Laboratory Parameters after Treatment for *Loa loa* and *Mansonella perstans*: The Experience of a Single Referral Center for Tropical Diseases in a Non-Endemic Area

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Abstract. Infections due to *Loa loa* and *Mansonella perstans* are common yet elusive neglected filariases. Parasitological cure after treatment is very difficult to assess, as adult parasites are not accessible. Therefore, outside transmission areas, patients require a long follow-up period to ascertain the therapeutic outcome, which is impractical for non-sedentary populations such as migrants. We studied the change over time of microfilaremia, eosinophil counts, and antifilarial antibodies tested with a commercial ELISA test (Bordier Affinity Products, Crissier, Switzerland), in a retrospective cohort of patients with confirmed *L. loa* and *M. perstans* infections, to evaluate the role of serology in clinical practice. After treatment, all 22 eligible patients diagnosed in our center between 2015 and 2017 reached amicrofilaremia, with microfilarial counts decreasing sharply within 2 months. Paralleling eosinophil counts, antibodies decreased in all patients, 36% of whom reached sero-reversion or near-sero-reversion in < 20 months. These findings suggest that positive serology is not just residual from a past infection, and may be used for diagnosis even when microfilaremia is negative or cannot be performed. Interestingly, antibodies and eosinophil counts increased following some, but not all, re-treatment courses. If the rise in these parameters reflects death of macrofilariae, caution is required in interpreting high eosinophil counts and antibody titers shortly after treatment, as these may reflect no need for further treatment. To optimize patients' management, it is now pivotal to ascertain the interval between treatment and macrofilarial death and therefore whether re-treatments are required for complete clearance of parasites.

INTRODUCTION

Infection with the filarial nematodes *Loa loa* and *Mansonella perstans* are among the most common yet elusive and neglected filarial diseases. An estimated 10 million people are infected with *L. loa* in the 10 endemic countries of West Africa¹ and more than 114 million with *M. perstans*, mostly in sub-Saharan Africa.²

Despite classical manifestations of loiasis having been reported as a major driver for people seeking medical advice in endemic areas, this infection has long been regarded as a benign condition.¹ Only recently has high *L. loa* microfilaremia been associated with an increased mortality risk, prompting reconsideration of the importance this parasitosis.³ Infection with *M. perstans* is considered one of the most prevalent human parasitosis in Africa, but clear definition of symptoms and quantification of associated health consequences are still lacking.²

Control of microfilaremia at the population level, aiming to interrupt transmission to arthropod vectors, is the goal in endemic areas where the burden of infection is present. This, however, is not adequate outside transmission areas, where the infection is imported and individual patient management should aim to eliminate adult parasites. Unfortunately, the general neglect of these common filariases has resulted in a paucity of scientific basis to support effective treatment and follow-up strategies.

Few studies have addressed therapy and outcome of loiasis and mansonellosis in patients treated outside of endemic areas.^{4–13} Effectiveness of treatment in terms of parasitological cure is very difficult to assess because adult *L. loa* and

M. perstans are not accessible. Generally, elimination of circulating microfilariae (mf), resolution of eosinophilia, and sustained absence of symptoms are used as indirect signs of therapy effectiveness. However, specific signs, such as Calabar swelling and the visualization of a worm migrating in the conjunctiva ("eye worm") for loiasis, are not always present clinically^{4,10,13}; patients are often asymptomatic or present unspecific complaints.^{4,9,10} Eosinophilia is unspecific and is not observed in all infected patients.^{4,9,13} In loiasis, microfilaremia may be undetectable by microscopy-based techniques, especially in early infection, in roughly 40–80% of cases,^{4,14–17} and therefore its absence cannot be used as a sure sign of an absence of adult worms. Although less frequent, amicrofilaremic *M. perstans* infections are also documented.⁶ Conversely, after treatment, presence of microfilaremia cannot be used to determine residual presence of adult worms, as mf may persist in the circulation for several months.²

Serology, although confounded by variable levels of cross-reactivity depending on the antigen and assay used, is also positive only in a proportion of cases.^{6,9,10,13,18} Nevertheless, the evolution of antifilarial antibodies may be an additional, complementary tool for the follow-up of infected patients. Very few clinical studies have investigated longitudinally the evolution of laboratory parameters, including antifilarial antibody titers, after treatment for *L. loa*,^{11,19–22} and only one after treatment for *M. perstans* infection.²² In these studies, however, in-house ELISA assays were used to monitor the evolution of antifilarial antibody titers after therapy. Moreover, only two of these studies were carried out outside transmission areas,^{11,19} none of which, to our knowledge, investigated the follow-up parameters after treatment for a *M. perstans* infection.

