Use of a *Mycobacterium leprae* dipstick to classify patients with leprosy

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Achievement of the goal of the World Health Organization (WHO) to eliminate leprosy as a public health problem by the year 2000, to be reached when the global prevalence falls below 1 per 10,000, has recently been deferred to the year 2005. In itself, this postponement already signifies the difficulty of controlling and, especially, of eliminating leprosy. Even when this goal is reached, elimination does not signify the end of the problem. Because of the present numbers of unevenly distributed leprosy cases and an incubation time of several years, many more leprosy patients can be expected to emerge in leprosy-endemic countries after the year 2005.

The current strategy of multidrug therapy (MDT) is based on passive case detection and treatment. However, the effectiveness of this approach to the control of leprosy has been questioned. Indeed, it can be at best only partially effective. Because of logistic limitations, active-case detection, if it is performed at all, is limited mainly to the population of household contacts, whereas, in leprosy-endemic areas, the majority of new leprosy patients are not household contacts of known patients. Treatment of patients can be administered only after diagnosis, but patients, especially whose with multibacillary (MB) disease, who shed *Mycobacterium leprae* from the nose until diagnosis and treatment, are important sources of transmission of the organism, which cannot be effectively controlled.

In addition, under the pressure to achieve the elimination goal, a number of measures were taken that were aimed at accelerating reduction of the registered prevalence. However, the rationale for these actions is not always clear. Treatment schedules for MB patients have been reduced to 1 year, without the necessary evidence of efficacy, and even shorter durations of therapy are being considered. Classification criteria are no longer based on bacteriological findings, but only on clinical findings. Combined with a loss of expertise that may be expected when leprosy control activities are incorporated into the general health services, these measures may have an effect opposite to that for which they were intended.

It is clear from epidemiological investigations that a number of organism-, host- and environment-related factors may be incriminated in the dynamic process of the development of leprosy, the disease, but many features of the epidemiology of leprosy remain to be elucidated. This is a prerequisite for success in controlling the disease.

Especially in this period of uncertainty, during which it is unclear in which direction trends of incidence are moving, continued recording of the descriptive aspects of the epidemiology of leprosy is much needed. Considering that diagnostic criteria are being loosened and treatment schedules shortened, the use of modern molecular and immunological tools could be valuable aids to efforts to control leprosy.

The diagnosis of leprosy is based on clinical, bacteriological and histopathological

findings. However, facilities for bacteriological examination of slit-skin smears and histopathological examination of skin or nerve biopsy specimens are often not available, and, if they are available, the results of the examinations may be erroneous or biased; concentrations of *M. leprae* are underestimated, or the organisms are not detected at all, with the outcome that patients are misclassified or missed entirely. Discrepancies in histopathological diagnosis of skin biopsy specimens, even when performed by experienced pathologists, have been reported, illustrating the difficulty of diagnosing the disease correctly.

Early diagnosis and effective treatment are very important in preventing deformities and transmission of the organism. In an attempt to overcome the problem of the absence of adequate diagnostic facilities, the WHO has adopted a simplified method of classification, which is based upon counting the number of lesions. Patients with fewer than six lesions receive PB MDT, and patients with six or more lesions are considered to be MB patients. However, more accurate, yet easily applied methods of diagnosis are available.

Several studies have shown that the presence of antibodies to the *M. leprae*-specific phenolic glycolipid-I (PGL-I) correlates with the bacterial load of a leprosy patient. The great majority of patients with paucibacillary (PB) leprosy are seronegative. Studies of changes of serum antibody levels during treatment have further demonstrated the correlation of serum anti-PGL-I antibodies with the bacterial load; during treatment, antibody levels decrease in parallel with the declining bacterial indices. The observation that increases of the levels of anti-PGL-I antibodies in patients have been associated with the onset of relapse also indicates a relation between levels of anti-PGL-I antibodies and the presence of *M. leprae*. Detection of these antibodies may thus be a useful tool for confirming the diagnosis of MB disease.

