC, et al. Audit of the community-directed treatment with ivermectin (CDTI) for onchocerciasis and factors associated with adherence in three regions of Cameroon. Parasit Vectors **2018**; 11:356.
30. Kershaw WE, Chalmers TA, Duke BO. Studies on the intake of microfilariae by their insect vectors, their survival, and their effect on the survival of their vectors. IV. The survival-rate of *Chrysops* under laboratory conditions, and the effect upon it of *Loa loa*. Ann Trop Med Parasitol **1954**; 48:329–39.
31. Whittaker C, Walker M, Pion SDS, et al. The population biology and transmission dynamics of *Loa loa*. Trends Parasitol **2018**; 34:335–50.
32. Proust-Lima C, Philipps V, Liquet B. Estimation of extended mixed models using latent classes and latent processes: the R package *lcmm*. J Stat Soft **2017**; 78:1–71.

APPENDIX

AVERAGE TREND ANALYSIS OF *LOA LOA* MICROFILARIAL DENSITY AFTER IVERMECTIN TREATMENT

The effect of a single dose of ivermectin was illustrated by the change in the pooled geometric mean of MFD per milliliter of blood (mf/mL) from baseline (pretreatment) values. We defined GM_{ti} as the GM of MFD at time t in study i and $a_{ti} = \frac{GM}{GM_{0i}}$ the ratio of GM at time t in study i with respect to the pretreatment GM of MFD (GM_{0i}). The pooled GM of this ratio at time t was calculated as a weighted GM (A_t), in which the sample size of each study was used as a proxy measure for the inverse of the variance (Equation 1):

$$A_t = \left\{ \left[\prod_{i=1}^{k_t} (a_{ti} + 1)^{n_{ti}} \right]^{1/N_t} \right\} - 1 \qquad (1)$$

where n_{ti} is the sample size (number of participants) at time t of study i; k_t is the number of studies that reported MFD at time t; and N_t is the total number of patients eligible for analysis at time t (the sum of sample sizes across k_t studies). The weighting by the sample size implicitly assumes that all studies in the analysis had the same underlying variance for changes in the MFD.

LATENT CLASS MODELING TO TEST FOR THE EXISTENCE OF DIFFERENT GROUPS OF RESPONDERS

Latent trajectory analysis assumes heterogeneity in a sample where unobserved homogeneous subpopulations (latent classes) exist. The first step of this analysis was to determine the number of latent classes that best fitted the data. For this, we compared the Bayesian Information Criterion between 4 different models based on Equation (2), where k, the number of latent classes, was successively set at 1, 2, 3, and 4.

$$Ln\,(MFD_i) = \sum_{l=1}^{k} \left[f\,(a_{il}, t_i)\, 1_{class=l} \right] + f\,(u_i,\ t_i) + e_i \qquad (2)$$

where $f(.,)$ function f represents orthogonal polynomials of degree ≤5 of time t_i; a_{il} is the parameters vector of the orthogonal polynomial for latent class l, $l = 1,\ldots,k$; u_i is the vector of individual parameters; and e_i is the vector of residuals.

We examined the stability of the latent classes determined when the covariates were successively added to the model. Indeed, the addition of significant covariates to the model did not widely modify the percentage of subjects in each trajectory.

Table A1. Hypothetical Results for Determination of the Optimal Number of Latent Classes Using BIC

Models	BIC	Percentage of Sample Size Per Class Based on Most Likely Class Membership
Model with 1 class without covariate	11 386.13	100/0
Model with 2 latent classes without covariate	10 058.23	55.2/44.8
Model with 3 latent classes without covariate	10 437.2	53/20.9/26.1
Model with 4 latent classes without covariate	10 220.8	39.7/24.6/17.7/17.9
Model with 2 class with covariate (final model)	8992.4	50.9/49.1

The model without a covariate with the lowest BIC was the model with 2 latent classes. Thus the optimal number of latent classes chosen was 2 latent classes. When we added a significant covariate in the model, we observed little variation of the percentages of subjects in each latent class: 7.1% of the subjects were reassigned from latent class 1 to latent class 2, and 2.8% of the subjects were reassigned from latent class 2 to latent class 1.

The second step consisted of estimating, for each individual, (1) the probability of belonging to each latent class by maximum likelihood estimation and (2) the latent trajectory of MFD over time in each homogeneous latent cluster. The model estimated the membership probabilities for each patient, and a unique latent trajectory was assigned to each patient based on the maximal membership probability. This model assumes that repeated observations of the same individual are independent, conditional on latent trajectory, meaning that the within-person correlation structure is explained completely by the estimated trajectory curve for each person in a given latent cluster. The association between the probability of belonging to each latent class and individual risk factors, namely age, sex, and initial microfilaremia, was assessed by logistic regression. Different models were fitted and compared with the BIC. This analysis was performed using the latent classes mixed model (*lcmm*) package from R-Cran software [32].

Abbreviation: BIC, Bayesian Information Criterion.