In this work, we assessed the change over time of antifilarial antibodies in a retrospective cohort of patients with confirmed *L. loa* and *M. perstans* infections, treated in a single Italian referral center for tropical diseases and followed up using parameters from a single laboratory and a single commercial

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antifilarial antibody test, with the aim of preliminarily evaluating the role of this test in clinical practice.

MATERIALS AND METHODS

Ethics statement. The study was conducted under the provisions of the Declaration of Helsinki, and in accordance with the International Conference on Harmonization Consolidated Guideline on Good Clinical Practice. Because this study was retrospective and non-pharmacological, written informed consent was not provided. In Italy, ethical review board authorization for these studies is not required (see Italian Guidelines for classification and conduction of observational studies, established by the Italian Drug Agency, “Agenzia Italiana del Farmaco-AIFA” on March 20, 2008).

Patients selection. This is a retrospective longitudinal observational study. Patients with detectable circulating microfilariae of *L. loa* or *M. perstans* diagnosed and treated in 2015–2017 in the Centre for Tropical Diseases, IRCCS, Sacro Cuore-Don Calabria Hospital, Negrar, Verona, Italy, were identified in the medical files of the center’s clinical records. Patients’ demographic data, nationality, signs and reported symptoms, and coinfections, were extracted from medical files, as well as antifilarial treatments, microfilarial counts, eosinophil counts, and antifilarial serology results, at baseline and at follow-up visits.

Eosinophilia was defined as an eosinophil count ≥ 450 eosinophils/ μ L blood. Circulating mf were detected in 9 mL

peripheral blood collected at daytime using a modified Knott technique followed by Giemsa staining for species identification. Antifilarial antibodies were detected with a commercial ELISA kit using *Acanthocheilonema vitae* as source of antigens (Bordier Affinity Products, Crissier, Switzerland) as per manufacturer instructions. This test is not specific for single filarial species, and detects IgG against various filarial nematodes affecting humans. A serological index (SI) of ≥ 1 calculated as per the manufacturer’s protocol was considered positive.

Patients with *L. loa* were treated with albendazole 400 mg BID for 28 days followed by 1-day ivermectin 200 μ g/kg SID. Patients with *M. perstans* were treated with mebendazole 500 mg TID for 14–28 days followed by doxycycline 100 mg BID for 6 weeks. Amicrofilaraemic individuals with positive antifilarial antibodies (not included in this study) were empirically treated with albendazole 400 mg BID for 5 days followed by 1-day ivermectin 200 μ g/kg SID.

Clinical and laboratory follow-up was carried out at variable intervals. As a rule, patients were re-treated in the case of persistence/reappearance of symptoms or evolution of laboratory parameters that could suggest persistence of infection. Re-treatment for *M. perstans* was carried out with mebendazole 500 mg TID for 14–28 days and for *L. loa* with diethylcarbamazine 6 mg/kg SID for 21 days. Patients were considered cured on obtainment of normal eosinophil counts, negative microfilaremia, and absence of symptoms.

FIGURE 1. Overview of individuals screened for filarial infection in 2015–2017.

TABLE 1

Demographic characteristics, laboratory parameters at diagnosis, treatments effectuated, and change in laboratory parameters over the follow-up of patients included in the study

