



Influence of *Mansonella perstans* microfilaraemia on total IgE levels in Gabonese patients co-infected with *Loa loa*



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ABSTRACT

Mansonella (M.) perstans filariasis is widely found in Africa, including Gabon where *Loa loa* is also endemic. This study reports the total IgE titres according to different bioclinical forms of single or co-infection with *L. loa* and *M. perstans* in 138 patients and 20 healthy controls. The median parasite density was significantly higher in cases of loiasis. IgE titres were higher in patients with microscopic dual-infection and in the group of patients with occult loiasis plus *M. perstans* microfilaraemia (8425 [5292–20,679] KUI/L and 6304 [1045–10,326] KUI/L, respectively), compared to individuals with either microfilaraemic *Loa loa* (3368 [1414–7074] KUI/L) or *Mansonella* (4370 [1478–7334] KUI/L) single infections ($p < 0.01$). IgE levels were positively correlated with *M. perstans* microfilaraemia ($\rho = 0.27$; $p < 0.01$). Compared to single infections, dual *M. perstans*–*L. loa* infection induces very high total IgE titres. Studies correlating IgE titres and clinical symptoms are needed to confirm the involvement of this immunoglobulin in the pathological processes during filariasis.

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1. Introduction

Loiasis, a neglected tropical disease, remains a public health problem and an obstacle for onchocerciasis and lymphatic filariasis control in co-endemic areas. The clinical manifestations of *Loa loa* infection are commonly characterized by acute allergic reactions caused by excreted antigenic substances of migrating adult filaria or by newly released embryos in the dermis (Pinder et al., 1992). *L. loa* is also responsible for general pruritus, subconjunctival migration of adult worms and Calabar swellings (Boussinesq, 2006). The geographic distribution of *L. loa* is limited to the central African rain forest block, where nearly 14.4 million people are exposed to the risk of infection (Zouré et al., 2011).

Mansonella perstans is endemic in 33 countries of sub-Saharan Africa where more than 100 million individuals are estimated to be infected (Simonsen et al., 2011). Formerly considered to be non-pathogenic, *M. perstans* is now suspected to be responsible for significant clinical symptoms, including allergic reactions, such as acute swelling and itching with or without rash or urticaria. This infection also induces hypereosinophilia (Fux et al., 2006; Bregani et al., 2006).

Although the pathophysiology of clinical symptoms due to helminth infections is not clearly understood, immune mechanisms are supposed to be the primary cause. Helminths induce a type 2 immune response characterized by eosinophilia, high antigen specificity and high polyclonal IgE levels. In fact, filaria-infected patients' pathological profiles are reflected by their immunoglobulin response (Nutmans et al., 1984; Klion et al., 1991); and IgG4 and IgE are considered markers of immune response in patients suffering from filariasis. Antigen-specific IgE production has been also shown to be associated with allergic reactions and parasite clearance and to parallel total IgE synthesis (Nutman et al., 1984; Blackwell et al., 2011). As opposed to IgG4, IgE levels are high during loiasis infections, and their levels were shown to drop after treatment (Mitre and Nutman, 2006; Johnson et al., 1998). In addition, a relationship between high IgE titres and patent infection, parasitaemia and clinical expression was recently reported elsewhere (Arndts et al., 2012).

In areas such as Gabon where *L. loa* and *M. perstans* are co-endemic, studies on filaria-induced immune response often focused on the *L. loa* species, although dual infection is not uncommon (Kombila and Richard-Lenoble, 1998; Akue et al., 2011). It was recently reported that *M. perstans* infection is associated with an increase of *Wuchereria bancrofti* microfilaraemia during co-infection, and a reduction of anaemia and pro-inflammatory response during clinical malaria (Uttah and Ibeh, 2011; Dolo et al., 2012). Furthermore, it has also been suggested that immune

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regulatory responses are modified during *Mansonella* infection (Simonsen et al., 2011). Therefore, clarifying the clinical and immunological significance of dual infection will lead to a better understanding of the pathophysiology issue. Examination of the range of IgE levels during single or concomitant infection may help to clarify this issue, as well as to establish the usefulness of IgE levels in identifying patients with dual infections, and to evaluate post-treatment strategies to cure persistent infections.

