Relevance of the eosinophil blood count in bancroftian filariasis as a screening tool for the treatment

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Backgroud: Lymphatic filariasis constitutes a major public health issue in French Polynesia. Diagnosis has been revolutionized with the availability of circulating filarial antigen (CFA) tests which are easy to perform but are costly. Filariasis is responsible for acquired eosinophilia and eosinophil blood count (EBC) is commonly used as a screening tool.

Methods: We retrospectively analysed all the results of EBCs and CFA tests performed in our laboratory over a 2-year period. We calculated the prevalence of antigenemia for nine different eosinophil cutoffs. We calculated the number of patients detected by CFA testing and the number of estimated CFA-positive patients according to their EBC.

Results: Over a 2-year period, we detected 7503 eosinophilic patients. For an EBC above 500/mm^3, the prevalence of positive CFA was 25.78% and the estimated number of positive CFA patients was 1934. During the same period, as CFA determination is not routinely performed, only 141 patients were detected and treated.

Conclusion: Our current strategy against lymphatic filariasis which combines annual mass drug administration, systematic treatment of antigenemic and microfilaraemic patients, and vector control; failed to reach the target of 1% prevalence. Unfortunately, mainly for economical reasons, the antigenemia cannot be determined for all patients. In complement to existing strategy, we propose an additional action based on the treatment of eosinophilic patients in order to reduce the filariasis prevalence in our country.

Keywords: Eosinophilia, Filariasis, Microfilariae, *Wuchereria bancrofti*

Introduction

Lymphatic filariasis (LF) is caused by three species of nematodes, *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*.[1] Infection is common in many areas of the tropics and subtropics and constitutes a major public health problem in several regions including the Pacific Island Countries (PICs). Infection often begins during childhood but clinical filariasis is mainly a disease of adults. LF is a major cause of disability, social stigmatization, and psychosocial and economic reductions in life opportunities. It is also a major burden on health and hospital resources. It may present as asymptomatic, acute, or chronic infections.[2] The majority of affected subjects show a clinically asymptomatic infection and harbour microfilariae or circulating filarial antigens (CFAs) in their peripheral blood. The adult worm lives within the human lymphatic system during about 4–6 years.[3] Fertilized females discharge microfilariae via the lymphatic system into the bloodstream.[4]

World Health Organization (WHO) considers LF to be a global health problem affecting over 120 million people in 73 countries in 2012.[5] At present, over 1.4 billion people are at risk of being infected. In 1993, the International Task Force of Disease Eradication declared LF one of six potentially eradicable diseases.[6] In 1999, the 'Pacific Programme to Eliminate Lymphatic Filariasis' (PacELF) was created with the goal of eliminating LF by 2010 in the PICs.[7] In 2000, the WHO established a global plan to eliminate LF: the 'Global Programme to Eliminate Lymphatic Filariasis in order to achieve the global goal of LF elimination as a public health problem by the year 2020'.[8] The programme consists of annual mass drug administration (MDA) using a two drugs combination to treat the entire at-risk population.[9]

Economic impact of LF is important. It has been estimated that filariasis in India costs over US$1 billion each year in lost productivity and it is now established that every dollar invested in filariasis

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control in China has produced more than US$15 benefits. Of the 81 countries considered LF endemic in 2000, approximately 22 million people have been protected from LF infection and disease with saving of US$24.2 billion.[10]

Microscopy remains the cornerstone of diagnosis laboratory testing for the diagnosis of LF. Microscopy is performed on thick and thin blood smears or buffy coat films stained with Giemsa.[11] Concentration using centrifugation or millipore membrane filters increases the sensitivity of light microscopy. The main problem is the labour-intensiveness of preparing and examining microscope slides. The sensitivity of microfilariae detection depends on the volume of blood sampled, the time of blood collection, and potential introduction of bias depending upon the skill and dedication of the microscopist.[12] Unfortunately, microfilariae are frequently absent from the blood during both the early and late stages of the disease. Microscopy is not sensitive enough to identify many infections, especially those of low density and those where adult worms are present but produce no microfilariae. Serological testing is not specific[13] and not sensitive enough.[14] It does not differentiate between past and current infection. Real-time and conventional polymerase chain reaction have been developed for the detection of *W. bancrofti* in blood, but they are not routinely performed.[15]

