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Early detection os subclinical leprosy

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Introduction

Expanded implementation of multidrug therapy (MDT) for Hansen's Disease (HD) has resulted in a dramatic reduction in
estimated global prevalence of the disease. Recent WHO figures estimate that the worldwide prevalence of HD is 1.26 million cases unevenly distributed among approximately 60 countries[18,19]. As prevalence drops and fewer resources are required for treating HD it is anticipated that health ministries around the world will begin integrating HD care with other health care programs. Mainstreaming HD services could prove beneficial by helping dispel the stigma surrounding HD, however, this strategy may also result in inferior medical care for new and old HD patients, who often require prolonged and diverse treatment and rehabilitation for successful recovery.

In contrast to falling prevalence, global incidence of HD has not changed during the MDT era. Approximately one-half million new cases of leprosy are diagnosed each year. Many of these cases are detected at a stage where permanent and progressive nerve damage has occurred and may lead to significant deformities and disabilities. For example, the proportion of patients presenting with severe disabilities ranged from 2.5% in India to a high of 23.9% in Pakistan in 1996[18]. While it is likely that the predominant type of HD is related to the degree of disabilities found in a given region, the statistics compel us to consider new disease management strategies that will help reduce the development of severe disabilities. Among these are vaccines to prevent infection, efficacious drug regimens minimizing spread of the bacteria to nerves and improved diagnostic criteria to identify patients at the earliest time point following infection.

Overall patient management can be improved as diagnosis of HD becomes more precise. Diagnosis of HD in the earliest stages should mean fewer bacteria in the patient and a better prognosis with effective treatment. Studies comparing relapse rates following MDT show that patients with high BI are at much greater risk for relapse than those with lower BI[20,6] Timely and effective therapy should reduce dissemination of *M. leprae* and potentially reduce nerve involvement and related disabilities. Finally, while there is little data detailing modes of transmission in HD, it is assumed that humans constitute the primary reservoir for dissemination. Therefore, early diagnosis and delivery of effective treatment of HD should shrink the *M. leprae* reservoir and reduce transmission of the disease.

Can early case detection be improved?

The simplest strategy for improving early case detection is through education. First, the public must be convinced that HD is curable and that access to medical care for HD is available. Second, mass campaigns must be mounted and maintained to educate the public to recognize early signs and symptoms of HD. With public acceptance of the facts surrounding treatment and cure of HD earlier diagnosis will become a reality. Because HD is a disease of low incidence, active surveillance is cost-prohibitive and, therefore, not sustainable in most endemic countries. This reality makes self-diag-

nosis even more critical in attempting to identify and treat infected individuals at the earliest time point.

Support for maintaining a well trained medical work force, experienced in diagnosing and managing HD, is also critical for improved case detection in the future. The combination of. decreasing prevalence and integrating HD services with the general health care system will surely result in fewer medical workers trained in diagnosing HD. This is not a trivial matter since leprosy workers have little in the way of simple, objective diagnostic tools. With changing case loads and few objective tests available to aid diagnosis it will be critical for health care workers to maintain expertise in leprosy diagnosis.

To support future improvements in diagnosis and management of HD we need to learn more about the epidemiology of HD as well as the risk factors for developing the disease. Out of this work may come a better understanding of who needs to be monitored for infection and what types of tests need to be applied to help diagnose HD in its early stages. For example, intensified education and regular surveillance of at risk individuals, such as household contacts (HHC) of previously cured HD cases, could be attempted as a focused, early diagnosis strategy. While this approach won't help detect new cases arising from individuals not living with an index case, it should minimize advanced disease in this group.

Objective laboratory tests for diagnosing HD over the past two decades have held much promise but delivered little for the field worker. In general, tests developed to aid diagnosis of HD have been based on detecting a specific immune response to M. *leprae* or identifying *M. leprae-specific* molecules in tissues. In the latter group of tests the most promising approach has been the detection of *M. leprae* DNA or RNA following amplification from patient samples [5,15,17] . These tests have found limited utility for diagnostic purposes, primarily due to expense and degree of sophistication required to implement the tests. They are being applied, however, as epidemiological tools in an attempt to learn more about transmission of *M.* leprae and risk factors for HD.