The present study aimed to explore the magnitude of the immune response, specifically the polyclonal IgE titres, during *L. loa* and *M. perstans* single or dual infections among symptomatic patients in Gabon.

2. Patients and methods

2.1. Study population

In the Department of Parasitology Mycology (DPM) at the Faculty of Medicine of Libreville, among the sixty patients seen weekly, approximately one half undergo a biological testing due to the presence of clinical signs suggestive of filariasis. Almost one-third of these patients are infected, they receive treatment and they are followed-up from three to 12 months until biological and clinical recovery is achieved.

A subset of patients seen at the DPM between June and July 2008 who presented with symptoms suggestive of loiasis and who were diagnosed with *L. loa* and/or *M. perstans* infections were selected for this study. Criteria for enrolment included the following: living in an onchocerciasis non-endemic focus, having an available serum sample and recorded age and sex, having the results of all the parasitological tests. Samples from patients with the following criteria were excluded: living in the well-defined onchocerciasis endemic area (Richard-Lenoble et al., 1980; Kombila and Richard-Lenoble, 1998), presenting suggestive symptoms of onchocerciasis, having any other helminth infection (nematodes, trematodes) detected in stools or urine. Clinical symptoms were classified as objective (i.e., Calabar swelling, adult worm subconjunctival and subcutaneous migration) or subjective (pruritus, urticarial and pricking sensations). Twenty individuals from Libreville, without helminth or malarial infections were matched by age and sex to serve as a control group.

2.2. Laboratory examinations

Seven millilitres of venous blood were collected between 10:00 am and 2:00 pm prior to the administration of treatment. A volume of 4 mL was stored in a tube containing EDTA, 20 µL served for microscopic examination and the remaining blood was stored in a dry tube. Blood samples were processed within four hours. After centrifugation of the blood kept in the dry tube, the serum was conserved at -20°C for *L. loa* serological diagnosis and total IgE measurements.

2.2.1. Microfilaria detection and quantification

The detection of microfilaria was performed using two different methods with increasing sensitivities to prevent false negative results in the case of low microfilaraemia. The first step for microfilaria detection and quantification was the direct microscopic examination of ten microlitres of fresh, uncoagulated blood. All of the microfilariae present in the slide were identified and counted. Parasitaemia was expressed as the number of microfilaria per millilitre (mf/mL) of blood. The limit of microfilaria detection with this method is 100 mf/mL. Then, the leucoconcentration was performed following a standard protocol (Ho et al., 1963). This technique has a higher sensitivity compared to the direct blood examination. Because the *M. perstans* parasite density is often

low, the leucoconcentration was performed for all of the patients, regardless of their *L. loa* microfilaraemia. The principle of leucoconcentration involves the lysis of red blood cells with saponin to obtain a concentration of leukocytes and microfilaria in a pellet. Briefly, 4 mL of blood collected into the EDTA tube was centrifuged. The plasma was removed, then two mL of 0.9% NaCl and 1 mL of 2% saponin were added. The reagents were mixed with the pellet and the solution was centrifuged during 10 min. The supernatant was removed, and the same operation was repeated twice with the pellet and 2 mL NaCl plus 1 mL saponin. After a final centrifugation, the pellet containing the leukocytes and microfilaria was collected and examined microscopically. Parasitic species were identified according to their size, motility, presence of a sheath and shape, and they were quantified as described above.

2.2.2. Eosinophil count

This was performed in 10 µL of blood spread on slides consecutively stained with Giemsa. The percentage of eosinophils was calculated, taking into account 100 white blood cells (WBC). The normal mean of the WBC count in the Gabonese population is generally equal to or lower than 5000/µL; the normal eosinophil count, between 0% and 10% of the WBC; and the normal absolute eosinophil count, below 500/µL of blood. Thus, hypereosinophilia was defined as an eosinophil count higher than 10% of the WBC.