The diagnosis of *W. bancrofti* has been revolutionized with the availability of CFA tests.[16,17] CFA tests are more sensitive than thick smear or membrane filtration and are not dependent upon the presence of microfilariae. Moreover, CFA tests rely on blood that can be drawn at any time. Its simplicity of use, high sensitivity and specificity to *W. bancrofti*, and compatibility with finger-prick blood collected either during night or day are important advantages of CFA. The test also shows to be positive in early stages of the disease when the adult worm is alive but becomes negative once the worms are dead. CFA detection can be performed by immunochromatographic card test (ICT)[18] or Og4C3 enzyme-linked immunosorbent assay. Og4C3 enzyme-linked immunosorbent assay has been reported to be more sensitive than ICT;[19–21] however, ICT may be more appropriate in remote areas because of its ease of use. The major disadvantage of ICT is its cost. ICT is the main diagnosis tool used in the PICs particularly in community-wide surveys[22] according to the PacELF guidelines for stopping LF. Nevertheless, the CFA sensitivity is not 100%; consequently, caution should be applied when the tests are used for individual diagnosis.[23]

Eosinophils are multifunctional leukocytes with functions in innate immunity, inflammation, and other likely homeostatic host responses. Typically eosinophils in peripheral blood are $<500/mm^3$.[24,25] Worldwide, multicellular helminth parasites are most commonly associated with significant eosinophilia, followed by adverse reactions to medication, toxins, allergic disorders, idiopathic/autoimmune inflammatory conditions, and malignancies. Eosinophil blood count (EBC) is highest among parasites with a phase of development that involves migration through tissue including schistosomiasis, visceral toxocariasis, strongyloidiasis, filariasis, ancylostomiasis, fascioliasis, trichinellosis, and paragonimiasis.[26]

French Polynesia (FP) joined PacELF in 1999 and has carried out eight rounds of MDA between 2000 and 2007. In 2008, the overall prevalence of antigenemia in FP was 11.3%, and the target of <1% of prevalence of LF has not been attained.[27] The expert group (WHO, Louis Malarde Institute, and FP Directory of Health) recommended during his meeting in 2008 to change the distribution strategy. To ensure a better assessment of the distribution coverage, FP was asked to implement a new strategy based on directly observed treatment. This new strategy started in 2010. The observed drug coverages in 2010–2012 were 71, 87, and 84%, respectively among the target population. The next assessment survey will be conducted in sentinel sites and spot checks in late 2012.

A study was carried out to determine the predictive value of the EBC in the detection of CFA-positive patients and to determine if the systematic treatment of eosinophilic patients would be a valuable strategy in addition to the WHO recommendations.

Results from this study may be helpful to design in policy making to strengthen diagnosis and treatment components of the FP filariasis control strategy.

Materiel and Methods

Study population and data sources

The study took place in FP. Our laboratory is located in Papeete, Tahiti. We routinely perform blood tests for the inhabitants of the five archipelagos of FP. Historically our laboratory works in the field of endemic infectious diseases including filariasis, dengue, leptospirosis, leprosis, and tuberculosis. We work in collaboration with the 'Office of Health Surveillance' and the 'Prevention Programs Department' of the FP Directory of Health.

We retrospectively analysed all the results of blood counts and CFA tests performed in our laboratory over a 24-month period between January 2010 and December 2011, irrespective of clinical presentation of the patient and the diagnosis suspected. All blood tests were performed in accordance with a medical prescription.

CFA tests were performed when prescribed by physicians and were not related to an initial EBC

result. We extracted from our database all available EBC and ICT results. All results were anonymized before statistical analyses.

Blood collection

All blood samples were collected from venous blood obtained by standard venipuncture, stored at 8–10°C before testing, and preceded within 24 hours after reception.

Blood count

All blood counts were performed with an automated haematology analyser Sysmex XT-2000*i*. White blood cell differentiation and EBC were systematically determined.

Nine different EBC cutoff values were considered: 200, 300, 400, 500, 600, 700, 800, 900, and 1000/mm^3. For each cutoff, we analysed the number and the percentage of blood count performed.

CFA detection

CFA tests were done with the immunochromatographic BinaxNOW® filariasis test (Alere North America, Orlando, USA) (Inverness Medical), according to the manufacturer instructions. Results were reported as 'positive', 'negative', or 'indeterminate'. The results were recorded 10 minutes after specimen application to avoid false-positive reactions.[28]

Correlation between EBC and CFAs

When EBC and ICT results were available for the same patient, we reported for each cutoff the number of ICT tests performed and their results. For each cutoff, we calculated the percentage of positive CFA (calculated as the number of ICT-positive individuals divided by the number of individuals on whom ICT tests were performed). For the relationship between EBC and CFA, we considered only cases with the two results available on the same sample and cases with EBC result available within 15 days after CFA determination. In order to avoid bias due to prescription of CFA after eosinophilia detection, when CFAs were determined after detection of an elevated EBC, cases were not included.

