REPORT OF THE CONSULTATION ON THE EARLY DIAGNOSIS OF LEPROSY


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1. INTRODUCTION

The Consultation on the Early Diagnosis of Leprosy was opened by Dr Najera-Morrondo, Director, Division of Control of Tropical Diseases (CTD). Welcoming the participants, he emphasized how much multidrug therapy (MDT) had contributed to leprosy control in recent years by reducing the reservoir of infection. He also emphasized the need to strengthen early case detection and improve diagnosis of early leprosy. Newer techniques, which also have some promise for general operational use, are becoming available more widely for the detection of *M. leprae* infection. The established techniques are, of course, still vitally important.

Professor Lechat was elected Chairman of the meeting, Dr Job, Co-Chairman and Dr Lucas, Rapporteur. The list of participants is attached as Annex 1.

2. OBJECTIVES OF THE MEETING

The objectives of the meeting were:

1. To review problems in leprosy control with regard to early diagnosis.

2. To review the state of the art in the early diagnosis of leprosy from both clinical and histopathological aspects.

3. To review the state of the art on assays for immunodiagnosis of latent or subclinical leprosy, including the predictive values of immunogenic tests for future disease and their applicability in disease control programmes.

4. To review the progress and prospects of newer diagnostic tools such as DNA probes, including Polymerase Chain Reaction (PCR).

5. To identify cost-effective methods for early case detection and diagnosis that could be applied within leprosy control programmes.

6. To identify research needs for early diagnosis.

3. PRESENTATIONS ON CLINICAL AND HISTOPATHOLOGICAL ASPECTS OF EARLY DIAGNOSIS OF LEPROSY

The first set of presentations dealt with a review of the state of the art on early diagnosis from the clinical and histopathological perspectives.

DR CONVIT described the experience of early leprosy in the Venezuela immunoprophylaxis trial. He distinguished between 'initial lesions' and 'early' leprosy lesions. The former spontaneously regress and are regarded as temporary signs of infection with *M. leprae*. Clinically they include popular erythematous lichenoid lesions, erythematous plaques and nodular erythematous lesions. Histologically, they show tuberculoid granulomas or macrophage clusters, with occasional AFB found in macrophages but not in nerves. These lesions heal to leave only a site of anaesthesia. (These 'initial' lesions correspond to the 'suspect leprosy' lesions delineated in section 5.1.1.B.)
The 'early' lesions persist or progress to determined leprosy unless treated. Clinically, they include hypopigmented macules and plaques, and anaesthetic areas. The histamine test is useful to pick up partial sensory impairment, as is the sweat test with intradermal injection of acetylcholine. Histology shows tuberculoid granulomas or perineural lymphocytosis. AFB, if present, are found inside nerves. Those cases of early leprosy which are Mitsuda skin test negative frequently evolve to multibacillary leprosy; those with positive Mitsuda become tuberculoid.

DR LOMBARDI said that currently 3000 new leprosy cases are detected per annum in Sao Paulo State. Indeterminate leprosy cases have formed 20-30% of new cases for over 50 years. Agreement between clinicians and histopathologists on 'indeterminate' leprosy has historically been uniformly good, and AFB are found in 20% of these cases on biopsy. To pursue this, a retrospective analysis was undertaken of skin biopsies from 70 clinically diagnosed indeterminate leprosy cases. Three histopathologists (Drs Ridley, Leiker and Cohen) reviewed the slides, in addition to the original diagnostic pathologist. Overall, 15% of the cases had AFB in the biopsy sections. The four histological categories were: (A) no evidence of leprosy; (B) possibly leprosy; (C) indeterminate leprosy; (D) tuberculoid leprosy.

The comparable diagnoses of the 70 cases were:

<table>
<thead>
<tr>
<th>Histopathology Category</th>
<th>Pathologists</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>42.9%</td>
</tr>
<tr>
<td>B</td>
<td>34.3%</td>
</tr>
<tr>
<td>C</td>
<td>7.1%</td>
</tr>
<tr>
<td>D</td>
<td>15.7%</td>
</tr>
</tbody>
</table>

The proportion of tuberculoid cases was the same among the four pathologists, but agreement on 'indeterminate' leprosy was very poor. The histopathological diagnosis of indeterminate leprosy is highly subjective, with lack of reproducibility even when several experts examine the same sections. Nonetheless, it is believed that indeterminate leprosy is a well-defined entity and synonymous with the diagnosis of early leprosy. Further histological studies, including newer immunohistochemical techniques, should improve the reliability of diagnosing indeterminate leprosy, and thus help with disease control. Use of immunostaining with anti-S100 antibodies for evidence of cutaneous nerve damage is being analysed in Sao Paulo.

Discussion: In Dr Lombardi's study, one Fite-stained slide was available for identifying AFB. In Venezuela, up to 20 Fite-stained slides are studied in suspect cases. Dr Neerdeen pointed out that this was the first proper study of clinical indeterminate leprosy to show how inadequate the existing diagnostic tools are.
DR PONNIGHAUS presented data from total population surveys carried out in Malawi since 1979. All individuals found with signs of possible infection with M. leprae are reviewed by a medical officer who grades the clinical certainty of the diagnosis of leprosy using a 5 point grading scale. Skin biopsies of all suspicious lesions are taken and the histopathologist reports using a certainty scale for leprosy (i.e., grades 1 = definite; 2 = probable; 3 = possible; 4 = non-specific findings; 5-8 = other skin conditions). It was shown that there is good agreement between clinical and histopathological certainty grades.