2.2.3. Serological diagnosis

L. loa specific antibodies were detected in the sera from patients by indirect immunofluorescence (IIF) using homologous antigens of *L. loa* microfilaria prepared in the DPM, as described elsewhere (Walker-Deemin et al., 2004). The antibody – *L. loa* specificity threshold was a titre higher than 1:200.

2.2.4. Serum total IgE determination

The IgE titres in the patients' sera were measured by an immunoenzymatic sandwich ELISA using an automat, the Minividas® Biomerieux. The normal value was less than or equal to 150 KU/L.

2.3. Ethical considerations

Apart from the total IgE measurements, all of the tests performed were part of the routine diagnosis of filarial infection in the DPM. IgE measurements did not require any additional sampling or intervention. Oral informed consent for the use of data and total IgE measurements was therefore obtained from each participant after the study had been explained.

2.4. Statistical analysis

Data were double entered in Excel, and Statview 5.0 software (SAS Institute, Cary, NC, USA) was used for the descriptive and comparative analysis. A control of data entry was performed before the statistical analysis. Medians with interquartile ranges [IQR] for both non-normally distributed and means ($\pm$ standard deviation, SD) of normally distributed continuous variables were calculated, and parasite densities were assessed among the microscopically infected patients only. Continuous variables were compared using the Mann–Whitney *U* test or the Kruskal–Wallis test. The Spearman Rank test allowed the analysis of correlations. Differences between the proportions were tested with the Chi-square or Fischer's exact tests.

According to the parasitological test results (i.e., microfilaraemia), the presence of *L. loa* specific antibodies and the clinical symptoms, the population was divided into six subgroups: ML, only *L. loa* microfilaria detected; OL, patients with occult loiasis (clinical symptoms, absence of *L. loa* microfilaria and presence of *L. loa* specific antibodies, IFI $\geq$ 1:200); MP, presence of *M. perstans*

Table 1

Distribution of the population characteristics according to the detected filaria.

	Patient group						Control group
	ML	OL	MP	MLMP	OLMP	All	
Number (% of all)	26 (18.8)	27 (19.6)	49 (35.5)	18 (13.0)	18 (13.0)	138 (100.0)	20
Females, n (% in group)	14 (53.8)	16 (59.3)	28 (57.1)	9 (50.0)	12 (66.7)	79 (57.2)	11 (55.0)
Age in years ($\pm$ SD)	45 ($\pm$ 14)	40 ($\pm$ 14)	49 ($\pm$ 14)	52 ($\pm$ 19)	52 ($\pm$ 26)	46 ($\pm$ 16)	42 ($\pm$ 16)
Objective symptoms, n (% in group)	10 (38.5)	20 (74.1)	15 (30.6)	11 (61.1)	8 (44.4)	64 (46.4)	0 (0.0)
Eosinophils, ^a median (IQR)	18 (13–24)	27 (24–37)	11 (4–21)	20 (12–29)	25 (20–40)	20 (11–28)	3 (2–3)
<i>L. loa</i> antibody positive, n (% in group)	4 (15.4)	27 (100.0)	0 (0.0)	3 (16.7)	18 (100.0)	52 (37.7)	0 (0.0)

^a Per 100 WBC.

microfilaria without any loiasis infection; MLMP, detection of *L. loa* microfilaria plus *M. perstans* microfilaria; OLMP, specific dual infection involving occult loiasis plus the presence of *M. perstans* microfilaraemia; and the control group.

3. Results

3.1. Characteristics of the population

The total number of screened patients during the study period was 232. Of these, 138 met the inclusion criteria. Complete data were available for 79 (57%) females and 59 males. The age varied from seven to 81 years old, with a mean age of 46 ($\pm$ 16). The control group was comparable to the infected group in both age [42 ($\pm$ 16) years] and sex distributions (55% female).

3.2. Types of filarial infection

Among the 138 infected patients, 53 (38%) were infected with *L. loa* only (26 ML and 27 OL), while 49 (36%) had a single *M. perstans* infection. One-quarter of the patients ($n = 36/138$; 26.1%) had a dual infection (Table 1). A comparison of the six subgroups according to sex and age showed that patients infected with *M. perstans* were significantly older than patients infected with loiasis or the control group (50 versus 43 and 42 years, respectively) ($p = 0.03$).