Standard treatment procedure

According to PacELF recommendations, a single-dose, once-yearly MDA using a combination of albendazole (ALB, 400 mg) and diethylcarbamazine (DEC citrate, 6 mg/kg) is distributed to the target population (whole population except pregnant women, less than 2 years infants, and severely ill people including epileptic patients for whom a medical advice is recommended before swallowing the tablets). This MDA must be implemented during at least 5 years. Additional strategy consists of all CFA and microfilaremic-positive individuals' treatment. In those cases, the regimen is different with a single-dose treatment repeated every 3 months during 1 year.

Data analyses and statistical methods

For each eosinophil cutoff, we calculated the sensitivity, specificity, positive predictive value, and negative predictive value of EBC for the CFA positivity. Sensitivity and 1-specificity results were reported on a receiver operating characteristic (ROC) curve. We calculated the overall prevalence of positive CFA, and the prevalence for each EBC cutoff. We considered true positives as patients with EBC above 500/mm^3 and positive CFA test; false positives as patients with EBC above 500/mm^3 and negative CFA test; true negatives as patients with EBC under 500/mm^3 and negative CFA test; and false negatives as patients with EBC under 500/mm^3 and positive CFA test.

Estimate of the number of probable CFA-positive patients was calculated by multiplication of the number of blood counts performed by the prevalence of positive CFA for the cutoff. Sensitivity, specificity, positive predictive values, negative predictive values, and prevalence rates were determinate with contingency tables. Confidence intervals (CIs) were reported at 95% (probability of a type 1 error at 5%). ROC curve was used by plotting sensitivity and 1-specificity at various signals to cutoff value of eosinophils/mm^3 and using Microsoft Excel®. Prevalence rates were compared using Chi-square test.

Cost of CFA tests and antifilarial treatment

For cost analyses, we considered the ICT price in FP laboratories (including transportation, custom taxes, and laboratory labour). For the treatment price, we considered ALB and DEC prices when delivered in our public health structures.

Results

We performed 37 066 blood counts. Number and percentage of EBC for different cutoffs are reported in Table 1. The mean eosinophil count among all subjects was 365/mm^3 and the median was 265/mm^3, extreme values ranging from 0 to 24 161/mm^3. There were 7503 patients or 20.24% (CI: 19.84–20.66%) who had eosinophilia (EBC>500/mm^3).

We performed 1184 CFA tests. For 1071 tests, the EBC results were available. For those 1071 patients, ICT was positive for 141, indeterminate for 10, and negative for 920. The results of the patients with

Table 1 Number and percentages of blood count classified for different eosinophil cutoffs

Eosinophil cutoff	Total blood counts	% of blood counts
>1000	1777	4.79
>900	2253	6.08
>800	2969	8.01
>700	3945	10.64
>600	5380	14.51
>500	7503	20.24
>400	10 877	29.34
>300	16 103	43.44
>200	23 783	64.16

indeterminate ICT were excluded from the subsequent analyses, and then 1061 participant specimens had valid results for the two tests (Table 2). The overall prevalence of positive CFA was 13.29% (CI: 11.25–15.34%). This prevalence was not significantly different with the prevalence calculated at 11.3% in the study conducted in 2008 (Chi-square test; $P<0.05$).

Prevalence of positive CFA was calculated for the different cutoff values. This prevalence increases with the eosinophil counts. For EBC above 500/mm^3, the prevalence of positive CFA was 25.78% (CI: 21.21–30.35%).

The results of sensitivity, specificity, positive predictive value, and negative predictive value of EBC antigenemia positivity are reported in Table 3. Results of sensitivity and (1-specificity) are reported on an ROC curve (Fig. 1). The shape of the curve confirms the association between antigenemia and eosinophilia but it does not find an optimal threshold eosinophil count relative to antigenemia. The point on the ROC curve which is farthest from the line of equality (Youden index) is close to the value of 500 eosinophils/mm^3.

The number of estimated CFA-positive patients (CFA E) is reported in Table 4, for each eosinophil cutoff. We compared this value to the number of CFA-positive patients detected (CFA D). For example, above 500 eosinophil/mm^3, the prevalence of positive CFA was 25.78%, the estimated number of positive CFA for the 7503 patients was 1934 (CI: 1591–2277). We also detected only 91 positives CFA patients but within this group we estimated that 1843 (CI: 1500–2186) patients were CFA positives.