Suspects in whom neither the clinician nor histopathologist found sufficient evidence of leprosy to initiate treatment were reviewed during subsequent routine surveys. Depending on the initial clinical certainty grade, up to 22% of suspects had developed leprosy. Overall 33/268 (12.3%) of these suspects had developed the disease over several years.

There were rather disturbing figures showing considerable interobserver variation between four histopathologists studying the same biopsy slides from suspect cases. If two out of four histopathologists consider that a biopsy represents definite leprosy, then the patient is assumed to have the disease. There is a group of 24 patients who were not treated because of lack of initial clinical and/or pathological evidence, but later review of the biopsies indicated leprosy. Ten of these suspects thus had leprosy; four had healed (suggesting a self-healing rate of 40%), and six had progressed, at later clinical review.

The implications for a vaccine trial were discussed. The role of histopathology needs to be seen in the light of the need for maximum sensitivity during the intake phase, and maximum specificity during the follow-up phase.

DR RAMU described the clinical features of early leprosy. Neurological abnormalities are characteristic, may precede skin lesions and are the only manifestations in 18% of patients. Paresthesia may precede visible skin lesions. Then numbness, burning pains, paraesthesia, shooting pains along trunk nerves, blistering due to heat and friction when sensory loss is not appreciated by the patient, and muscle weakness may be found. Arthritis and epistaxis are occasional initial features of multibacillary leprosy.

Early evolved leprosy lesions do not present diagnostic problems if cardinal signs are easily elicited. These are: (1) skin lesions with sensory impairment; (2) thickening of cutaneous or peripheral nerve with impaired sensation in the skin supplied by that nerve; and, (3) APB in the skin. The concordance rate of two clinical observers in diagnosing the type of leprosy depends on the specific type, as shown following based on a study in South India.

<table>
<thead>
<tr>
<th>Leprosy type</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indeterminate</td>
<td>42%</td>
</tr>
<tr>
<td>Maculo-anæsthetic</td>
<td>32%</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>67%</td>
</tr>
<tr>
<td>Polyneuritic</td>
<td>71%</td>
</tr>
<tr>
<td>Borderline</td>
<td>73%</td>
</tr>
</tbody>
</table>

In spite of the limited concordance on the type of leprosy, concordance on the diagnosis of disease itself was quite high.

Monofilament nylon is used to test to touch sensory impairment, with beneficial results from standardization of this test. In very early lesions when touch sensation is intact, impairment of pain and thermal sensation is diagnostic in the majority of cases. Of 52 suspect cases, monofilament nylon provided diagnostic evidence of anaesthesia in 21 cases. In the other 31, immunohistology [see following] was confirmatory.
Preclinical disease has been investigated by means of a serum antibody competition assay for MLO4 antibodies. 37.5% of leprosy contacts positive for MLO4 develop the disease later.

Immunohistochemical identification of M. leprae antigens using anti-BGC and MLO4 monoclonal antibodies in paraffin sections (with PAP immunoperoxidase) has given good results. These immunostains are positive in leprosy cases even when AFB cannot be found on histology.

Discussion: Dr Ponnighaus considered that many of Dr Ramu's cases were advanced, not early leprosy. How does Dr Ramu classify patients with macular anaesthetic lesions as definite leprosy even without nerve thickening? Dr Lombardi said that in Sao Paulo, nerve enlargement, whether of cutaneous or trunk nerves, was not considered 'early' leprosy. Dr Noordeen commented that the use of 'early leprosy' as a diagnosis depended on the purpose for making that diagnosis. In vaccine trials, clinical patient management and leprosy control, there are different demands for sensitivity and specificity in the diagnosis of leprosy. The thresholds of diagnostic criteria and the certainties required for intervention vary. For the purposes of leprosy control, a case of leprosy is one who needs chemotherapy. Professor Lechat said that new definitions of leprosy may be needed since new tools for control have now become available. Operational definitions are imperative.

Dr Job discussed the problems of correlation between clinical and histopathological diagnosis in early leprosy. If there is clinical doubt, it is mandatory to take a biopsy; this may also establish a non-leprosy diagnosis which can vastly alter the patient's life.

The specific pathological changes sought in skin biopsies of early leprosy are:
1. Intraneural AFB; 2. Granulomatus inflammation; and, 3. Neuritis. The evaluation of perineuritis and nerve destruction may be subjective rather than absolute.

During a survey in Micronesia of suspect leprosy cases with a hypopigmented lesion, 259/519 patients' biopsies had 'non-specific' chronic inflammation. They showed inflammation limited to skin appendages, or the superficial perineuromuscular plexi, or to blood vessels through the dermis. Obviously some of these people probably had leprosy, though the diagnosis could not be established.

In established leprosy, there is good agreement between clinical and histopathological diagnoses. However, in early disease there is much disagreement. Leprosy should be diagnosed only when it is 100% certain. In Western countries, clinicians are most reluctant to diagnose leprosy without a positive pathology report. Other criteria for supporting a diagnosis of leprosy that could be used include Phenolic Glycolipid-I (PGL-I) antibodies and PCR for identifying M. leprae DNA. A majority of patients with indeterminate leprosy self-heal with no sequelae, do not need chemotherapy, and run the risk of stigmatization. There is a danger that new tools for identifying M. leprae infection could be incorrectly interpreted to be equivalent to diagnosing the disease leprosy.