Objective symptoms (89% represented by Calabar swelling) were found in 64 infected patients, occurring more frequently among the OL (74.1%) and the MLMP (61.1%) compared to the OLMP (44.4%), the ML (38.5%) and the MP (30.6%) groups ($p < 0.01$) (Table 1). Subjective symptoms were observed in 87 patients, among whom 30 (34%) also presented objective symptoms. The main subjective manifestation was a general pruritus ($n = 73$; 84%). Subjective symptoms were also more frequently presented by patients with either single MP infections or MLMP dual infections, compared to the other groups ($p < 0.01$).

Among the 26 infected patients with *L. loa* microfilaraemia, the median [IQR] parasitaemia was 9500 [3000–30,000]/mL. There was a positive correlation between parasite density and age: $\rho = 0.26$ ($p = 0.02$). The parasitaemia among patients infected with *M. perstans* was low (25 [8–68]/mL) and was not correlated with age ($\rho = 0.12$; $p = 0.3$).

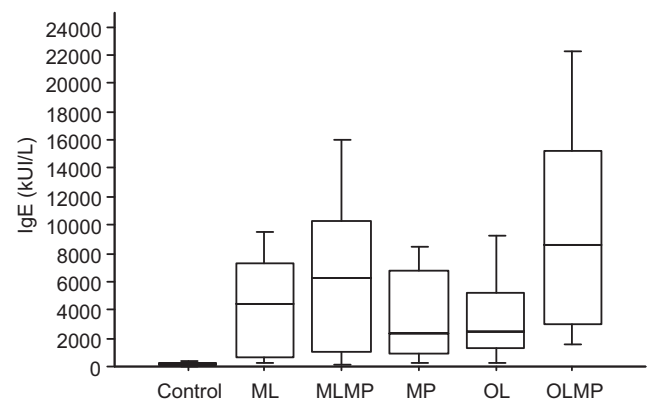
L. loa specific antibodies were detected in 52 infected patients: all the OLMP and OL patients, 4 ML patients and 3 MLMP patients had specific antibodies (Table 1). Values varied from 1:400 to 1:1600: two patients from the OLMP group had a serological index of 1:1600; an index of 1:800 was observed in 15 patients (7 OL and 8 OLMP); and the others had an index of 1:400. Specific reactivity to *L. loa* antigens was not detected among the individuals from the control group, nor in the MP group.

3.3. Hyperesinophilia

The median eosinophil rate was the lowest in the control group and the highest in patients with occult loiasis, either as single infection or associated with *M. perstans* infection (Table 1). In the control group, no individual had a high eosinophil count, whereas 100% of those from the OLMP group and 81 to 96% of the patients with other types of loiasis infection had hyperesinophilia. Half ($n = 24$; 49%) of the patients with *M. perstans* single infection had eosinophil counts higher than 10%. However, eosinophil counts were not correlated with microfilaria density, with a ρ of 0.15 for MP and 0.01 for ML.

3.4. Total serum IgE

The median total IgE titre was the lowest in the control group (154 [67–264] KUI/L) and the highest in the OLMP (8425 [5292–20,679] KUI/L) ($p < 0.01$). Overall, there was no overlap between IgE levels in the control group and in any of the infected groups. Co-infected patients had higher IgE levels compared to single infected ones ($p < 0.01$) (Fig. 1). Titres were comparable among the ML patients (4370 [1478–7334] KUI/L), the MP group (3368 [1414–7074] KUI/L) and the OL group (2461 [1247–5178] KUI/L) ($p = 0.53$). Median IgE level was higher in the OLMP group compared to the MLMP group (6304 [1045–10,362] KUI/L) ($p = 0.01$). IgE levels were positively correlated with parasite densities in the MP subgroup alone ($\rho = 0.27$; $p < 0.01$).



Legend

Control: Control group.

