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Wuchereria bancrofti infection and disease in a rural area of Papua New Guinea

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SUMMARY

The relation between infection and disease in lymphatic filariasis is still imperfectly understood. This paper presents baseline data on microfilaraemia, oedema and acute episodes from a drug trial against bancroftian filariasis in Papua New Guinea. Among 2187 people with data on these manifestations, 1144 were microfilaraemic, 78 had leg oedema, 356 had acute episodes and 919 were negative for all three. All possible combinations of the three manifestations were observed. The relationships between microfilaraemia and disease are found to be time-dependent. Microfilaraemia is a positive risk factor for leg oedema at lower ages, but a negative one at higher ages. Acute disease over a whole year is found to be positively associated with a point estimate of microfilaraemia. However, when considering incidence within a month of the time of the blood sample, a curvilinear relationship is found and people with low but positive microfilarial counts have the lowest incidence. Possible explanations for these findings are discussed.

Introduction

Lymphatic filariasis is a major cause of morbidity on a world scale (1,2), yet some aspects of its pathogenesis remain controversial. In particular, the extent to which oedema and acute adenolymphangitis (ADL) are due purely to filarial, rather than bacterial, infections has been debated throughout the past century (3). This paper reviews clinical and parasitological data from Papua New Guinea which have contributed to our understanding of these issues and presents new analyses with the aim of shedding light on some of the remaining complexities.

Methods

A cluster-randomized trial of diethylcarbamazine (DEC) versus ivermectin plus DEC was carried out in parts of Urat and Urim census districts of East Sepik Province, Papua New Guinea. The area is in the rural southern foothills of the Torricelli Range, with the steeply forested ridges being used for subsistence shifting agriculture and some cultivation of cash crops. Transmission of bancroftian filariasis is predominantly by *Anopheles punctulatus* s.s. (4).

The clinical and parasitological methods have been described elsewhere (5,6), as has the system of village-based reporting of acute episodes (7). To summarize: annual surveys of the population (aged over 5 years) were carried out, in order to determine the prevalence of oedema of the limbs and breast and of hydrocele. 1 ml venepuncture blood samples were also taken, passed through a Nuclepore (Pleasanton, California, USA) filter and *Wuchereria bancrofti* microfilariae counted microscopically.

For acute disease, local people were employed as fulltime reporters, whose schedule involved visiting each person in their area once a week. If a person reported having been sick in the previous week, then a questionnaire was completed regarding painful swelling in the leg, groin, arm, armpit, breast and scrotum. If these coincided with self-reported fever, then the episode was considered indicative of acute

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filarial disease. Demographic surveillance was used to maintain the currency of the population data on which these procedures were based.

This paper presents pretreatment data from the 1993 and 1994 annual surveys and the first year's acute disease surveillance from 1 June 1993 to 31 May 1994. In most analyses, the 1994 annual survey is used because it was the first to contain 2 villages added to the study after the 1993 survey. However, the 1993 survey is used in the analysis of time-dependence of acute episodes, because it occurred during the first year of morbidity surveillance.

Statistical analysis is by chi-squared test for contingency tables and by generalized linear models. In particular, logistic and Poisson regressions are used, with allowance for overdispersion where necessary. The p values quoted are two-sided and 95% confidence intervals (CIs) are presented.

Results

Figure 1 shows the status of microfilaraemia, leg oedema and acute episodes in the leg (including groin). This figure gives us an idea of the range of manifestations of filarial disease: all 8 possible combinations occur, including 'overlap syndromes' (8) such as a combination of oedema and acute episodes. In particular, the idea that people with leg oedema are generally amicrofilaraemic (9,10) is not borne out. This figure gives a simple summary, but for a more thorough understanding we need to take account of factors such as the age dependence of all manifestations. This paper concentrates on the relationships between microfilaraemia and disease and particularly on time-dependent changes in them.

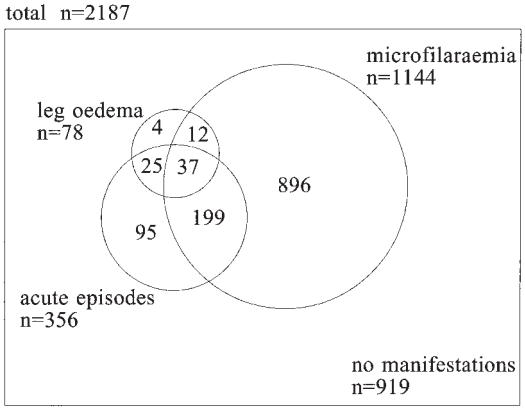

Figure 1. Venn diagram showing numbers of people with combinations of microfilaraemia, oedema of the leg and acute episodes in the leg. 2187 people had data on all three manifestations. Of these, 919 people had no manifestations, 1144 were microfilaraemic, 78 had leg oedema and 356 had acute episodes. All the possible combinations of the three manifestions were observed, as shown by the numbers inside the overlapping circles.