Patient	Age (years)	Origin	Coinfections	Filarial infection	At diagnosis			Treatments after the first T0 course ‡	Follow-up time points (months)	Follow-up time point in months when reaching		
					ELISA SI*	Eosinophils (n/μL)†	Microfilariae (mf/mL blood)			ELISA negative/borderline (SI)	Eosinophils < 450/μL	Amicrofilaremia
#1	28	Senegal	–	<i>Mansonella perstans</i>	1.73	750	0.50	–	19, 22	–	19	19
#2	21	Ghana	Sch; Hkw	<i>M. perstans</i>	1.63	1,080	3.60	–	16, 21	21 (1.02)	16	16
#3	21	Senegal	Str	<i>M. perstans</i>	1.22	500	103.00	T1	1, 13, 18	13 (1.08)	13	13
#4	35	Senegal	–	<i>M. perstans</i>	2.33	1,010	6.10	–	13, 17	17 (1.02)	13	13
#5	18	Senegal	–	<i>M. perstans</i>	3.67	1,100	1.47	–	2, 3, 10, 13, 20	–	10	3
#6	55	Italy§	Str	<i>M. perstans</i>	3.68	9,400	5.60	–	3, 19	19 (0.68)	19	3
#7	23	Guinea Bissau	Sch; HIV	<i>M. perstans</i>	1.43	1,300	789.00	T2, T4, T5, T6	2, 4, 5, 6, 10, 13	–	10	13
#8	19	Guinea Bissau	Hkw; HBV	<i>M. perstans</i>	2.57	900	11.90	T2, T5	2, 5, 15	15 (0.27)	2	15
#9	18	Senegal	HBV	<i>M. perstans</i>	2.41	1,000	0.33	–	1, 4, 10	–	10	1
#10	19	Guinea Bissau	–	<i>M. perstans</i>	1.83	800	6.33	T1, T2, T4, T5	1, 2, 4, 5, 6, 10	–	2, 10	6
#11	25	Ivory Coast	–	<i>M. perstans</i>	1.47	200	2.20	–	2, 4	–	0	2
#12	25	Guinea	–	<i>M. perstans</i>	2.39	600	19.50	T1	1, 5	–	5	5
#13	26	Togo	Str; Sch; HBV	<i>M. perstans</i>	2.56	490	0.14	–	1, 5	15 (1.06)	5	1
#14	25	Ivory Coast	Sch	<i>M. perstans</i>	2.07	600	53.80	T1, T2	1, 2, 3	–	1, 3	3
#15	18	Guinea Bissau	–	<i>M. perstans</i>	3.17	100	1.50	–	3	–	0	3
#16	18	Guinea Bissau	Hkw	<i>M. perstans</i>	5.30	1,000	30.80	T1	1, 2	–	2	2
#17	30	Ivory Coast	Sch; Hkw	<i>M. perstans</i>	3.27	900	511.00	T1	1, 2	–	2	2
#18	21	Cameroon	–	<i>M. perstans</i> + <i>Loa loa</i>	1.39	400	12.00 Mp; 9,800.00 LI	T1	1, 4, 5	5 (0.69)	0, 4	4 (Mp)
#19	24	Nigeria	Hkw	<i>L. loa</i>	2.15	1,300	160.00	T1	1, 2, 3, 4, 6, 7	7 (0.74)	3	2
#20	18	Nigeria	–	<i>L. loa</i>	2.57	1,100	476.00	–	2, 3, 4	–	4	4
#21	29	Cameroon	Sch	<i>L. loa</i>	2.85	590	980.00	–	1, 3, 7	–	3	1
#22	27	Cameroon	Sch; Hkw	<i>L. loa</i>	1.53	670	491.00	–	1, 5, 6, 7, 8	5 (0.68)	5	8

Hkw = hookworm; Sch = schistosomiasis; SI = serological index; Str = strongyloidiasis.

* Patient #6 was Italian and most likely acquired *M. perstans* infection in the Democratic Republic of the Congo.

† Cutoff for eosinophilia $\geq 450/\mu\text{L}$.

‡ Tn = follow-up at n months after first treatment (T0).

§ ELISA SI cutoff value ≥ 1 .

RESULTS

In 2015–2017, our laboratory screened 1,170 migrants, expatriates, and travelers using the antifilarial antibody ELISA test (Figure 1); 149 (12.7%) resulted positive. Of these, 93 (62.4%) individuals who attended a follow-up visit after the

screening were investigated by modified Knott test; mf were found in 29 (31.2%) ($n = 24$ *M. perstans*; $n = 4$ *L. loa*; $n = 1$ mixed *M. perstans* + *L. loa*). Of these microfilaremic individuals, follow-up visits after the first treatment were attended by 22 patients: 17 with confirmed *M. perstans*, the four patients with *L. loa*, and the patient with mixed *L. loa* and *M. perstans*

FIGURE 2. Trends of microfilaremia (mf/μL) over time of the 22 patients included in this study. This figure appears in color at www.ajtmh.org.

