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THE SENSITIVITY OF STANDARD CIRCULATING FILARIAL ANTIGEN TESTS AND ULTRASONOGRAPHY FOR INDIVIDUAL DIAGNOSTICS AND EPIDEMIOLOGICAL SURVEILLANCE OF BANCROFTIAN FILARIASIS

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Significant advances were made in the diagnosis of lymphatic filariasis (LF) in the past years using two tests for the detection of circulating filarial antigen (CFA) in individuals with LF: the Og4C3-ELISA (TropBio®) and the immunochromatographic test (ICT; NOW® Filariasis). These tests have been mainly used in microfilariae (Mf) carriers and both resulted in high sensitivity. To verify parasitic infection also in amicrofilaremic individuals, ultrasonography (USG) of the scrotal area is frequently used. In this study Mf-load and CFA-status (Og4C3) were assessed in healthy adult volunteers (n=1976), 535 samples were additionally analysed with ICT. All men (n=1132) underwent ultrasound examination of the scrotum. Altogether 324 were Mf+ and 1652 were Mf-. Both tests, Og4C3 and ICT, showed a high sensitivity for detection of CFA in the Mf+ samples (99% and 100% respectively) but there was a significant difference between both tests regarding the Mf- samples (consistency only in 410/483 (85%) cases). USG revealed that 201 men were FDS+/Mf+, 151 FDS+/Mf-, 74 FDS-/Mf+ and 706 FDS-/Mf-. The sensitivity of Og4C3 and ICT was high in microfilaremic patients (99% or 100%). The sensitivity of the Og4C3 for FDS+/Mf- men was 91%, that of the ICT 82%. There was a significant difference between both tests in the assessment of the FDS-/Mf- patients (consistency in 113/140 (81%)). In 74/275 (27%) Mf+ men, life adult worms could not be detected by USG. In conclusion, confirmative to a former trial, in 73% of the Mf+ individuals life adult worms were detected by USG. The lower detection of the USG is presumably caused by adult worms located in sites of the body other than the scrotum. Og4C3 and ICT both show a high and comparable sensitivity in the detection of Mf+ individuals while in FDS+/Mf- cases the sensitivity of antigen detection is lower. Particularly in absence of Mf and FDS, Og4C3 and ICT show a lack of consistency. Therefore antigen results from Mf- individuals should be interpreted taking this caveat into account.

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IDENTIFICATION OF A *WUCHERERIA BANCROFTI* LARVAL STAGE SPECIFIC STAGE PROTEIN THAT IS BOTH SENSITIVE AND SPECIFIC IN DETECTING ANTIBODIES IN *W. BANCROFTI* INFECTED PATIENTS

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The current antibody tests used for mapping the distribution of lymphatic filariasis (LF) and for monitoring progress in elimination programs suffer from poor specificity because of considerable cross-reactivity with antibodies induced by other filarial infections such as *Loa loa*, *Onchocerca volvulus*, and *Mansonella* spp. Using the dCAS bioinformatics package, we assembled 2048 expressed sequence tags (EST) from the L3 infective larvae of *W. bancrofti* into non-redundant contigs which were then assessed for homology to protein and nucleotide databases as well as head-to-head against contig sets assembled from L3 larval ESTs of *B. malayi* (Bm - 5068 ESTs), *O. volvulus* (Ov - 4166 ESTs), and *Loa loa (*Ll- 3315 ESTs). Nineteen potential L3- and Wb-specific antigens were identified and expressed as fusion proteins with Renilla luciferase in mammalian cells. Screening of cell lysates by a Luciferase Immunopreciptation System (LIPS) assay revealed that only 1 of the 19 antigens (Wb-123) was both highly immunogenic and Wb-specific. Using a broad panel of well-defined sera from normal North Americans (n=53) and patients infected exclusively with Wb (n=43), Ll (n= 70), Ov (n=43), or intestinal helminths (n= 21), the Wb-123 based LIPS assay could identify sera from all of the Wb-infected individuals (MF+ or CAg+ from diverse geographic regions) with 100% sensitivity and 100% specificity compared to sera from uninfected controls and those with intestinal helminths. When specificities and sensitivities were assessed using sera from Ll-infected or Ov-infected individuals as the comparator, the sensitivities ranged between 98-100% and the specificities between 97-98%. Thus, we have identified an L3- and Wb-specific antigen that can be used not only as a rapid and specific tool to diagnose individual *Wb* infections but also as a sensitive, high-throughput, and potentially point-of care method for early detection of recrudescent infections in areas of control and for mapping new areas of Wb transmission.