Cost of unitary ICT in FP laboratories is US$50. We have had discounted prices for purchasing generic ALB and DEC. A one-dose treatment with ALB 400 mg and DEC at 6 mg/kg for a 70-kg patient is 0.17 USD.

Table 2 Results of circulating filarial antigen detection*

Eosinophil cutoff	CFA pos	CFA neg	tot CFA	prev CFA pos
>1000	40	77	117	34.19
>900	46	102	148	31.08
>800	57	133	190	30.00
>700	69	170	239	28.87
>600	82	222	304	26.97
>500	91	262	353	25.78
>400	106	360	466	22.75
>300	120	486	606	19.80
>200	128	668	796	16.08
Total	141	920	1,061	13.29

Notes: CFA, circulating filarial antigen; pos, positive; neg, negative; tot, total; prev, prevalence.
*Table from: Musso D, Vialette V. Predictive value of the eosinophil count in the biological diagnosis of lymphatic filariasis in French Polynesia. Med Mal Infect. 2012;42:585–90. Elsevier Masson SAS.

We performed 1184 CFA tests for a total amount of US$59 200. The total amount used for treatment by ALB and DEC of the 7503 eosinophilic patients with our protocol would total US$5102.

Discussion

MDA is currently the main strategy recommended by WHO to lead to interruption of LF transmission. This is based on the evidence of the effectiveness of a single dose of DEC (6 mg/kg) in the clearance of microfilaraemia and sustaining this over a period of at least 1 year. The addition of albendazole (400 mg) enhances this effect on microfilarial clearance. All endemic PICs have commenced MDA with DEC and ALB, targeting their whole populations. Since 2000, 1.5 million people have been reached by MDA in the Pacific. In 2005, 500 000 people were infected in the PacELF community with a prevalence of 6.5%. In 2010, seven of the PICs that require MDA have reached their targets and stopped MDA (American Samoa, Cook Islands, Marshall Islands, Niue, Tonga, Vanuatu, and Wallis and Futuna). These PICs have been implementing active surveillance. Six PICs continued to deliver MDA in 2011 (Fiji, Kiribati, FP, Papua New Guinea, Samoa, and Tuvalu).

From 1993 to 1999, FP had completed 14 semiannual MDA with DEC alone and, from 2000, eight annual MDA programmes using ALB and DEC combination according to the WHO recommendations. MDA was not performed because of the nation-wide survey in 2008 and concurrent influenza and dengue fever epidemics in 2009.

Sera collected during our prevalence study in 2008 were from a random sample population. CFAs were determined by ICT. Over the 1180 sera tested, the overall prevalence of positive CFA was 11.3%, and prevalence was not significantly different according to the age of the population above 10 years old. The positive CFA prevalence estimated in the present study on a non-random sample population (13.29%)

Table 3 SS, SP, PPV, and NPV results*

Eosinophil cutoff	SS	SP	NPV	PPV
1000	0.28	0.92	0.89	0.34
900	0.33	0.89	0.90	0.31
800	0.40	0.86	0.90	0.30
700	0.49	0.82	0.91	0.29
600	0.58	0.76	0.92	0.27
500	0.65	0.72	0.93	0.26
400	0.75	0.61	0.94	0.23
300	0.85	0.47	0.95	0.20
200	0.91	0.27	0.95	0.16

Notes: SS, sensitivity; SP, specificity; NPV, negative predictive value; PPV, positive predictive value.
*Table from: Musso D, Vialette V. Predictive value of the eosinophil count in the biological diagnosis of lymphatic filariasis in French Polynesia. Med Mal Infect. 2012;42:585–90. Elsevier Masson SAS.