Dr LUCAS reviewed the histology of indeterminate leprosy. Many but not all leprosy patients pass through a phase of clinical indeterminate leprosy, characterized by a few macular lesions of varying sensory impairment, but the clinicopathological correlation of this pattern is not reportedly constant around the world. 'Principles and Practice of Leprosy' (1966) quotes rates of indeterminate leprosy among new cases ranging from 0-43%. In South America, there has been much confusion with 'immature' or 'uncharacteristic' leprosy which has been said to comprise 50-70% of new cases and, in surveys of Burmese children with leprosy, 6-65% of cases are said to be indeterminate.
Histological studies have shown almost as much variance. In general, it seems agreed that indeterminate leprosy dermatopathology comprises mild perineurovascular and periappendageal lymphocytosis, without significant tuberculoid granuloma formation or macrophage accumulation. Scanty AFB may be found in the nerves and subepidermal zone, though some reports mention AFB mainly in perivascular macrophages. The significance of intra-epidermal lymphocytosis is uncertain, though some reports emphasize its diagnostic importance and as an indication that bacilli may enter the body via the epidermis. In published series, the proportion of cases that have demonstrable AFB in skin biopsies varies from 1-100%. In some series, up to 31% of biopsies show epithelioid cell foci, which to other observers might suggest a tuberculoid, rather than indeterminate histology. That observers vary [see Dr Lombardi’s presentation, above] is shown in the leprosy histopathology comparability trial using Malawi case material, where among three experienced pathologists, the proportions of slides labelled indeterminate varied from 1.5-21.3%. It is evident that both clinical and histological criteria for diagnosing indeterminate leprosy are not in reality consistent and reproducible.

A personal study of 1986-1989 biopsy material from the Marie Adelaide Leprosy Centre (MALC) in Karachi showed that if the 674 new patients are divided according to % body surface area involved by leprosy lesions, 76% of those with > 5% involvement (75% of the total patients) had AFB in skin biopsies; of those with < 5% area involvement (25% of the patients), only 18% had AFB in biopsies. In the small-area patients, only 20% had histological patterns of indeterminate leprosy (and only 15% of them had AFB); the rest had tuberculoid or lepromatous leprosy.

The histological diagnosis of leprosy in self-reporting patients, such as at the MALC, is far easier (and more certain) than that in surveyed patients, such as in the Malawi epidemiology trial [see Dr Ponnighaus’ presentation].

The confounding problem of contaminant AFB in histological sections (derived from environmental AFB in staining solutions and floating-out water baths) was illustrated. The finding of AFB in a biopsy section does not per se prove the diagnosis of leprosy.

In collaboration with Dr Klatser in Amsterdam, PCR has been used to identify M. leprae DNA in paraffin sections of fixed skin and nerve biopsies, probing for a 36kDa gene. The results have been disappointing in terms of sensitivity (half of untreated MB patients did not produce a positive signal with PCR), and specificity (1/6 non-leprosy dermatitides was positive). Perhaps with fresh tissue, and different probes, better results might follow.

In reporting on early/suspect leprosy cases, there is no agreement on the numbers of H&E and PTA-stained slides to be studied. It is recommended that histology be evaluated blind (without access to clinical data) and that a diagnostic certainty scale be used wherever possible.

Discussion: The usefulness of PCR for M. leprae on slit skin smears has yet to be evaluated. The question of optimal fixation of skin biopsies was raised. Dr Lucas recommended Ridley’s fixative, although its acidity impairs subsequent immunohistochemical staining.

Dr Job said that in Dr Cochrane’s series, 70% of indeterminate leprosy cases self-healed; but there was no proof that all those cases actually had leprosy.
4. PRESENTATIONS ON SEROLOGICAL ASPECTS OF EARLY DIAGNOSIS

DR BRENNAN reviewed the history of serology with PGL-I since its discovery by his group in 1980, and the various neo-antigens and assays that have been developed since then to optimize the immunological assays in terms of specificity, sensitivity and reproducibility. In this connection, he quoted the challenge raised in an earlier IMMLEP meeting where the desired test was expected to be "positive", not only in nearly all multibacillary leprosy but also in over 95% of untreated paucibacillary leprosy and yet "negative" in 99% of individuals from non-leprosy areas but who are BCG vaccinated, have tuberculosis, or have other mycobacterial infections. In terms of actual experience with contacts, studies in Cuba, French Polynesia, Philippines and New Caledonia have shown the positivity rate to vary between 9 to 14%. However, one study in Papua New Guinea indicated a rate of 32%. The predictive value of sero-positive results for future disease in comparison with sero-negative results have also varied from no difference in one study to over 20-fold additional risk for sero-positives in another. However, there is need for more longitudinal studies in large populations. In this connection, it is relevant to draw attention to the recommendations of the WHO Western Pacific Region's Working Group on rapid diagnostic tests for leprosy, which indicates the possibilities of PGL-based assays being applied, (i) as an epidemiological tool for monitoring effectiveness of leprosy control measures; (ii) as an additional tool for monitoring for occurrence of relapse after completion of MDT in MB patients; and (iii) where resources permit for monitoring contacts with its implication for chemoprophylaxis.