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CARDIAC LESIONS IN AN AREA HYPERENDEMIC FOR LOIASIS IN CAMEROON

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Although the majority of patients with loiasis are asymptomatic despite high levels of blood microfilariae, characteristic symptoms include migratory angioedema and subconjunctival migration of the adult worm. Serious complications, including endomyocardial fibrosis (EMF), have been described; however, the prevalence of such complications in endemic areas is unknown and likely underestimated. To assess the cardiac complications related to loiasis, we performed a cross-sectional, study of 297 adult (>15 years of age) residents of a hyperendemic focus of loiasis in Cameroon. Subjects with evidence of onchocerciasis or lymphatic filariasis, a history of cardiovascular disease prior to their settlement in the study area or any antifilarial treatment taken during the last two years were excluded from the study. All subjects underwent a detailed clinical examination, assessment of microfilaremia by calibrated thick smear of daytime blood, *Loa loa* serology (SXP LIPS), and echocardiography performed by an experienced cardiologist. Of the 297 subjects, 180 had detectable *Loa* microfilaremia, 39 had both *Loa* and *Mansonella perstans* microfilaremia and 63 had no serologic or parasitologic evidence of *Loa* infection. Echocardiography was abnormal in a high percentage (84.5%) of patients and included valvular or endocardial calcifications (70%), diastolic dysfunction (35.7%), cavity dilatation (34.3%), valvular insufficiency (18.5%), left ventricular hypertrophy (9.8%), pericardial lesions (2.4%) and EMF (1.01%). Although the frequency and distribution of these abnormalities was not statistically different between subjects with and without loiasis, the number of uninfected subjects was small. Of note, all 3 subjects with EMF had detectable *Loa* infection, negative stool examination for intestinal helminths and marked eosinophilia. Although these data are consistent with an increased prevalence of cardiac

abnormalities, including EMF, in an area hyperendemic for loiasis, the role of loiasis in the pathogenesis of these abnormalities remains to be elucidated.

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TARGETING *WOLBACHIA* ENDOSYMBIONTS IN *ONCHOCERCA VOLVULUS* EFFECTIVELY CLEARS PERSISTENT MICROFILARIAE IN THE SKIN OF ONCHOCERCIASIS PATIENTS IN WHOM REPEATED IVERMECTIN TREATMENT HAD FAILED TO CLEAR

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Ivermectin (IVM) has been the drug of choice for the treatment of onchocerciasis since 1987. However, there have been reports of persistent microfilariae (Mf) in the skin of some people after many rounds of IVM treatment in some districts in Ghana. These indications are consistent with the emergence of drug resistance or sub-optimal response to IVM. To assess the effect of targeting Wolbachia endosymbionts in O. volvulus on onchocerciasis patients in whom repeated IVM treatment had failed to mediate Mf clearance, 149 patients were recruited in 2 districts in Ghana where IVM resistance has been reported. They were treated with either 100mg/d doxycycline (Doxy) or matching placebo for 6 weeks. Three and 12 months after Doxy treatment, all patients took part in ongoing IVM mass treatment. Patients were snipped before, 12 and 20 months after treatment to assess the levels of Mf that IVM could not clear. Entomological work was also carried out in all the studied villages before and after Doxy treatment.