Microfilaraemia and chronic disease

We have already seen in Figure 1 that many people with leg oedema are microfilaraemic. Looking at this more closely, we find that 50 out of 80 people (63%) with oedema are microfilaraemic, as opposed to 1100 out of 2117 (52%) who are not oedematous. (These numbers are slightly larger than those in Figure 1 due to the inclusion of the few people with missing acute episode data). In fact, there is a tendency for microfilaraemia to be positively associated with oedema (odds ratio = 1.54, 95% CI 0.95-2.51, p=0.064). Similar results were obtained in the 1993 annual survey (5).

However, considering age-dependent changes reveals a more complicated pattern, as shown in Figure 2. The association between microfilaraemia and leg oedema changes with age (p=0.022 for interaction), being positive at younger ages but negative at older ones. Previous studies have found differing associations between sex and oedema (8). Here we see a tendency for a raised frequency in females. However, this was not statistically significant and has not been included in this analysis. The complex relationship with age highlights an issue that Michael et al. (11) raise as a limitation of their meta-analysis of the association between microfilaraemia and disease. The direction of the observed associations may be affected by the age profile of the study group, which can depend on either the sampling design or the underlying demography of the population. This effect could be further complicated if the 'crossover' age, at which there is no association, is itself affected by parameters such as transmission intensity.

Various hypotheses may help explain these age-dependent changes. Oedema could be the

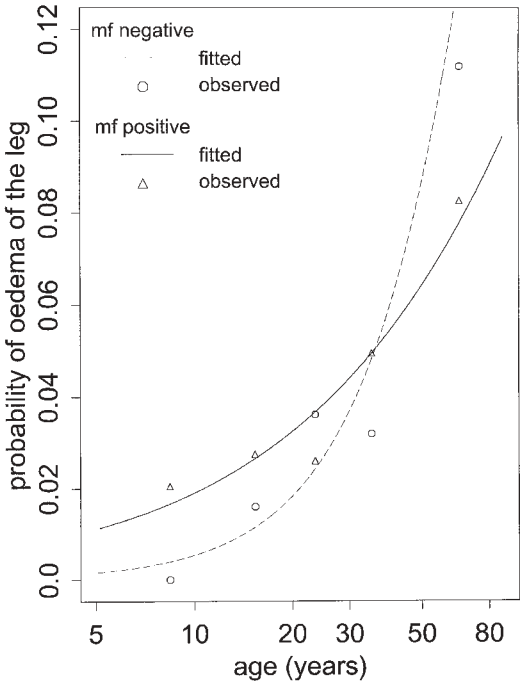

Figure 2. Probability of leg oedema as a function of age (logarithmic scale) and microfilaraemia (mf) status. Prevalences are shown for the quintiles of age: circles for mf-negative people and triangles for mf-positives. Values fitted from logistic regression are shown by a dashed line for mf-negatives and a solid line for mf-positives. The relationship between oedema and microfilaraemia is positive at younger ages, but negative at higher ages.

endpoint of a dynamic immunopathological process linked to the clearance of microfilaraemia (12). This could explain the excess oedema in older amicrofilaraemic people. However, it seems that another mechanism is relatively more important in younger people, hence causing the opposite relationship seen in them. This mechanism could be due directly to *Wuchereria bancrofti* (13,14), to secondary infections (3) or to environmental factors (15). In any case, it seems that a single static factor cannot explain the observed patterns.

We have concentrated on leg oedema, although the results can be contrasted with those for hydrocele. It is now well recognized that the pathogenesis of hydrocele is different from that of lymphoedema (3) and that it is not always useful to speak of them together as a single entity of 'obstructive lymphatic disease' (12). A simple contingency table indicates a strong positive association between hydrocele and microfilaraemia in our study and regression analysis shows that this relationship does not change significantly with age. The differing behaviour of the different types of chronic disease adds another layer of complexity when trying to understand their pathogenesis.