Recently, a dipstick suitable for use in the field that detects IgM anti-PGL-I antibodies (the ML dipstick) has been developed. Studies demonstrate 97·2% agreement between the dipstick assay and ELISA. In contrast to ELISA, the dipstick is a simple and rapid test, which is not dependent on equipment, and which employs highly stable reagents that do not require refrigeration.

A study in Brazil among 264 leprosy patients, 130 of whom had positive skin smears, found that classification of leprosy based only on the number of lesions demonstrated only 85% sensitivity and 81 specificity in detecting MB cases among the studied population, employing the BI as the standard. The sensitivity would have been increased had the patients been classified according to the number of lesions and the result of the dipstick assay (patients would be classified MB if they are dipstick-positive, have more than six lesions, or both; patients who are dipstick-negative and demonstrate fewer than six lesions would be classified PB). The combined method of ML dipstick and number of lesions demonstrated 94% sensitivity and 77% specificity. The classification based on the number of lesions alone classified 19 patients with BI >0 PB, whereas the combined method of ML dipstick and number of lesions classified only eight BI-positive cases (five BB, two BL and one TT) PB.

In another study, we found that, of 71 dipstick-negative PB patients, 25 (32·2%) were clinically cured at the end of treatment, compared to only two (9·5%) of the 21 dipstick-positive PB patients. Nine (5·3%) of 170 patients in the study population relapsed within the 5-year follow-up period; seven were MB patients, all of whom were dipstick-positive. Two PB patients relapsed, one dipstick-negative and one dipstick-positive.

In conclusion, introduction of the ML-dipstick as a tool in addition to clinical classification by the number of lesions can improve classification of leprosy patients for the purpose of treatment.

DISCUSSION

Professor Britton: The next step might be an intervention study, in which one would attempt to show that treatment of seropositive household contacts will prevent clinical expression of leprosy. Has such a study been carried out? And if it was, what treatment-regimen was employed?

Dr Klatser: Douglas showed in a study in Culion that treatment of seropositive contacts for 6 months reduces antibody levels. Additional studies are in progress, in which seropositive contacts are treated.

Professor Britton: It appears clear that MB leprosy is associated with high antibody levels. Does this mean that contacts with high antibody levels should be treated as MB leprosy?

Dr Klatser: That is a good question. I am uneasy about single-dose treatment of such individuals. I am not concerned about the individual who is incubating PB disease. But what about the individual who is incubating MB disease? He carries an enormous burden of *M. leprae*, and if he is infectious on the day of diagnosis, he probably was also infectious on the day before diagnosis, and probably also weeks or months earlier.

Dr Noordeen: With regard to the individual who is incubating MB disease, and who has no clinical features of leprosy, might he not have positive skin smears?

Dr Klatser: We have tried in a number of places to obtain skin smears, but it is not easy to obtain skin smears from healthy people, even though one may relatively easily obtain blood.

Dr Gupte: You stated that the specificity of the dipstick test in the diagnosis of MB leprosy was about 90%. The majority of patients in most endemic areas are PB, and the numbers of MB patients are small, no greater than 10–30% of the total. If you base your treatment on the serology, you will treat many PB patients as if they were MB.

Dr Klatser: It is not necessary to test serologically every PB patient, if the clinical diagnosis of PB is clear. However, in case of doubt, it would be useful to have an additional tool.

Professor Grosset: Even if the specificity of the serological test were 100%, the important question is to whom it should be applied. It's not a problem to apply the test to the leprosy patients. However, if the test is to be applied to healthy individuals, many false positives will result, if the specificity of the test is only 90%. A specificity of 90% implies that 10 of every 100 individuals tested will be false positives. Think what this means, if you are using the test to diagnose pre-clinical MB leprosy.

Professor Nath: I believe that the test should be used as serological tests are used in the diagnosis of other infectious diseases; one looks for rising antibody titres.

Dr Kaplan: I think you have made an interesting observation that supports your approach. When seropositive individuals were treated, antibody titres fell. Although it is arguable whether these individuals are shedding organisms and infecting contacts, the fact that they respond to chemotherapy by a reduction of antibody titre and, therefore, of antigenic load suggests that they are indeed infected, but that we are unable to detect the infection.