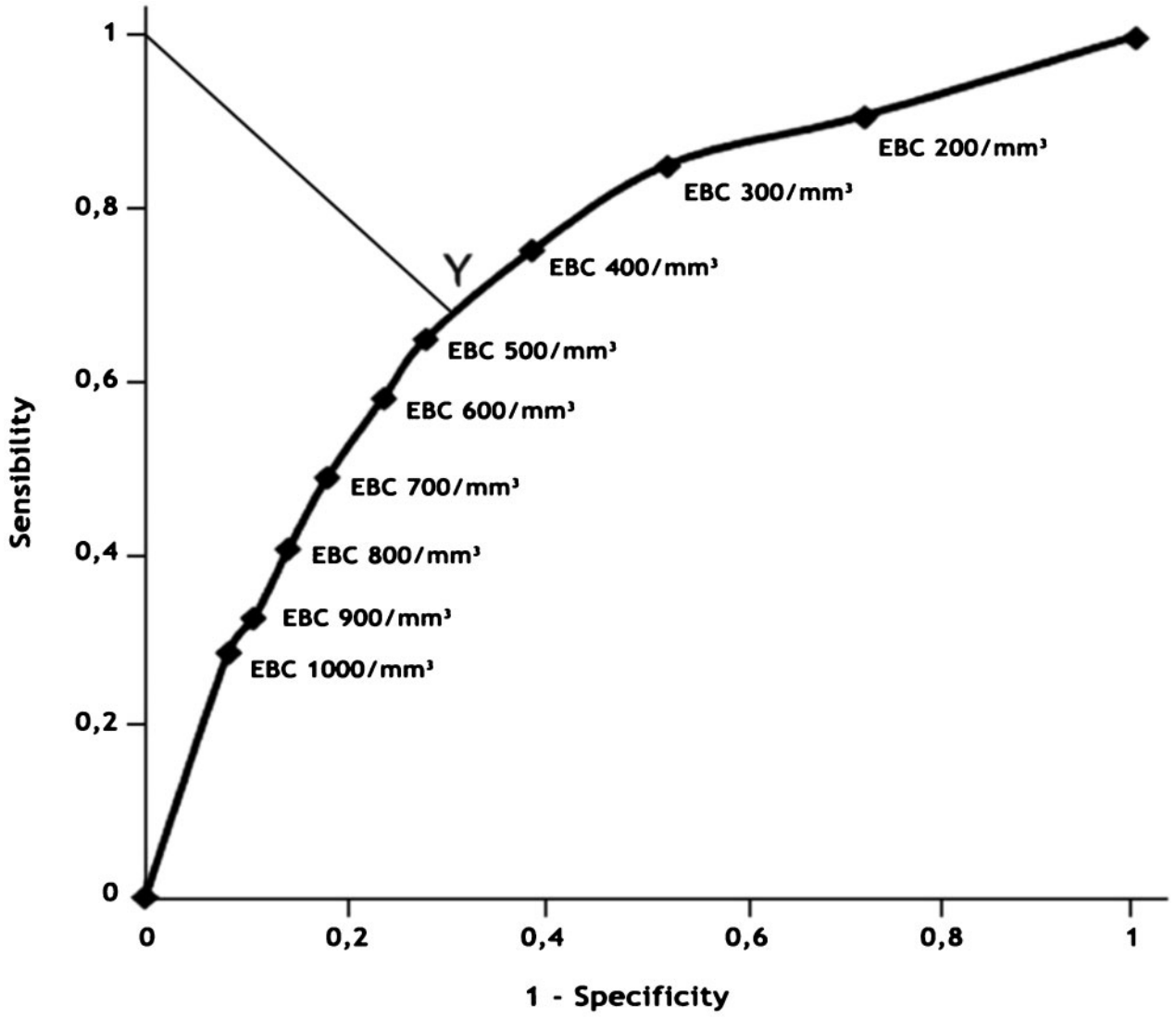

Figure 1 ROC curve, correlation between eosinophil count and antigenemia prevalence. EBC, eosinophil blood count/mm^3; Y, Youden index; ROC, receiver operating characteristic. (Figure from: Musso D, Vialette V. Predictive value of the eosinophil count in the biological diagnosis of lymphatic filariasis in French Polynesia. Med Mal Infect. 2012;42:585–90. Elsevier Masson SAS.)

was not statistically different compared to our previous study. We can speculate that the relationship between EBCs and CFA in our study can be extrapolated to the entire population. In addition, this confirms that despite numerous MDA campaigns, prevalence continues to be above the target of 1% in 2011. Such results make warrant additional strategies in order to reduce the LF prevalence in FP.