Microfilaraemia and acute disease

Microfilaraemia at the 1994 annual survey was associated with increased incidence of acute disease over the year's observation (7). This was the case for the leg, arm, breast and scrotum and was statistically significant for all of these locations except the scrotum. Microfilaraemia was categorized into three levels: negative; between 1 and 999 microfilariae per ml, inclusive; and 1000 or more microfilariae per ml. In the leg (including groin), 208 acute episodes were recorded over 849 person-years of observation in people who were negative for microfilaraemia, giving an incidence rate of 0.25 episodes per person-year. For those with 1-999 microfilariae per ml, 264 episodes were reported in 591 person-years, or 0.45 per person-year. For those with 1000 or more microfilariae per ml, the corresponding figures were 168/363, or 0.46 episodes per person-year. The rate ratios, relative to amicrofilaraemic people, were therefore 1.82 for those with 1-999 microfilariae per ml, and 1.89 for those with 1000 or more microfilariae per ml, with 95% CIs of 1.38-2.40 and 1.39-2.56 respectively. On adustment for age, sex and leg oedema, the rate ratios reduced to 1.35 and 1.59 respectively, but remained statistically significant, the 95% CIs being 1.06-1.72 and 1.20-2.09. There was a tendency for incidence to peak at an earlier age in microfilaraemic people. This interaction was not statistically significant, although similar patterns in the arm and scrotum were so.

These findings differ from those of Gyapong (16) who did not find microfilaraemia to be a risk factor. Dreyer and Piessens (3) emphasize the roles of secondary infections and adult worm mortality in the pathogenesis of acute disease, although this is not necessarily inconsistent with our findings. The role of microfilaraemia can be assessed in more detail by considering acute disease incidence near the

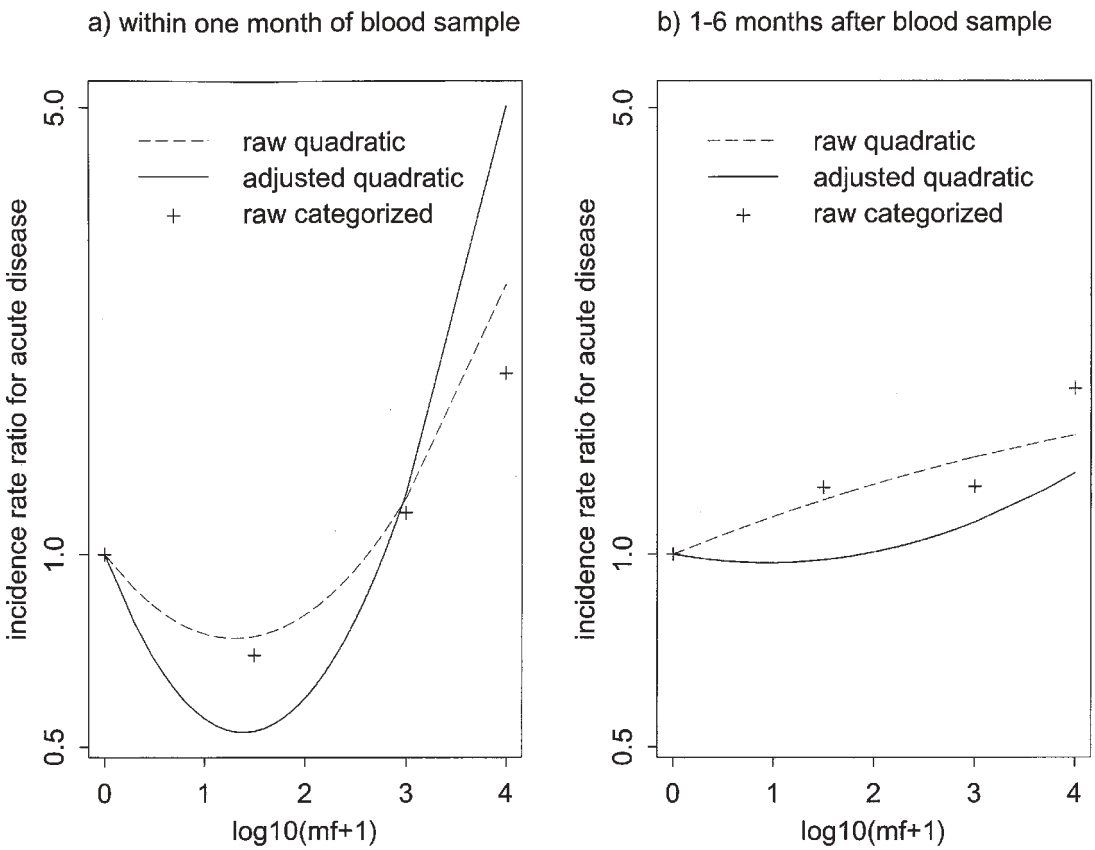

Figure 3. Relationship between microfilarial density and acute disease incidence, a) within a month of the microfilarial density measurement and b) 1-6 months afterwards. The + signs indicate the raw rate ratios categorized by age. The dashed lines indicate a quadratic regression equation relating incidence to microfilarial density. The solid lines are similar but include adjustment for age, sex and chronic disease status.