Dr van Brakel: The answer to the question of what degree of specificity is required is closely related to the consequences of a positive test. If you think of HIV infection, one demands a very high degree of specificity, because of the dramatic consequences of the diagnosis of AIDS. The consequences of a diagnosis of leprosy, or of predicting an increased risk of leprosy are much less dramatic.

Professor Ji: What is the primary purpose of developing an immunological test such as

yours? Is it to detect infection or disease in the individual, or is it to assess the epidemiologic status of the community?

Professor Brennan: This discussion goes directly to the heart of what we hoped to accomplish in this Workshop—to assess the current status of the available tools, and how closely they conform to our needs. I believe Dr Klatser has taken the serological test to enormous lengths, at least technically, in terms of its simplicity and applicability. Perhaps we should suspend the discussion on Professor Ji's question until we have considered some of the other tools that are available.

Dr Sengupta: Many studies have been carried out of the numbers of *M. leprae* in the nasal secretions of leprosy patients. Have you tried to determine the numbers of organisms in the nasal secretions of those in whose nasal swabs you detect *M. leprae* DNA?

Professor Ji: I believe that, if you find an organism in the nasal secretions of an individual, that individual is not someone who is subclinically infected; he is a patient, actually an MB patient.

Professor Britton: He may be an 'epidemiological patient', but if he has no clinical manifestations, is the presence of an organism in the nose sufficient to label the individual a patient with leprosy?

Professor Grosset: How can you identify the individual in whose nose you should look for *M. leprae*? You cannot perform nasal swabs on an entire population, just as you cannot examine an entire population serologically. There must be clinical indications to justify the test, at least a history of contact. It makes no sense to consider that there are many sources of infection in a population who have no clinical evidence of leprosy. Of course, there are patients who are unknown—so-called backlog cases. In tuberculosis, the duration between onset of symptoms and diagnosis is on average 3 months, during which time the patient excrete *M. tuberculosis* in his sputum and infects his contacts. Why should the situation in leprosy be different? Every clinician here knows that MB leprosy is not so easy to diagnose at an early stage. This difficulty of diagnosis allows patients plenty of time to spread their organisms. There is no need to speculate that there are many asymptomatic, healthy nasal carriers.

Dr Sow: In my experience, it is often more difficult on clinical grounds to diagnose MB than to diagnose PB leprosy. If there are *M. leprae* in the nose, there should be some clinical signs, at least enlarged nerves or difficult-to-detect skin macules.

Dr Noordeen: I think this discussion is interesting, in terms of how to progress in eliminating leprosy. New-case detection rates have not fallen; is this because of operational factors, or because cases are being missed? The difficulty with a serological test is knowing whom to test. We could focus on contacts, but we know that contacts contribute only a minority of the new patients. Therefore, we don't know whom to subject to our nasal swabs, serological tests, or skin smears. Yet, screening the entire population is impossible.

Professor Brennan: Dr Noordeen, your comment is a good one, which you have made in the past. In the past, only serological testing was available. Time has passed, and research is being done. Where should our priorities lie now?

Dr Kaplan: I'd like to address the point that, because contacts of patients contribute only a minority of new cases, it is not useful to target them as a high-risk group. I believe that contacts represent an ideal group in which to test an immunological assay that might be capable of detecting those at high risk of leprosy. Why not test the assay among contacts, and then apply it to screening the entire population?

Dr Klatser: Earlier, I presented data showing that contacts represent a large fraction of

new patients. Certainly, leprosy is an infectious disease, and the causative organism must be transmitted from patient to contact, unless there is some environmental source.

Professor Ji: No one denies that leprosy is an infectious disease. Of course, every new patient must have had some kind of contact with an infectious patient. However, not every new patient can identify the source of his infection.

Dr Gupte: We have conducted serological studies. In our population, the rate of seropositivity is very low. I must also point out that lepromatous leprosy *de novo* is very rare; most cases of lepromatous leprosy evolve by downgrading. Thus, at least in our population, the individual who is incubating MB leprosy, and who is shedding organisms although he is apparently healthy, is rare.

Dr Gillis: The issue here is how to apply the available tests. Serological tests cannot be applied to entire populations.