Before treatment, of the 73 patients allocated for doxycycline, 66% had persistent Mf in the skin and 34% had only nodules but no skin Mf, and of the 76 patients allocated for placebo, 63% had persistent Mf in the skin and 37% had only nodules (P=0.74). However, at 12 months after Doxy treatment, of the 72 Doxy-treated patients snipped, 10% still had low numbers of Mf in the skin and 90% had no Mf at all. Of the 71 placebo patients snipped, 58% still had Mf in the skin while 42% had no Mf (P<0.001). At 20 months post therapy, only 3% of the 69 Doxy patients had low Mf and 97% were Mf negative. In contrast, of the 71 placebo patients, 69% still had Mf while only 31% had no Mf. This difference between the Doxy and placebo groups was significant (P<0.001). Doxy cleared Wolbachia significantly compared to placebo group and shows embryostatic effect in the adult worms compared to placebo patients. A comparison between pre-treatment and post treatment transmission parameters indicated a significant reduction after intervention in most areas. Doxycycline clears Wolbachia from O. volvulus worms, and resulted in embryogenesis blockade. Therefore, targeting Wolbachia in O. volvulus is effective in clearing Mf in the skin of onchocerciasis patients in whom repeated standard treatment has failed to clear; thus strategies may be developed including anti-Wolbachia l treatment to control the re-emergence of onchocerciasis in areas where infections persist despite the use of IVM.

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HIGH DOSE BIANNUAL ALBENDAZOLE AND IVERMECTIN SUPPRESS *WUCHERERIA BANCROFTI* MICROFILARIAL LEVELS MORE EFFECTIVELY THAN STANDARD DOSE ANNUAL TREATMENT

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Annual mass treatment with albendazole and ivermectin is the mainstay of current strategies to interrupt transmission of *Wuchereria bancrofti* (Wb) in Africa. More effective microfilarial suppression could reduce the time necessary to interrupt transmission and ease the economic burden of such programs in countries with limited resources. To determine the effect of increased dose and frequency of albendazole/ivermectin (A/I) treatment on microfilarial (mf) clearance, 40 Wb microfilaremic residents of an endemic area in Mali were randomized to receive three doses of standard annual A/I therapy (400 mg/150 mcg/kg; n=21) or six doses of twice-yearly increased dose A/I therapy (800 mg/400 mcg/kg; n=19). Mf levels were assessed by Nuclepore filtration of 1 ml of blood and circulating antigen (CAg) levels by TropBio™ ELISA. We have previously reported increased efficacy of twice-yearly high dose treatment in reducing mf counts at 12, 18 and 24 months as compared to standard dose annual therapy with no mf detected in subjects in the twice-yearly group at any time point after 6 months. At 30 months, only 1/17 subjects in the annual group and 0/17 subjects in the twice-yearly group had detectable mf (p=NS). As at prior time points, a significant and comparable decrease in CAg levels was seen in the annual and twice-yearly treatment groups at 30 months with geometric mean (GM) % pre-treatment levels of 74% and 54%, respectively. Thirty-six month followup is planned for July 2010. These findings suggest that increasing the dose and frequency of A/I treatment leads to more rapid suppression of microfilaremia than standard annual therapy and that this effect is not due to an enhanced adulticidal effect. Consequently, twice-yearly high dose treatment is likely to have the greatest benefit in accelerating transmission interruption in regions where mass treatment has been non-existent or suboptimal. Additional studies examining the independent effects of dose and frequency are clearly needed.

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A HYDROCELECTOMY PROGRAM FOR LYMPHATIC FILARIASIS IN LÉOGANE, HAITI: CLINICAL INFORMATION AND SURGICAL OUTCOMES

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Lymphatic filariasis (LF) has been endemic in Haiti for over 250 years with a current estimate of 8 million people at risk of infection. In Léogane Commune, up to 30% of adult males suffer from hydrocele, the most common manifestation of chronic LF. Since 2001, a surgical program providing hydrocelectomy has been in operation at Hôpital Sainte Croix and Hôpital Cardinal Légère in Léogane. We assessed clinical data and surgical outcomes for 491 men who underwent hydrocelectomy between 2001 and 2008. Patients ranged in age from 14-85 years (mean, 42 years) and reported an average of 5.6 years with the hydrocele (range, 3 days