Data on anti-neural antibodies is potentially promising, with 19-30% of leprosy patients in Korea seropositive, compared with 7% of TB meningitis patients and 7% of healthy controls. Whether the antibodies really indicate host response to nerve damage, or an auto-immune production of cross-reactive antibodies is uncertain.

In terms of both specificity and sensitivity, tests based on PCR appear to be the only realistic hope for the future. However, there is a need to address reagents for skin tests, especially with the new 14 kDa and 35 kDa proteins. As for the future of assays with PGL-I antibodies themselves, they are likely to be of most value in epidemiological studies and unlikely to be of great benefit for case finding or monitoring chemotherapy.

DR IZUMI presented data on serology using synthetic antigens of PGL-I as studied in leprosy patients and contacts in various sites. The methodology is based on an M. leprae gelatin-particle agglutination test (MPLA). After experimenting with different semisynthetic neoglycoconjugates, the most suitable antigen, at least in the particle agglutination test, was the one with the natural trisaccharide with the linker arm of phenylproprionate conjugated with bovine serum albumin, designated as NT-P-BSA, which had comparable sensitivity and specificity with conventional indirect ELISA (concordance rate more than 90%). The test was found to be positive in leprosy cases, ranging from 33% of tuberculoid to 80% of lepromatous patients. Seven per cent of noncontact (normal) people had low titre positive serology. In a highly endemic area in Indonesia, 39% of the contacts were positive. Preliminary clinical experience with MPLA showed that the test was useful in early diagnosis or differential diagnosis of indeterminate leprosy, particularly if combined with immunopathological staining techniques. In a population where leprosy has a low prevalence, the reliability of a positive PGL antibody is low, unless a high cut-off value is chosen.

DR CELLOMA presented seroepidemiological data from the Philippines using the semi-synthetic antigen ND-O-BSA to represent the PGL-I molecule of M. leprae. 95% of leprosy patients with BI > 4 were seropositive; 54% of those with BI < 2 were seropositive. Of paucibacillary leprosy patients, 84% were seronegative. Of leprosy contacts, 92% were seronegative, irrespective of the index patients' type of leprosy. On follow-up of contacts, only 12% of those who developed leprosy were initially seropositive, i.e., had been predicted. 1.2% of the normal population is seropositive.
Reactivation of paucibacillary disease (bacillary relapse) was predicted by persistent seropositivity or re-seroconversion in some cases, but the numbers were small. Seropositivity declines in treated multibacillary patients, though they remain persistently positive whilst the BI declines to zero.

Of newly discovered leprosy cases, only 20% had index cases in the household (i.e., were contacts). The great majority of normal seropositive people do not develop leprosy.

Dr. Smith gave a review of the global literature on PGL-I serology and presented some results from the Venezuelan immunophylaxis trial. The development of serological tests in leprosy has been conducted with several objectives. Among the aims have been the development of tests to help with the diagnosis of doubtful cases; to identify cases before they manifest clinically and to distinguish those who have been infected subclinically. In theory, the use of such tests to identify very early cases of leprosy for treatment may have an impact on transmission of leprosy.

The problems concern who to screen, the sensitivity and specificity of the tests, and what to do with "positives." A very high specificity is needed. For example, the annual incidence of leprosy is typically of the order of 1/1000 people/year, thus a test with a sensitivity of 100% but a specificity of 95% would identify one true positive for every 50 false positives. It would seem reasonable to expect that a test that is likely to identify early or latent leprosy should be positive in those people with overt disease.

Unfortunately, this is not the case in published studies on antibodies to PGL-I. While in most studies a high proportion of multibacillary patients are "positive," this is not the case for paucibacillary patients, of whom 50% or more may be serologically "negative." In people who have never been exposed to M. leprae, a small proportion are found who have antibodies to PGL-I.

In many populations, if individuals are skin tested with tuberculin, a characteristic bimodal distribution is obtained of the size of tuberculin reactions enabling separation of individuals into infected and non-infected groups. The distribution of PGL-I antibody titres in large studies in Malawi and Venezuela were found to be unimodal. This suggests that either the test is not useful for separating out infected and non-infected groups, or that only a small proportion of those populations had been infected. Also the average antibody titres were found to be higher in females than males and declined with age. These phenomena are unexplained.

Most studies of the predictive value of PGL-I serology have been cross-sectional but a few prospective studies have been reported and in those it is possible to determine if PGL-I antibody positivity predicts later disease. Prospective studies in Tahiti, Philippines, Polynesia and Venezuela all show an increased relative risk (RR) of developing leprosy in people who have raised antibodies to PGL-I, the RR ranging from 2 upwards to over 200 if high titres are selected.

An important observation in the Venezuelan study was that a longer time risk of subsequent leprosy was strongly related to the PGL-I antibody titre, most cases of leprosy arose in individuals whose titres were not greatly elevated. Thus serology was only of very limited value for the early diagnosis of leprosy.