Dr Kaplan: Why not? Once the test has been shown effective among contacts, why not apply it to entire populations?

Dr Klatser: It would not detect the majority of PB patients.

Dr Kaplan: Then let's add another test that does.

Professor Ji: Missing PB patients would not present a problem, because these are not the infectious patients. Moreover, the clinical diagnosis of PB leprosy is relatively easy.

Dr Sow: How does the serological test perform in predicting relapse?

Dr Klatser: Usually, antibody titres fall during treatment. When titres do not fall, or actually increase, you should consider the possibility of relapse. These serological phenomena precede clinical evidence of relapse.

Dr van Brakel: How simple is the assay?

Dr Klatser: No laboratory facility is required. Similar tests have been developed for leptospirosis, leishmaniasis and other diseases. One only needs to do a finger-prick to obtain a drop of blood. The test is then interpreted on the spot.

Professor Brennan: The antigen employed in this test is synthesised in my laboratory under the terms of a contract with the NIH. We supply the antigen to Dr Klatser, who coats his dipsticks with it.

Dr Gillis: If I remember correctly, some of Douglas' seropositives did not express clinical leprosy, whereas some of his seronegatives did. This appears to yield the true measure of sensitivity and specificity.

Dr Klatser: You are correct. However, it is important to point out that the great majority of seronegatives who developed leprosy were PB. Also, we now see that seropositives who did not develop leprosy early did so later.

Professor Smith: I sometimes feel that we are in too much of a hurry. We now appear to have some interesting tools; perhaps the next step is to put them into population studies. Such studies will not only teach us much about these tests, but also about the epidemiology of leprosy. We appear to have exposed a great deal of ignorance, particularly about transmission. We should know a great deal more than we do about transmission if we hope to develop strategies to interrupt it. Rather than attempting to decide now whether or not to employ certain tests on a routine basis, we should employ them in prospective studies, and in this way learn more about the tests and also about the disease.

Dr Kaplan: To return to cell-mediated immunity, in the context of the skin testing you are doing with Dr Roche in Nepal, and of some of Dr Dockrell's work, I wish to point out that there are available today a number of assays that require only small volumes of blood that give us different views of CMI, including antigen-specific CMI. And to the extent that it is

possible, I wish to emphasise the importance of using more than one assay. We should build onto existing studies of CML vaccine trials and diagnosis to exploit 'high-tech' assays in relatively 'low-tech' environments.

Dr Ottenhoff: I wish to ask Dr Kaplan of what assays she was thinking when she was speaking of assays that required only small volumes of blood.

Dr Kaplan: The whole-blood assay can be carried out at the level of FACS analysis. If you obtain whole blood, stimulate it and then freeze the sample, you can later carry out a FACS-based analysis of phenotype, cytokine content, and other parameters, including thymidine incorporation, if you incubate the cells with BUdR before fixing them.

Another new assay is a FACS-based ELISA for cytokines in plasma. The kit enables one to measure six cytokines in 50 μl of plasma or blood. The assay is based on a bead-conjugated antibody for which you use a fluorescent tag. You can freeze the cells, ship them anywhere, and perform quantitative assays months later. Workers are now developing micro-cytotoxicity assays.

Dr Dockrell: I agree with Dr Kaplan's proposals. At the same time, I believe that it is a three-step process. The first step is a more sophisticated analysis, which requires FACS and the new techniques about which she spoke. At this step, one identifies which antigens and which cytokines are most appropriate for study. The second stage is one of simplification, as I am attempting with the whole-blood assays. The third step is the further development and simplification of the technique, as Dr Klatser has done for the anti-PGL-I antibody. I agree with Dr Kaplan, that once the assay has been carried to the point of a dipstick assay, it deserves to be used in the field. Once we have a dipstick assay for the cytokine, then we can perform two dipstick assays, and then we'll have proper coverage.

Dr Modlin: I agree with Dr Kaplan that we should carry out a larger number of tests. However, I believe that, despite all of the research that has been carried out on the immunology of leprosy, we have yet to identify a single assay that correlates with protective immunity. We shouldn't be satisfied with IFNγ as the single, correct target.