Diagnosis of Hansen's disease

Diagnostic criteria for HD consist of characteristic dermatological lesions associated with neurological sequelae and acid-fast bacilli in the skin. Symptoms range from a slightly hypopigmented or faintly erythematous ill-defined macule(s) with possible impaired sensation, as seen in indeterminate HD, to multiple symmetrically distributed erythematous or shiny, macules, papules or nodules with loss of eyebrows and eyelashes as seen in advanced cases of lepromatous HD. Skin scrapings for acid-fast bacilli aid in diagnosing cases with large numbers of bacilli in the skin, however, this insensitive tool is relatively ineffective for detecting bacilli in early lesions where few bacteria exist. Availability of pathologists in HD centers generally improves specificity and sensitivity of diagnoses and leads to accurate staging of the disease. Integration of HD services could jeopardize the availability of this valuable resource further intensifying the need for developing simple, effective diagnostic tests which can complement clinical diagnostic criteria for HD.

If we are to diagnose HD in its earliest stages we must understand what happens in the patient prior to clinically apparent signs and symptoms. To answer these questions we need new studies in the following areas: 1) characterization of early dermatologic changes associated with *M. leprae* infection, 2) characterization of initial and chronic changes in nerves following M. *leprae* infection, 3) a better understanding of how *M. leprae* evades innate immunity, and 4) a better definition of early events associated with *M. leprae* stimulation of acquired immunity. Clarifying these events may lead to improved diagnostic tools for early diagnosis and improved treatment strategies capable of reducing nerve damage and interrupting transmission of HD.

Diagnostic tests for Hansen's Disease

Immunological studies have given us defined antigens for use in serologic tests and tests associated with cell-mediated immunity (CMI) to detect infection with *M. leprae*[1,4,9,12,13,14]. Due to constraints related to testing correlates of CMI, emphasis on immunological tests for

diagnosis has been on serologic tests. Genetic studies have described numerous genes and DNA fragments from *M. leprae* which have been used to develop DNA amplification-based tests[2,16,17] to detect the presence of *M. leprae* in tissues. Serologic and DNA-based tests generally have proved effective at detecting multibacillary (MB) disease but paucibacilllary (PB) disease, including early HD of any type, has proven more difficult. Since early disease remains a major obstacle for the diagnostician, improvements in these tests or development of other tests are urgently needed. It would be prudent to keep in mind that extant tests may become more useful as a control measure in the future if clinical diagnostic expertise begins to diminish as a result of perceived eradication of HD as a public health problem.

The primary DNA-based test developed for use in detecting *M. leprae* has been PCR. Early enthusiasm for PCR's use as a diagnostic test for HD were based on impressive levels of sensitivity and specificity. After several years of testing we have concluded that the sensitivity of PCR tests for *M. leprae* DNA in tissues is extremely high (95-100%) but specificity can be quite variable depending on the type of HD or patient material being studied. Many PCR studies using biopsies of untreated PB cases have reported a lower end of specificity of around 40-50%. The lowest specificity we have reported came while reviewing biopsies sent to our institution over the last four years for special study[11]. In a subset of nine AFB-negative biopsies tested for *M. leprae* by PCR only one (11%) biopsy tested positive. These were unique referral cases sent to our center because of their unusual nature at presentation and may not represent AFB-negative PB caseloads in other parts of the world.

In a separate study we examined the role of PCR in the diagnosis of early HD in India[7]. In this study 39 patients suspected of having HD presented with one (n=37), two (n=1), or three (n=1) hypopigmented skin lesions. Final clinical diagnosis by an experienced leprologist ruled out twenty-five of the suspected cases, leaving 14 clinically diagnosed HD patients. The patients were classified by clinical criteria as 1 indeterminate (I), 4 tuberculoid (TT) and 9 borderline tuberculoid (BT) HD cases. Histopathological examination of the 39 suspected cases identified 26 HD patients. Six were indeterminate, 3 TT and 17 BT HD cases. PCR evidence of *M. leprae* DNA was found in 11 biopsies from the HD patient group defined by histopathological criteria (PCR specificity = 42%). Two of the 11 PCR-positive biopsies were reported as nonspecific chronic inflammation by histopathology. A second biopsy was taken from the two patients and one was confirmed by histopathology as indeterminate HD.

