WORKSHOP 6: PATHOLOGY

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Summary and recommendations. Pathology continues to make an important contribution to the study and control of leprosy. Although the histopathology of leprosy is well known, there are a number of areas in which recent advances have been made and some in which the pathologist should respond to advances in other disciplines. In leprosy pathology, as in many other fields of leprosy research, there is a need to distinguish cause from consequence. The Workshop makes the following recommendations:

1. We recommend the application of standard diagnostic criteria for early leprosy.

2. Planning and implementation of all new trials of treatment should include a pathologist.

3. For the distinction between relapse and reaction, demonstration of solid-staining acid-fast bacilli (AFB) appears to be the most reliable histological criterion.

4. There is an urgent need for detailed pathogenetic studies of the Lucio phenomenon.

5. In view of the global pandemic of HIV infection, the relationship between AIDS and leprosy requires careful clinico-pathological study.

Early leprosy. The diagnosis of early leprosy is often uncertain clinically and histologically. However, there are many patients in which histology either confirms the suspicion of leprosy or provides an alternative diagnosis. Histological examination of clinically indeterminate lesions and lesions suspicious of leprosy is an important diagnostic tool. However, individual pathologists differ widely in the certainty with which they diagnose leprosy, and there is clearly a need for standardization of diagnostic criteria. The following criteria should be applied for the diagnosis of biopsies from clinically suspicious lesions of leprosy:

a) A diagnosis of early leprosy can be given if one or both of the following criteria are satisfied: i) presence of convincing AFB: The participants agreed that a minimum of six serial sections of every biopsy should be stained by an appropriate modified Ziehl-Neelsen method, such as the Wade-Fite or Fite-Faraco for AFB. Certain sites are more likely to harbor AFB and should be searched in the following order: nerve bundles, subepidermal zone, arrector pili muscle, and areas of inflammation. ii) presence of endoneurial inflammatory cells (lymphocytes and macrophages usually predominant), preferably with disruption of neural architecture.

b) A report of findings compatible with leprosy should be given if the following suggestive features are present: i) a chronic mononuclear cell inflammatory infiltrate with a superficial and deep dermal pattern surrounding nerves, vessels and adnexae, without neural disruption or AFB. In such cases, re-examination for AFB and possibly further biopsy is indicated.

Procedures which may be of help include the use of sections cut at deeper levels to determine the relationship of inflammatory foci to nerve. Special staining procedures (e.g., S100 and neuron-specific enolase for nerves) and the demonstration of mycobacterial antigens may be helpful with appropriate controls, but require further evaluation.

Biopsies should generally be taken from the edge of the lesion, but in lesions less than 1.5-cm in diameter, a central biopsy

is more likely to be diagnostic. The diagnosis and classification of established leprosy is not usually problematic. In evaluating the differential diagnosis of granulomatous dermatitis the above principles apply. Clinical consultation is an important part of the diagnostic process.

Evolution of disease. Several presentations addressed the issue of disease progression in skin, nose and nerve. There is scope for further research in this area and for sequential biopsy studies. Bacilliferous lesions may be present in the nasal mucosa and nerve prior to the development of skin lesions. It is possible that early nerve damage occurs in the absence of bacilli, but further work on the pathogenesis of nerve destruction before, during and after treatment is required, ethical considerations permitting. In particular, the pathogenesis of progressive neural deficit and fibrosis following cessation of therapy requires elucidation. The interaction between *Mycobacterium leprae* and endothelial cells may be an important determinant of localization and lesion development. Further work is also required in this area.

Reactions. The basic criterion for diagnosis of erythema nodosum leprosum (ENL) (infiltration by neutrophils) is well known but in a proportion of cases with clinical evidence of ENL, no neutrophils are present. Participants felt that this discordance might be due to timing of the biopsy and the importance of changes in vascular dynamics which are not seen by the pathologist. ENL has an appreciable mortality rate and in these severe cases, ENL lesions are often found in internal organs at necropsy. The pathogenesis of ENL requires clarification in relation to neuritis, glomerulonephritis, iridocyclitis, arthritis, testicular involvement, amyloidosis, immune complexes and reversal reaction.

The clinical and histological features of the Lucio phenomenon have been fully described, but the pathogenesis of this reactional state remains obscure. Clearly, vascular bacillation and associated thrombotic phenomena are important. However, the genetic and immunological factors involved need substantial research investment.

The histological diagnosis of reversal reaction is often difficult and does not appear to correlate well with clinical signs. This may reflect the lack of histological features associated with erythema and induration. However, immunological changes such as increased IL2R or HLA-DR expression and CD4+ lymphocyte infiltration can be seen and quantified in histological sections. Reversal reaction may represent qualitative or quantitative immunological differences among patients. An effort should be made to distinguish between these two possibilities. The relative importance of other factors such as disease load, treatment, timing of biopsy, sex and age has yet to be determined.

The participants were unanimous in their opinion that relapse could only be reliably distinguished from reversal reaction following MDT when solid-staining AFB are demonstrated. The most difficult biopsies are those in which there are no bacilli and the appearances are of the tuberculoid type.

Monitoring treatment. In clinical trials, the response to therapy should be monitored by histological as well as clinical, bacteriological, and immunological means. There was concern at the lack of histological evaluation in several current chemotherapeutic trials. The inclusion of a pathologist at the planning stage of these trials is a necessity.

Recent studies have demonstrated the utility of sequential biopsies to measure many parameters including granuloma fraction, bacterial index, antigen load, and cell surface antigen expression. These can be used as surrogate markers of response to chemotherapeutic and immunochemotherapeutic regimens.

After completion of MDT, there is often persistence of foam cells without solid bacilli (MBL) or epithelioid granulomas (PBL) in the absence of clinical activity. The significance of these changes is not understood, and there is no consensus as to whether histological normality should be a condition for the release of patients from treatment.

Systemic involvement. Systemic involvement in leprosy is common, particularly in lepromatous patients. Regression in the skin may occur during treatment without complete clearance of visceral, ocular and neural bacilli, leading to later relapse. Involvement of the larynx and testis are of

particular importance. Secondary amyloidosis occurs, can best be diagnosed by the biopsy of minor salivary glands, and may regress following treatment.

Eye involvement is common, but usually seen by the pathologist at a late stage in its development. There is a need to follow ocular changes by regular examination during treatment and to obtain material for pathology where possible.

The pandemic of HIV infection and AIDS involves many leprosy-endemic areas. The effects of HIV on leprosy are not yet clear and require further clinico-pathological study.

There is need for all tissue removed from leprosy patients to be sent for pathological examination and for further necropsy studies to be performed.

Training, quality assurance and audit. The Workshop identified a lack of trained leprosy pathologists, particularly in endemic countries. There is an urgent need to educate practicing and trainee pathologists in the diagnosis and classification of leprosy. One method is the organization of regular training workshops. In many countries technical standards of skin section preparation require improvement. This could best be achieved by visiting senior technical staff from existing laboratories. Achievement of these aims could be assisted by the development of quality assurance and audit schemes for histopathology laboratories.