ML: only *Loa loa* microfilariaMLMP: *Loa loa* microfilaria associated with *M. perstans* microfilariaMP: only *M. perstans* microfilaria

OL: occult loiasis

OLMP: occult loiasis associated with *M. perstans* microfilaria

Fig. 1. IgE levels, according to the type of filarial infection. Control, control group; ML, only *Loa loa* microfilaria; MLMP, *Loa loa* microfilaria associated with *M. perstans* microfilaria; MP, only *M. perstans* microfilaria; OL, occult loiasis; OLMP, occult loiasis associated with *M. perstans* microfilaria.

There was a positive correlation between the IgE titre and eosinophil count: $\rho = 0.48$ ($p < 0.01$). Indeed, 96% ($n = 102/106$) of patients with hypereosinophilia had a high IgE titre. The median IgE level was higher in patients presenting objective symptoms (4369 [1692–8784] KUI/L) compared to patients with subjective symptoms (2531 [815–6838] KUI/L) ($p < 0.01$).

4. Discussion

M. perstans and *L. loa* are endemic in several countries. These species often coexist in areas where onchocerciasis or lymphatic filariasis are also endemic. Onchocerciasis and lymphatic filariasis control with mass ivermectin treatment increases the risks of complication in cases of co-infection with *L. loa* (Uttah and Ibeh, 2011). So, identifying ways to interrupt filaria transmission is a priority. In Gabon, co-infection with *M. perstans* and of *L. loa* is frequent (Kombila and Richard-Lenoble, 1998; Richard-Lenoble et al., 1988). The treatment of single or concomitantly infected patients, although leading to parasite clearance, is not always followed by the disappearance of unpleasant or troublesome clinical symptoms. Highly specific methods able to detect low microfilaraemia levels exist, but they are expensive and not easily affordable in endemic areas (Burbelo et al., 2008; Fink et al., 2011). Most of the infected patients live in rural areas where molecular methodologies and ELISA cannot be performed. Specific immunological markers will be helpful for the identification of dual infected patients, at least by correlating the presence of clinical symptoms with that of microfilaria, especially when parasite densities are below the threshold for detection by microscopy.

This study is the first reporting the total IgE titres according to different bioclinical forms of single or co-infection with *L. loa* or/and *M. perstans*. Prior studies have evaluated IgE levels in filaria-infected individuals, even in Gabon (Richard-Lenoble et al., 2010). However, they have all focused on *L. loa*. None of these studied IgE titres in *M. perstans* patients with dual infections, in clinically well-defined groups, or took into account infected patients with occult loiasis, a clinical status frequent in our settings.

The contribution of *M. perstans* to the disabilities observed in parasitized patients is difficult to determine because of the high prevalence of dual infections and the lack of specificity in many clinical symptoms. However, all patients included in this analysis were symptomatic. *M. perstans* was the single filaria identified in 36% of them, suggesting that mansonellosis is associated with the occurrence of subjective symptoms such as pruritus and pricking sensation.

As previously reported, the total IgE levels were elevated among infected patients, compared to the control group (Akue et al., 1996; Arndts et al., 2012). These unusual levels are due to both filaria species. Indeed, none of the patients had other helminthiasis detected by microscopy. Helminth infections with low parasite density can be excluded because self-treatment with one or three days of albendazole is common in the country. This short treatment does not clear filarial infections, although it is sufficient to clear nematodes such as *Ascaris lumbricoides* or *Trichuris trichiura* which induce high IgE responses (Cooper et al., 2008; Hagel et al., 2008). The present results also confirm a significant total IgE response in the case of MP mono-infection, like reported 16 years ago (Akue et al., 1996).

Higher IgE titres are observed in dual-infected patients compared to patients with a single *L. loa* or *M. perstans* infection. This difference suggests an important hyper-responsiveness during co-infection. The positive correlation between IgE titres and *M. perstans* microfilaria density ($\rho = 0.27$) also supports this hypothesis. Considering that immunoglobulin responses reflect the clinical profiles of filarial-infected patients, in the presence of the two parasites, due to the high IgE titres, allergic symptoms would be

more pronounced and persistent. The smallest IgE titres found in the MP and OL groups compared to the others can be explained by the low microfilaraemia in MP patients and the amicrofilaraemic status in the OL group. In both cases, IgG4 levels are known to be high, with a low ratio of IgG4/IgE, as IgG4 counter-regulates high IgE levels (Arndts et al., 2012; Garraud et al., 1996; Kurniawan et al., 1993). Furthermore, if IgG4 levels are elevated in asymptomatic microfilaraemic patients and lowered in those with significant symptoms, IgE levels should be opposed to those of IgG4 in both situations (Kurniawan et al., 1993; Akue et al., 1994).