FIGURE 3. Trends of antifilarial antibody ELISA serological index (SI) over time of the 22 patients included in this study. The thick black line shows ELISA cutoff for positivity (≥ 1). This figure appears in color at www.ajtmh.org.

infection. Demographic characteristics, laboratory parameters at diagnosis, treatments effectuated, and change in laboratory parameters over the follow-up of patients are summarized in Table 1.

At diagnosis, all but one of the patients were young male migrants from sub-Saharan Africa, aged 18–35 years (median 23.5 years). Among migrants, the country of origin of patients with *L. loa* was Cameroon ($n = 2$) and Nigeria ($n = 2$); the patient with mixed *L. loa*–*M. perstans* infection was from Cameroon; patients with *M. perstans* infection came from Guinea Bissau ($n = 5$), Senegal ($n = 5$), Côte d'Ivoire ($n = 3$), and Ghana, Guinea, and Togo ($n = 1$ each). The only Italian patient of the cohort was a male expatriate aged 55 years who most likely acquired infection with *M. perstans* in the Democratic Republic of the Congo. Fourteen patients were also affected by one or more coinfections, including hookworm infection, schistosomiasis, strongyloidiasis, hepatitis B, and HIV. No patient with loiasis had/reported having had specific signs of loiasis such as Calabar swelling or “eye worm” before diagnosis or during the follow-up.

At diagnosis, antifilarial SI ranged from 1.22 to 5.30 (median 2.36); 19 (86%) patients had eosinophilia (range 490–9,400 eosinophils/ μ L, median 900 eosinophils/ μ L in patients with eosinophilia). Microfilarial counts before treatment ranged from 0.14 to 789 mf/mL blood (median 6.21 mf/mL) for *M. perstans*, and from 160 to 9,800 mf/mL blood (median 491 mf/mL) for *L. loa*. Three patients with *M. perstans* infection were coinfecting with *Strongyloides stercoralis*, and hence received a single dose of 200 mcg/kg of ivermectin. Ten patients were re-treated one or more times after the first treatment, for a total of

18 re-treatments, in all cases within the first 6 months from the first treatment. Antibody SIs were tested within the 2 months after (re)-treatment on 20 occasions, in 11 patients. In nine (45%) of these occasions, an increase in antibody SI was observed.

Figures 2–4 show the trend in each patient of microfilarial counts, antifilarial antibodies, and eosinophils counts from baseline values pretreatment to the last follow-up, respectively. Microfilarial counts decreased sharply in all patients within the first 1–2 months after treatment, with all patients having achieved amicrofilaremia at last follow-up (Figure 2 and Table 1). An overall trend toward antibody SI decrease was observed in all patients over the follow-up period, with five (23%) patients having reached sero-reversion ($n = 5$; 23%) or near-cutoff SI values ($n = 3$; 14%) within 20 months from first treatment (Figure 3 and Table 1). As shown in Figure 4 and Table 1, eosinophil counts followed the trend of antibody SI but achieved normalization in shorter times.

DISCUSSION

In our cohort, the trend over time of eosinophil counts and antibody SI were similar to those observed in the study by Klion and colleagues in *L. loa*-infected patients.¹¹ In the latter, the authors examined patients treated with diethylcarbamazine, a drug known to be only partially macrofilaricidal.²³ Eosinophil counts were reported to decrease sharply in the first 6 months after treatment, and to remain low or normal also in patients with relapses; conversely, their increase at 6 months was not always predictive of relapse. A similar behavior was observed for antifilarial IgG titers detected by an in-house ELISA based on

FIGURE 4. Trends of eosinophil counts (eosinophils/ μ L) over time of the 22 patients included in this study. The thick black line shows cutoff for eosinophilia (≥ 450 eosinophils/ μ L). This figure appears in color at www.ajtmh.org.