By microscopy only 2 biopsies showed AFB, while PCR detected *M. leprae* in 11 specimens. This shows an increased rate in detecting *M. leprae* in PB specimens 5 to 6 times that of histopathology. Sensitivity for PCR in this study was 100% as all nonspecific chronic dermatitis specimens were negative for *M. leprae* DNA. Since histopathological diagnosis of early lesions of HD depend entirely upon the finding of one or more AFB, PCR may provide a useful adjunct in diagnosing early HD.

With the availability of effective antileprosy drugs HD is being rapidly controlled throughout the world. In addition, the profile of leprosy patients presenting in some outpatient clinics is changing with more early lesions encountered. This trend may continue with broader MDT coverage and increased self-diagnosis. Therefore, additional tools like PCR may become important in the field of early diagnosis. Because of the current expense and sophistication required to perform PCR it may be possible to implement its use in reference laboratories with experienced workers. Finally, we suggest that appropriate use of PCR for diagnostic purposes at this time may best be utilized as an adjunct to histopathological studies in patients suspected of having HD from very low endemic areas and from non-endemic countries.

Epidemiological studies using PCR detection of *M. leprae* DNA

A major obstacle facing HD control strategies is our limited understanding of transmission of the disease. If humans constitute the primary reservoir of *M. leprae,* then global coverage of most patients with MDT should begin to affect transmission and eventually lower incidence of new cases. Assuming global MDT coverage can be achieved and maintained

and disease incidence does not fall, other possibilities concerning primary reservoir and disease transmission must be considered. Alternative explanations include but are not limited to 1) the existence of a significant animal or environmental reservoir(s) of *M. leprae* or 2) the existence of an *M. leprae* carrier state (transient or chronic) in humans capable of transmitting the disease to susceptible individuals in the community. Strong evidence exists for an animal reservoir of *M. leprae* with the armadillo in the Americas. However, the degree of significance related to the armadillos role in infecting humans on a large scale is weak. Examples of environmental isolates of *M. leprae* have been reported but little evidence has been provided for the authenticity of the bacteria found or for the universality of the findings.

Studies have been performed to determine the rate at which contacts of index cases test positive by PCR for *M. leprae* in nasal secretions'a'° Our study in Cebu, PI detected *M.* leprae in 3.3% of the household contacts of MB index cases only'. A second study in the capital region of Manila, PI confirmed the absence of PCR-positive contacts associated with PB disease and gave a rate of 6.9% positivity among household contacts of MB index cases. Interestingly, some of the positive contacts were associated with index cases who had either completed MDT or were in the midst of completing treatment. A study by Pattyn, et contacts of MB patients and 1.9% for PB index cases. Klatser, et al.' reported a 2.4% rate of positivity in nasal secretions from contacts of leprosy patients in two villages in Indonesia.

Taken together these reports suggest the possibility that contacts of HD patients carry *M. leprae* in their nasal secretions at some point during the index cases disease. It will be interesting to determine whether *M. leprae* carriage is transient, as in the case of environmental contamination of nasal secretions, or whether in some contacts *M. leprae* carriage reflects actual colonization of tissues resulting in *M.* leprae-laden nasal secretions. These types of studies could help elucidate epidemiological issues concerning transmission of HD and diagnostic issues, should it turn out that true carriers of *M. leprae* may be at risk for developing HD or important in spreading the infection.

Future emphasis for improved early case detection should focus on patient education, training of health care workers, focused surveillance and improved diagnostic tests for HD. Driven by optimistic projections of decreasing incidence and prevalence of HD by the year 2000, potential changes in HD management, such as integration of HD services, underscore the need for new strategies for diagnosis and follow-up of patients. Objective, simple and inexpensive test for early diagnosis and prognosis are crucial to reaching truly effective HD control programs in the world.

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