IgE titres are known to drop after anti-filarial treatment and are associated with the presence of clinical symptoms. This Ig isotype could therefore be used as marker of drug efficacy in *L. loa* and/or *M. perstans* treated patients who still present clinical symptoms without detected microscopic parasitaemia during their post-treatment follow up (Nutman et al., 1984; Blackwell et al., 2011). Thus, a decrease of these titres after treatment would be expected with the disappearance of filariasis specific symptoms and the decrease or negativity of microfilaria density. Longitudinal studies are needed to clarify the kinetic profiles of IgE titres in patients with different bioclinical forms of these filariasis.

Since IgE antibodies influence the host immune response and immunopathology during filariasis, it can be suggested that, during multiple filarial infections, the shift towards a Th2 response may be more accentuated after the raise of IgE antibody response (Bakiri and Mingomataj, 2010). Thereby, the duration of clinical symptoms in dual-infected patients and the risk of modulating other pathogen disease courses may be modified. This study reveals that *M. perstans* infection has an influence on the immune response against loiasis like observed during *M. perstans* and *W. bancrofti* dual infections (Uttah and Ibeh, 2011). It has been suggested that *Mansonella* infection can influence the regulatory responses of infected individuals, and thereby interfere with the outcome of other endemic infections, such as HIV and malaria, in areas where they coexist (Simonsen et al., 2011). The possible association of the two filaria with anaemia in a malaria-endemic area was also recently reported (Dolo et al., 2012).

O. volvulus infection and lymphatic filariasis can be confounders against the correct interpretation of IgE titres. However, none of the included patients came from *O. volvulus* foci, which have been well identified in the country for more than 20 years, and no patients had onchocerciasis objective symptoms (Richard-Lenoble et al., 1980; Kombila and Richard-Lenoble, 1998). Over the past few years, less than 1% of the patients consulted at the DPM have been infected with *O. volvulus*. Moreover, the serological method used for *L. loa* antibody detection is specific when a threshold of 1:200 is used for positivity (Walker-Deemin et al., 2004). *W. bancrofti* parasites have never been found in Gabon, neither during the prospective surveys performed across the whole country from the 1980s to the 1990s, nor during the last survey, which was taken two years ago in the areas of the country that were supposed to be endemic by some authors. During this survey, parasitological examinations and immunological tests were performed on 852 individuals (unpublished data).

The major limitation of this work was the lack of antigen-specific IgE tests. Nevertheless, the associations found between total IgE titres and the different forms of filariasis are convincing and statistically significant. Moreover, the total IgE parallels the specific IgE response (Nutman et al., 1986; Kurniawan et al., 1993; Blackwell et al., 2011).

5. Conclusion

Even though IgG1 and IgG4 are considered to be constant markers of loiasis infection, the present results highlight a potential role

of IgE as a marker of the patient's immune response during *M. perstans* and loiasis infections and, most likely, after the disappearance of parasites in the blood. These filariases often coexist; thus, it is necessary to perform further studies on clinical and biological interactions, concomitantly with specific immune responses in single- and dual-infected patients. Indeed, chronic filarial infections are responsible for immune modulation and the alteration of the host's response towards intracellular pathogens, such as *Plasmodium* parasites and *Mycobacterium tuberculosis*; these pathogens are co-endemic in Gabon and may also coexist with *M. perstans* and *L. loa* parasitaemia (Metenou et al., 2012; Li and Zhou, 2013). Efforts must be made to identify non-expensive sensitive methods to aid for the diagnosis and classification of infected patients (even at low parasite density), to evaluate the treatment efficacy, as well as to understand the immune interactions between multiple filarial infections and other pathogens. Measurements of total or specific IgE levels could help elucidate this issue.

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