Discussion: Dr. Lucas asked whether all the leprosy patients on whom these basic studies on immunodiagnostics were made actually had leprosy beyond doubt, given the established uncertainty in diagnosis as discussed earlier. Would a 10% diagnostic error rate significantly alter the predictive statistics? Dr. Smith answered that this might affect the apparent sensitivity of the test but would not be expected to affect the specificity of the test.
PROFESSOR LECRAT emphasized the fact that epidemiology requires precise definitions and end-points as well as corresponding measurements. Be it for research or control purposes, different definitions may be acceptable provided they respond to what one proposes to do. One of the major issues is the identification of high risk groups that could be targeted for surveillance, early detection and treatment. It is wrong to talk about subclinical infection and clinical overt disease as two different stages since it is a continuum. The cut-off line will depend on the definition of what one decides leprosy is for a given purpose and on the sensitivity and specificity of diagnostic criteria. The real issue is rather an operational one: who has to be closely watched (who is at risk), and who has to be treated. The matter is further complicated by the fact that in a number of people the suspicious signs spontaneously revert and even self-heal. The predictive values of a positive ELISA or of an indeterminate macula have not been fully assessed. Even operational procedures may influence these predictive values. Self-reporting patients are more likely to present lesions that require treatment than patients who were detected through active surveys. It is therefore questionable whether leprosy needs to be diagnosed at the preclinical stage, and whether it is useful to establish the presence of infection before signs and symptoms appear. The earlier the diagnosis, the less specific and more costly it is. For control purposes, leprosy should be defined as the stage of the leprosy infection when MDT is needed. For research purposes, it can be different. A single AFB in the skin could then be relevant.

Discussion: Dr Ponnighaus questioned deferring the diagnosis of leprosy until it justified MDT. In Malawi, patients in the Leprosy Control Programme (LCP) have a disability rate of 18%, whereas in the Leprosy Evaluation Project (LEP) the disability rate is only 3%. In the latter programme there is greater emphasis on diagnosing leprosy early, and so overdiagnosis of leprosy may be beneficial if it prevents disability. A single dose of rifampicin in suspect leprosy cases may be tried in a trial.

DR LEE (WPRO) mentioned that the regional goal of the Western Pacific Region is the elimination of leprosy as a public health problem by the year 2000. The operational definition of ‘elimination’ is reduction of leprosy prevalence to less than 1/10,000 people, and of incidence to less than 1/100,000 per year. For countries with less than 100,000 population, this means no more than one new case per year. The definition of a case of leprosy is a person requiring chemotherapy (i.e., excluding inactive cases).

The interim goal is to place all cases on MDT by the year 1995. The WHO Regional Office (Manila) is providing drugs, manpower training, epidemiological surveillance, evaluation of leprosy projects in Member States, research support, and development of educational materials. Operational difficulties include: problems of the diagnosis and treatment of leprosy, which is primarily performed by paramedicals; unreliable slit skin smear services; and the follow-up of cases on MDT.

For several years, a research programme of PGL-I serology has been instituted, and recommendations have been made [see Dr Brennan’s presentation, section 4]. The future role of such serology is still unclear: is it operationally useful or useless? Guidance is required.

Discussion: Dr Smith asked what happens to anti-PGL levels in an asymptomatic person with high titres, if given rifampicin. Dr Izumi quoted a Japanese case whose high anti-PGL titres did not change on dapsone, but did decline after daily [sic] rifampicin for a year.

DR ENGERS presented data on new monoclonal antibodies against M. leprae antigens, the current list of cloned genes for mycobacterial antigens and on PCR techniques for identifying M. leprae DNA. These had been discussed at the recent IMMELP meeting.

PCR was now able to identify M. leprae at densities of 100 organisms, and perhaps down to a single bacillus. The optimum primers had not yet been established, but all primers so far tested had given comparable results.
The advantages of PCR in detecting *M. leprae* are:

1. specificity - possibilities exist for distinguishing mycobacterial species;
2. sensitivity;
3. rapidity at 8-18 hours for processing;
4. reproducibility - primers are stable;
5. versatility - the same technology can be used for detecting many different infectious agents, as well as heritable gene components, cancer genes, etc.

The disadvantages of PCR are (currently):

1. cross contamination - dedicated rooms and disposable equipment is needed; the use of nested primers may improve this;
2. quantitation of infectious agents is difficult;
3. sophistication - compared to slit smears and serology;
4. expense - but costs will come down in the future.

The potential research applications of PCR for *M. leprae* detection are:

1. detection of viability, which will help chemotherapy studies in determining duration of treatment and evaluating the efficacy of different drugs and drug combinations;
2. epidemiology of transmission.

The potential applications for disease control are:

1. identification of drug resistance;
2. diagnosis of early relapse;
3. monitoring of *M. leprae* infection in individuals.

So far, PCR appears optimally sensitive for *M. leprae* detection in fresh, unfixed samples. Although it has worked well for other infections in fixed, paraffin-embedded material, preliminary evidence suggests that for leprosy it may lack sensitivity and specificity with fixed tissues [see Dr Lucas' statement above].

Dr Noordeen considered the impact of new tests for leprosy control by asking several sets of questions:

1. Can the test assist in confirming the diagnosis in difficult-to-diagnose early cases? Some related questions are:
   - What proportion of individuals reporting to leprosy clinics are cases with equivocal signs of the disease?
   - In such cases, to what extent is diagnosis capable of being confirmed through histopathology?
   - What proportion of difficult-to-diagnose cases are capable of being confirmed through serology?
2. Can the test identify latent disease, i.e., individuals harbouring *M. leprae* with no clinical evidence but with a very high chance of coming down with clinical disease in the future? Some other related questions that arise are:

What proportion of individuals in endemic areas are sero-positive?