A possible explanation of the high prevalence in 2008 was a poor MDA coverage: the percentages of the population who received medication and took it were 85.8% in 2006, 77.9% in 2007, and 75.8% in 2008. Another possible explanation was the vector *Aedes polynesiensis*. In the south Pacific, only *W. bancrofti* is found[29] and is especially transmitted by the mosquito *Ae. polynesiensis* which exhibits a trait

Table 4 Probable positives antigenemic patients for the different cutoffs

Eosinophil cutoff	Total blood counts	prev CFA pos	CFA E	CFA D
>1000	1777	34.19	608	40
>900	2253	31.08	700	46
>800	2969	30.00	891	57
>700	3945	28.87	1,139	69
>600	5380	26.97	1,451	82
>500	7503	25.78	1,934	91
>400	10 877	22.75	2,474	106
>300	16 103	19.80	3,189	120
>200	23 783	16.08	3,824	128

Note: CFA, circulating filarial antigen; pos, positive; E, estimated; D, detected.

called 'limitation', meaning that the mosquito becomes more efficient for transmission of LF when the prevalence within the population is low.[30]

Our current strategy is based on MDA, vector control, and systematic treatment of CFA positives and microfilaremic patients. CFA tests and microfilariae detection cannot be routinely performed because of their cost and an additional strategy based upon the EBC is to be considered.

The relation between EBC and filariasis is well known but the predictive positive value of the EBC for antigenemia has never been evaluated.

In the case of FP, when eosinophilia was defined as an eosinophil count above 500 mm^3/ml, the prevalence rate of antigenemia was estimated at 25.78%. Nevertheless, as the positive predictive value is proportional to disease occurrence, the positive predictive value of EBC is expected to be lower in countries with a lower prevalence of LF and higher in a country with higher prevalence. In addition, we rarely identify helminths from stool examinations in FP. This is probably due to the annual distribution of ALB and its impact on other intestinal helminths (hookworm, ascaris, enterobius, and trichuris). So we can assume that the main cause of eosinophilia in our population is LF.

Over a 2-year period we detected 141 positive antigenemic and 7503 eosinophilic patients. According to the prevalence of positive CFA patients for an EBC above 500/mm^3, we speculate that 1934 patients could have been found ICT-positive, while only 141 CFA-positive had a recommendation to be treated as their CFA was found positive. Keeping in mind that CFA detect antigens secreted from adult worms indicate active infection and that such people can be implicated in the transmission of LF. According to WHO, ALB, and DEC to use in national programmes to eliminate LF are both safe and well tolerated. Recent studies have confirmed that co-administration of these drugs does not enhance their toxicity. The adverse reactions are usually self-limited and resolve without any action, although symptomatic treatment with analgesics or antipyretics is helpful.

Then, there is no risk in the treatment for all eosinophilic patients, with respect to exclusion criteria including pregnant women, children under 2 years old, infirms, and sick (especially epileptic patients according to our experience, unpublished data). Treatment can be given to the patients when they return to health centres to withdraw blood test results having a 'direct observed therapy' to ensure a proper observance and administration of the drug under the supervision of a health care worker. In addition, local practitioners and health centres are familiar with the symptoms that might occur and are prepared to treat adverse reactions.

In our laboratory, only patients with severe eosinophilia (EBC above 1500/mm^3) had additional blood tests to determinate the cause of eosinophilia. Consequently, laboratory tests for LF diagnosis are rarely performed, leaving these patients undetected.

With our proposed additional strategy, the total cost for the treatment of all the 7503 eosinophilic patients would be US$5102. With our current strategy, we only treated 141 patients for a total amount of US$59 200 due to the cost of CFA determination.

There is no additional cost for eosinophil determination (including transportation of samples and blood count determination) because our proposed strategy relies on the optimization of all the blood count results.

Testing of all eosinophilic patients by ICT and treatment of the positives would be the 'gold standard' protocol but is not financially possible due to our high eosinophilia prevalence. This 'test and treat' strategy is recommended by PacELF guidelines in special conditions, usually to complete MDA in remote and low inhabited areas.

Systematic treatment of all eosinophilic patients could lead to a higher number of antigenemic patients treated and in a reduced health care cost compared to a test and treat strategy.

We demonstrated that more than 25% of our eosinophilic patients are CFA positives. With our additional strategy we can ensure treatment of all of these patients. It is important because we know that MDA coverage is not 100%. Members of the community with normal eosinophil count are expected to be treated during the annual MDA.

Additional strategies can be discussed such as treatment of all persons that interact with the health system (without blood screening) or to use a lower EBC cutoff. It should be kept in mind that our entire population, which includes the population not screened for eosinophilia, is expected to be treated during MDA.

The likelihood of success increases when a multifaceted strategy is cohesively employed using a variety of tools such as MDA, vector control, helminth control, health education, and home treatment. Additional strategies could help to reach the goal of LF elimination in FP. One of these strategies would be the systematic treatment of eosinophilic patients. The proposed screening strategy alone cannot be sufficient to achieve transmission interruption of LF but it should be used as a complementary tool to MDA, treatment of diagnosed filaremic patients, and vector control.

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