Brugia malayi adult extract, which declined rapidly between 3 and 12 months posttreatment and either remained low/negative or increased again transiently at 3 months independently of cure or relapse outcome. In that study, relapse was defined as reappearance of symptoms, whereas asymptomatic patients after treatment with persistently high/increasing eosinophil counts and/or antifilarial antibodies were considered “uncertain” and excluded from the analysis. In our study, no appearance of specific symptoms and no new

or increasing mf/mL, eosinophil counts, or antibody SI clearly indicating relapse were observed, likely due to the adoption, in our center, of a treatment schedule with plausible high macrofilaricidal effect.^{1,24,25}

When re-treatment was decided for patients in our cohort, a posttreatment rise in eosinophil counts and antibody SI was observed in some but not all occasions. Interestingly, in two patients (#7 and #10), a rise in these laboratory parameters could be observed after one re-treatment (at T2 and T15,

FIGURE 5. Trends of antifilarial antibody ELISA serological index (SI) and eosinophil counts (eosinophils/ μ L) over time of patient #7 (A) and patient #10 (B). Arrows indicate the time of treatment: first treatment was mebendazole 500 mg TID for 14 (patient #7) or 28 (patient #10) days followed by doxycycline 100 mg BID for 45 days; the following re-treatments were with mebendazole 500 mg TID for 14 days.

respectively), but not after previous courses. Figure 5A and B shows the particular cases of patients #7 and #10, respectively. It can be speculated that the rise in immune-related parameters may have been stimulated by killing of residual adult worms; hence, it could have been an indirect sign of effectiveness of treatment. The interval (and its variability) between treatment administration and macrofilarial death remains unclear. Therefore, whether additional re-treatments were required for complete clearance of parasites or whether initial damage caused by first course of therapy could have been enough to induce parasitological cure over time is not clear. In the latter scenario, patients will have received unnecessary additional treatments when their infection was eradicated with the first course. This hypothesis would fit with data from Klion et al.,¹¹ reporting increase in laboratory parameters in patients fulfilling criteria for cure, and with the results of those patients in our cohort, who did not receive further re-treatments but nevertheless achieved amicrofilariaemia, normalization of eosinophil counts, and negativity (or near-negativity) of antibody SI. A delay between treatment end and adult death can be deducted for *L. loa* from results of Duke,²³ and is known after doxycycline treatment for *Onchocerca volvulus* and *Wuchereria bancrofti*.²⁶ The retrospective design and heterogeneity of treatment and follow-up time points of individual patients, two limitations of our study, prevent answering this important question and encourage the design and implementation of prospective clinical trials to address it.

Another question of pivotal practical importance concerns the required minimum length of follow-up of treated patients to reasonably declare cure, which is crucial when dealing with non-sedentary populations, such as our patients. It cannot be excluded that late relapses were not detected in our cohort because of a very short follow-up time. However, our results suggest that a 12- to 18-month observation period should be enough to evaluate plausible cure. This is also supported by the results of Klion et al.,¹¹ who observed that 70% of relapses occur within the first year of follow-up, and by the generally rather fast decline even of antibody titers to levels below or close to cutoff for positivity over time. Until the dynamics of parasite death are clarified, as discussed previously, our data suggest that the absence of early (1–2 months after treatment) rise in eosinophil counts or antibody titers cannot be reliably used as a sign of complete parasitological cure in patients re-treated because of persistence of circulating mf. Also, caution must be taken in interpreting high eosinophil counts and antibody titers in the months after treatment, as these may reflect adult parasite death and therefore no need for further treatment rather than the opposite. In general, the presence of microfilariaemia cannot be used as a sure sign of residual presence of adult worms after treatment, as mf may persist alive in the circulation for several months.² Longer intervals between treatment and follow-up of laboratory parameters may be warranted before re-treatment is implemented.

Finally, with the serological test used in our center, we observed a fast decrease in antibody SI fast after treatment, with sero-reversion or near sero-reversion in almost half of patients within a relatively short follow-up time. This observation may support the hypothesis that presence of clearly positive antibody titers in patients without microfilariaemia are not just residual from a past infection, and therefore may be effectively used to screen migrants/travelers from endemic areas to

diagnose filarial infections. Also, this may be particularly useful when microfilariaemia is negative or cannot be checked.

In conclusion, we describe the change over time of antifilarial antibodies in a retrospective cohort of patients with microscopically confirmed *L. loa* and, for the first time, *M. perstans* infections in a single Italian referral center for tropical diseases and followed up using parameters from a single laboratory and a single commercial antifilarial antibody test. Our results suggest that the continuous descending trend in antifilarial antibody titer parallels a similar, although faster, trend in eosinophil counts, and could be, therefore, used as an additional laboratory parameter for the diagnosis and monitoring of patients. Prospective multi-center studies with a standardized therapy and follow-up schedule are now needed to shed light on the dynamics of adult worm death after treatment, to safely shorten the follow-up of patients for whom long-term observation is not feasible.

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