What is the risk (absolute and relative) of future disease in such individuals?

What is the desired method of intervention for individuals at high risk? What are the costs involved?

What is the extent of false positivity and what is its implication in relation to intervention?

3. Can the test identify the end-point of disease for the purpose of stopping chemotherapy? Other related questions are:

Is it necessary to identify specific end-points of disease in individual patients in public health disease control programmes?

What is the experience with treatment regimens having fixed durations?

What levels of sero-positivity should be considered to decide on end-points of disease?

4. Can the test identify patients about to relapse? For instance,

What is the capability of the test to predict relapse with a high degree of reliability?

Can the test predict relapse in paucibacillary leprosy?

How early does seropositivity predict relapse before clinical and bacteriological findings become apparent?

Discussion: It was agreed that household contact tracing does not solve the problem of identifying all new cases. People must be encouraged to come forward with 'early' lesions.

5. GROUP DISCUSSIONS

The clinical and histopathological issues of early diagnosis, and the serodiagnostic issues of early diagnosis were discussed in separate groups and final reports on the subjects, as given below, were adopted at a plenary session.

5.1 Report on clinical and histopathological aspects of early diagnosis of leprosy

5.1.1 Definitions

The following definitions are recommended in relation to diagnosis of leprosy:

A. *Cardinal signs*: they include (a) single or multiple hypopigmented or erythematous lesions not typical of other skin diseases; (b) loss of sensation (thermal, pain and/or touch) with or without a skin lesion; and (c) enlarged nerve, either trunk or cutaneous.
In addition, the following features may be regarded as equivalent to cardinal signs: (d) AFB in slit skin smears after excluding contaminants/artefacts; and (e) definite histopathological evidence of leprosy (e.g., peri and intraneural inflammation and/or evidence of nerve destruction and/or AFB in typical sites).

B. **Suspect leprosy**: Leprosy should be suspected if only one cardinal sign of leprosy is found.

C. **Early leprosy** is present if two cardinal features but no disability are found. In early paucibacillary leprosy the number of lesions should be limited; in early multibacillary leprosy, infiltration should be mild.

D. **Advanced leprosy** is present if lesions are extensive and/or there are disabilities.

E. **Disability** includes nerve trunk anaesthesia; extensive limb anaesthesia; paralysis, visible deformity and/or neuropathic ulcers - i.e., Grades 1 and 2 of the WHO disability grading, as mentioned in the WHO Expert Committee Report on Leprosy, TRS 768.

5.1.2 **Diagnosis of early leprosy**

The aim is to diagnose leprosy as soon as two cardinal features are present.

A. **Clinical diagnosis**

On the clinical side there is room for improvement by standardisation of sensory testing to increase reproducibility.

B. **Bacteriological diagnosis**

Concerning skin smear examination, it is recommended (a) that reliability of slit smear service be improved; (b) that leprosy examination be integrated into other laboratory services, e.g., TB smear services and that centralization of services be explored; (c) that training of technicians and upgrading of laboratories be given priority as well as quality control.

C. **Histopathological diagnosis**

Concerning histopathology it is recommended (a) that it is important to strengthen histopathology by training histopathologists and identifying and setting up referral centres in order to provide diagnostic services for countries that cannot afford or justify local histopathology services, and to encourage histopathology research; and, (b) that research is undertaken to improve early diagnosis of leprosy through newer methods such as immunohistochemical techniques including detection of *M. leprae* antigens (e.g. LAM, glycolipids), and nerve antigens (e.g., S100), and PCR techniques to identify *M. leprae* DNA.

5.1.3 **Diagnosis of suspected leprosy**

A. **Action in control programmes**

In control programmes, if only one cardinal sign can be found, suspects should be put on surveillance. There is a choice between (a) ‘passive’ surveillance (encouraging the suspect to report for review examinations regularly); and (b) ‘active’ surveillance. Depending upon the resources available, surveillance of suspects should be carried out either through active or passive procedures.
B. Research on suspects

The following research is suggested for suspects with one cardinal sign: A double blind placebo controlled trial of a widely applicable intervention such as one dose treatment (for example 1500 mg Rifampicin) or vaccination. In such trials, participants should be lepromin tested and have their PGL-I serology performed. In addition, participants should be followed for at least five years to compare incidence rates of leprosy among treated and untreated groups. Various risk factors (age, sex, BCG scar status, serology, lepromin test result, etc.) should be considered in the analysis.

C. Individuals with vague signs:

If no cardinal sign is found but there remains a certain suspicion, meaning for example a non-typical lesion, a doubtfully enlarged nerve or suggestive symptoms even after thorough examination, including where feasible, histamine test, sweat test and histopathology, it may be necessary, depending on the resources, to have a programme of passive surveillance. Active surveillance for such individuals is not recommended.

5.1.4 Case detection

Early diagnosis should be a part of early case detection which should also be cost-effective. The aim is to encourage people to seek treatment at the stage of 'early leprosy', before disability occurs. This can be carried out through (a) health education; (b) identification of high risk groups; (c) training of medical and paramedical staff, including updating the teaching of leprosy in the curricula of medical and paramedical training; (d) improvement of delivery of service at the peripheral level, e.g., reduction of patient load, better conditions for examining patients.