time that it was measured, rather than over an entire year. Figure 3a shows that the incidence rate within a month of the 1993 survey was a nonlinear function of microfilarial density, with a quadratic term improving the fit significantly (p=0.008). Those with low, but positive microfilarial counts have reduced incidence relative to those with either high or zero counts. Adjusting for age group, sex and chronic disease increased the nonlinearity. This analysis includes episodes both before and after the survey; if they are considered separately, both show similar patterns but with borderline statistical significance (p=0.06 in each case). By contrast, considering incidence over a broader timescale, Figure 3b shows a linear increase, as expected from the results quoted earlier.

These findings suggest a more direct link between acute disease and microfilaraemia, perhaps via parturition of adult worms as suggested by Kar et al. (17). Those people with low microfilarial counts may harbour adult worms while being in a quiescent immunological state, while those with zero or high counts may experience acute episodes caused either by the worms themselves or an inflammatory immune response capable of suppressing microfilaraemia. This is speculative, but our data do suggest that we have yet to fully understand how the infection and disease processes develop and interact over time.

Discussion

The trial carried out in Urat and Urim has yielded community-based prospective data on filarial infection and disease which are more comprehensive than those in the few other comparable studies (16,18-20). The

occurrences of all three presentations reported here, namely, microfilaraemia, chronic disease and acute disease, are high compared to those in other filariasis-endemic parts of the world, which provides an opportunity to study their interactions in detail.

Microfilaraemia is not a complete measure of infection and other tests, such as those detecting circulating filarial antigen (21,22), have some advantages. However, although micro-filaraemia may not be a perfectly sensitive test of underlying infection, it is definitely highly specific. The system of lay reporting of acute episodes means that we cannot distinguish between syndromes such as the acute dermatolymphangioadenitis (ADLA) and acute filarial lymphangitis (AFL) identified by Dreyer et al. (23). Despite these limitations we can reach some conclusions about the relationship between infection and disease.

Firstly, all the possible combinations of the three presentations can occur. For example, there is no general pattern of people with chronic disease being amicrofilaraemic. In fact, we see a complex relationship between leg oedema and microfilaraemia which changes over time. This pattern may be due to, for example, immunological changes or secondary infections, although the current data cannot distinguish between these possibilities. Hydrocele shows a different relationship with microfilaraemia, in accordance with its different pathogenesis.

Acute disease over the course of a year is higher in those who were microfilaraemic at the annual cross-sectional surveys. Looking at a narrower time-frame around the time of the first survey shows a nonlinear pattern, suggesting a dynamic relationship with microfilaraemia. Again, we cannot identify the cause of this pattern from the current data, but it suggests that intensive short-term monitoring of infection status may advance our understanding of the aetiology of acute disease.

The long debate about the relative importance of *Wuchereria bancrofti* and secondary bacterial infections has recently shown increased emphasis on the latter (3). This has led to local hygiene and antibiotics being advocated (23,24) as treatment policies, with one suggested route being community-based 'Hope Clubs'. Shenoy et al. have carried out a series of randomized trials of antibiotic regimens against brugian filariasis (25-27), with only the last one finding differences between the allocation groups. Here, the change in ADL incidence was less in the placebo group than in the groups receiving DEC, penicillin, or DEC combined with penicillin. A foot care program was also extended to all the study groups and it was this, rather than antibiotics, which was identified as the most important treatment component (27). For bancroftian filariasis, Dreyer et al. (23) report success of measures such as local hygiene, postural drainage and antibiotics in reducing acute and chronic disease. However, the relative contributions of the various components are not clear. The above data do not convincingly demonstrate a key role for secondary bacterial infections. That is not to say they are unimportant, but there is a lack of supporting evidence. While more controlled trials are warranted, gaining a full understanding of the role of *Wuchereria bancrofti* infection in pathogenesis should also remain a priority.

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