The cost-effectiveness of the above mentioned methods for making people come forward with early leprosy in any given social context is not known; operational research into these methods must be encouraged.

5.2 Report on serological aspects of early diagnosis

5.2.1 Application of tests for antibodies to PGL-I in leprosy control and research

A. Current state of knowledge including some research issues

A variety of methods are now available to assay levels of PGL-I antibodies in serum samples. These include various forms of ELISA and agglutination assays. Cross-sectional studies of the prevalence of PGL-I antibodies in various groups have demonstrated that most patients (ranging from 100% in several studies down to about 70%) with multibacillary leprosy have elevated levels. In different studies, from 1% to 75% of paucibacillary patients and from 7% to 43% of household contacts have elevated levels. In contrast, elevated levels have been found in from 1% to 33% of those in leprosy endemic areas who have no history of close contact with leprosy cases, and levels between 1% and 5% have been found in those in non-endemic areas. There is considerable variability in the proportions of individuals in the different groups who have "positive" serology, due in part to the adoption of different criteria for positivity; in part to the small sample sizes in some studies and probably also because of differences in the epidemiological situation in different areas. Furthermore, the test procedures employed in different studies have varied considerably and there is a need for greater standardization in test methods to enable comparisons between different studies to be made with more confidence. On the basis of the cross-sectional studies it appears that testing for PGL-I antibodies has high sensitivity for multibacillary cases but only moderate sensitivity for paucibacillary cases. The extent to which the test detects those who are infected with leprosy is unclear and the failure to find a bimodal distribution of antibody levels in
population studies suggests that it may be a poor test for infection. The specificity of the test may be around 95% or less, making it unsatisfactory as a screening test for cases of a disease that, typically, have an annual incidence of 1/1000 or less. The specificity of the test can be increased by raising the cut-off level for "positivity" but this will also lower the sensitivity of the test.

Longitudinal studies of individuals with a relatively high risk of contracting leprosy (i.e., those living in highly endemic areas or the household contacts of cases) have shown that the risk of developing the disease within two or three years can be related to the PGL-I antibody level at the start of follow-up - those with the highest levels being at the highest risk of developing the disease, though in one study in Papua New Guinea no such association was found.

A review of the evidence on the value of PGL-I testing accumulated from studies conducted to date makes it possible to suggest recommendations as to the ways in which testing for antibodies for PGL-I might usefully be incorporated into leprosy control programmes. It is possible to consider situations in which leprosy case-finding is by "passive" and "active" means. In both situations it is considered that serological testing should only be contemplated if there are already good diagnostic facilities for leprosy, especially clinical, and there are good facilities for the treatment of patients. In programmes in which either of these two components is lacking, serological testing for PGL-I antibodies has no place.

B. Serology and passive case finding

In most leprosy control programmes the main way by which new cases are detected is through the examination of individuals who report to a medical facility with symptoms or signs which might be due to leprosy. These individuals are screened by someone experienced in the diagnosis of leprosy and are likely to be classified into one of three broad groups - (i) not leprosy, (ii) definite leprosy (early or advanced) and (iii) suspect leprosy. Serological testing may be of value with respect to those in the third group. The studies briefly summarized above have shown that paucibacillary patients are more likely to have a positive serological test for PGL-I antibodies than those without the disease, but a considerable proportion of such patients have no detectable antibodies to PGL-I. Thus, the diagnostic value of a negative test among those with possible signs of leprosy is not high. However, a positive test will increase the probability of leprosy being the correct diagnosis. Thus in someone who has symptoms and signs suggestive but not diagnostic of leprosy the finding of "high" antibody levels against PGL-I is likely to add to the confidence in the diagnosis of leprosy. If the antibody level is "intermediate" or "low", depending upon the clinical findings and histopathological findings (if available), the decision might be made to keep the individual under surveillance for the later development of clearer signs of disease (periodic serological studies might be included in such surveillance).

The definition of "intermediate" and "high" levels of antibodies is still a research issue. It would be of great value if studies were conducted in which suspected cases of leprosy were carefully followed and the proportions developing leprosy were related to their PGL-I antibody levels at different times (see section 5.1.3.B).

C. Serology and active case finding

In most leprosy control programmes active case finding in whole populations is not undertaken. It is done in some research studies and in some special situations. It is considered that screening whole populations for PGL-I antibodies has no value for leprosy control and is therefore an inappropriate way of using resources, except in very special situations.
Active case finding is undertaken in many control programmes among the household and other close contacts of cases. It would be inappropriate to screen all such contacts for PGL-I antibodies (except for research purposes) and it would only be worth undertaking serological testing for PGL-I in those contacts found to have signs which made leprosy a possible diagnosis. Action following the assay of antibody levels should be the same as outlined for passive case finding.

D. Other research issues in serology

It is suggested above that PGL-I serology may be useful in those in whom there are symptoms and signs of leprosy but for whom the diagnosis is uncertain. The prognostic significance of a high PGL-I antibody level in a person with no symptoms or signs of leprosy is unclear. Some studies have indicated that such persons are at increased risk of developing leprosy, but the specificity of the antibody test is such that these individuals cannot be separated from the many more individuals with high antibody levels who will not develop disease. There is a need for further longitudinal studies of populations in which PGL-I antibodies are assayed periodically and the results related to the subsequent risk of leprosy.

Monitoring the level of PGL-I antibodies in a population may be of value for assessing changes in the level of infection rates with leprosy. While it is clear that there is not a one-to-one correlation between the presence of antibodies to PGL-I in an individual and their history of infection with leprosy, it does seem that higher than average levels of antibodies to PGL-I are found in groups in which all, or a high proportion of individuals, are likely to have been infected (e.g., patients and contacts of patients). Thus, if in a population the incidence of leprosy declines or rises it seems reasonable to expect that the average level of antibodies to PGL-I will show similar directional changes. Of course, in most situations, the best way to monitor this will be to measure the actual incidence rates of leprosy over time, but in some situations this might be difficult or too expensive, or there may be a poor surveillance system. In such situations monitoring antibody levels in a sample of the population may be a viable alternative. Also, because the prevalence of those with antibodies to PGL-I is typically considerably higher than the prevalence of leprosy it may be possible using serology to detect changes in infection rates of a given magnitude using a smaller sample size than would be required to detect corresponding proportionate changes in disease rates.

At present, however, the value of such monitoring has not been validated and studies are required in which changes in the population incidence of leprosy are correlated with changes in the average level of PGL-I antibodies in random samples of the population. It may be that such monitoring would best be done in groups in which the greatest changes were expected (e.g., in young children).

The role of monitoring PGL-I antibodies among patients on or after treatment needs further research. A sudden rise in antibody levels in a patient may be predictive of a relapse. However, the extremely low incidence of relapses among patients on MDT to date makes such research an unattractive prospect.

An interesting finding from the cross-sectional studies is that a high proportion of paucibacillary patients do not have elevated antibody levels to PGL-I. This may be because the test has poor sensitivity in this group of patients, but another possibility is that there are other factors which differ between seropositive and seronegative patients (e.g., liability to relapse after chemotherapy; bacillary load). Further studies to identify such characteristics would be of interest.
5.2.2 Recent developments on new biological tools and techniques

In the next few years a range of new biological tools and techniques that may have value for research on leprosy and its control, are likely to become available. Many of these are at a relatively undeveloped stage and detailed speculation on their potential value may not be profitable. These include DNA and RNA probes with or without PCR, and synthetic, recombinant and native antigens; detected either directly or indirectly, for use in skin tests and for serological testing for antibodies and antigen. Research aimed at the development of more sensitive assays for PGL-I antigen offer considerable potential for a more specific test for the detection of infection. Continuing research on nerve antibody detection also seems to deserve priority. Tests based on the use of PCR are likely to be among those likely to become available soon and studies are necessary to evaluate their potential value for disease control - though it seems that for several years, at least, this will be only a research tool.

Among the most exciting recent developments has been the indication that it might be possible to develop a test for the viability of leprosy bacilli using PCR techniques. This would enable the cumbersome mouse footpad technique to be replaced for this assay, for example, when evaluating new chemotherapeutic compounds in clinical trials. For other organisms, PCR methods have been used to develop tests for resistance to drugs. Relapse rates under MDT are very low and the immediate need for such assays are not great for leprosy drugs, but resistance may become more of a problem in the future and the development of suitable tests for this will be of considerable value.

PCR and other methods may be developed that will provide good measures of infection, incidence and prevalence rates and assays for bacilli in different body tissues and in the environment. This will require that cross-sectional and longitudinal studies are set up in leprosy-endemic areas so that the relevant parameters can be measured in individuals and changes over time correlated with the occurrence of disease.

6. MAJOR RECOMMENDATIONS

1. With increasing coverage for treatment with MDT of already registered leprosy cases in many countries, high priority should now be given to bring under treatment patients not yet identified. Case detection efforts by various means therefore need to be intensified.

2. Case detection efforts should be combined with known and reliable diagnostic procedures applied by well-trained personnel, and should be part and parcel of leprosy control through effective MDT.

3. Individuals reporting to leprosy clinics should be clearly categorized into: (a) advanced leprosy; (b) early leprosy; (c) suspect leprosy; and (d) those with no evidence of leprosy, and dealt with appropriately.

4. Efforts should be made wherever necessary to improve the quality of skin smear services.

5. Although routine histopathology of all cases may not be necessary, histopathology is a useful additional tool for establishing the diagnosis in suspect cases when it is feasible.

6. With currently available information, it is recognized that PGL-I serodiagnosis has applications in leprosy control in limited situations, such as: (a) aiding in the diagnosis of suspect cases, and (b) monitoring trends of infection in communities under certain conditions.
Towards further promotion of better diagnosis and control of leprosy, research efforts need to be directed at: (a) investigation of predictors for development of leprosy amongst suspect cases, e.g., serology, skin testing; (b) investigation of possible interventions in suspect cases; (c) investigation of the significance of relatively high PGL-I antibody levels in normal individuals; (d) investigation of the determinants of PGL-I seropositivity among paucibacillary patients; and (e) further development of newer diagnostic tools such as PCR which offer prospects, among others, for a more sensitive and specific test for infection and a test for viability of M. leprae in specimens.
CONSULTATION ON THE EARLY DIAGNOSIS OF LEPROSY

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