This advanced course on histopathology of leprosy is based on the illustrated manual "Leprosy in the light skin" by D. L. Leiker and E. Nunzi, published by AIFO (Italy).

This course is dedicated to both of them, Prof. Dick L. Leiker and Prof. Enrico Nunzi, for all their work linked to leprosy.

The course has 38 slides and would require about one to two hours for completion.

Click on the "start" below to begin the course.
Most cases of leprosy can be diagnosed and classified without histopathological examination. However, examination of a biopsy specimen for histopathology can be a valuable aid to differential diagnosis and for detailed classification of the disease. It plays an important role in research as well.

Leprosy is one disease, yet it may like many diseases. It is caused by Mycobacterium leprae & manifests differently in different persons according to the immunological response of their bodies to the M. leprae. Ridley and Jopling proposed a classification system based on the different resistance of patients to M. leprae, shown in the picture here.
The different clinical forms through which leprosy manifests are called spectrum of the disease that goes from indeterminate (I), to tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL) and lepromatous (LL).

Each of these forms is accompanied by specific histopathological picture. Thus towards TT end of the spectrum, histopathology shows epitheloid cells, langhans giant cells and lymphocytes, while towards LL end of the spectrum, there are more foamy macrophages, foreign body giant cells and band of Unna.
| Local anaesthesia for biopsy | **BIOPSY TAKING:** The specimen should be taken from the most active part of the lesion after local anaesthesia. When no active looking lesions are present or in case of ill defined lesions, the biopsy should be taken from centre of the lesion and in well-defined lesions, from the edges of the lesion. |
BIOPSY TAKING: Although the taking of surgical biopsies is preferable, punch biopsies are acceptable, provided that the specimens include a part of the subcutis and are handled with great care.

Punch biopsy
BIOPSY TAKING: The tissue is fetched at the edge only, with a fine forceps, lifted, and cut as deep as possible with fine scissors.

Specimens should be handled with fine forceps at the outer edge only. In roughly handled, crushed specimens, the cells are distorted and they can not be identified properly.
A superficial biopsy specimen does not give all the required information.

**BIOPSY TAKING:** A superficial biopsy specimen may not be diagnostic because, not rarely, the typical signs of leprosy are absent in the superficial layers of the skin.

This picture shows the superficial part of a biopsy. It shows slight non-specific perivascular lymphocytic infiltration around neurovascular bundles and sweat glands in the deeper corium.

Because cellular infiltration of nerves was not found so this biopsy is not diagnostic of leprosy.
A deeper biopsy specimen containing deep cutis and subcutis is needed for diagnosis of leprosy.

BIOPSY TAKING: The typical signs of leprosy are clearly present in deep cutis and subcutis.

An examination of the deeper part of the same biopsy shown in the last page shows three enlarged and infiltrated nerves in the subcutaneous fat, which are diagnostic of leprosy.

The diagnosis of leprosy would have been missed if only a superficial biopsy specimen had been taken.
BIOPSY FIXATION & STAINING: It is possible to fix the specimens in 4% formalin (diluted 1:10, 40% stock solution) and then stain them with Ziehl Neelsen or Trichrome Masson-Fite-Faraco (TRIFF) method. In this way the bacilli are stained well but also cause shrinkage of tissue so examining cells and assessing edema are more difficult.

On the other hand fixation of specimen with Zenker's or Formaldehyde-mercury-glacial acid fixative (FMA) followed by Ziel Neelsen or TRIFF staining give much better results. However, to use these fixatives, it is necessary that specimens are transferred in 70% alcohol within 24 hours of the biopsy.
HISTOPATHOLOGICAL CRITERIA FOR DIAGNOSIS OF LEPROSY:

- Presence of cellular infiltration of nerve branches, often associated with thickening of nerve branches and destruction of nerve fibres & presence of intracellular acid-fast bacilli, in particular within nerve branches.
- However, in many cases of leprosy, acid-fast bacilli are not found. The distribution of cellular infiltrate around nerve branches and neurovascular bundles, hair follicles, sebaceous glands and sweat glands is sufficient to suspect leprosy but is not diagnostic.
- The finding of a few lymphocytes in a nerve branch, together with some proliferation of Schwann cells and a single intra-neural acid-fast bacillus is diagnostic of leprosy.

Abundant *M. leprae* in a hair follicle, which shows signs of degeneration
M. leprae in a smooth muscle (M. errector pili) without cellular reaction.

M. leprae in the endothelial cells and in the lumen of a blood vessel, showing haematogeneous dissemination of bacilli.
M. leprae in the lumen of a sweat duct. Such bacilli may reach the surface of the skin.

Indeterminate leprosy (I): The cellular infiltrate consists of lymphocytes only. They are located in or around nerve branches and around other appendages of skin.

In the slide, lymphocytic infiltration around a neurovascular bundle in the corium.
Indeterminate leprosy (I): Acid-fast bacilli are usually absent or few. The finding of higher number of bacilli indicates a development towards a lepromatous form of leprosy. The finding of epithelioid cells indicates a development towards a tuberculoid form of leprosy.

In the slide, the nerve shows slight proliferation of Schwann cells, a few perineural and intraneural lymphocytes and a single leprosy bacillus (arrow).

Tuberculoid leprosy (TT): The infiltrate consists of islands of epithelioid cells surrounded by a dense mass of lymphocytes. The infiltrates are located mainly in the superficial corium, pressing against the epidermis, with absence of an infiltrate free sub-epidermal zone and with flattening of the rete pegs. Langhans giant cells are often present.

In the slide, a dense epithelioid-tuberculoid infiltrate in the upper corium, not separated from the epidermis by a free zone. A few giant cells are present. The patchy parakeratosis and edema indicate a recent reversal reaction. Bacilli are absent.
Tuberculoid leprosy (TT): The infiltrate consists of islands of epithelioid cells surrounded by a dense mass of lymphocytes. The infiltrates are located mainly in the superficial corium, pressing against the epidermis, with absence of an infiltrate free sub-epidermal zone and with flattening of the rete pegs. Langhans giant cells are often present.

In the slide, similar type of infiltrate as in the previous slide but without any parakeratosis. Bacilli are absent.

Tuberculoid leprosy (TT): IN the slide, tuberculoid leprosy of a nerve in the subcutis. The nerve fibres are replaced by dense epithelioid cell infiltrate. The nerve is swollen and perineural infiltration is present. Bacilli are absent.
Borderline Tuberculoid leprosy (BT): The infiltrates consist of epithelioid cells and lymphocytes as in TT leprosy but the epithelioid tuberculoid infiltrates are often less well developed and often lymphocytes are scattered between the epithelioid cells. The number of lymphocytes vary, but are less than in TT leprosy.

The infiltrates are separated partly or completely from the epidermis by a zone free from infiltration. Frequently the superficially located infiltrates are largely lymphocytic, whereas in deeper corium, epithelioid-tuberculoid infiltrate may be present.

Nerve branches are infiltrated. Acid-fast bacilli may be present or absent, but large numbers are uncommon.
Borderline Tuberculoid leprosy (BT): In the slide, the dense epitheloid-tuberculoid in the upper corium is separated from the epidermis by a free zone. No bacilli were found in the infiltrates, but small number of bacilli were present in nerve branches.

Borderline Tuberculoid leprosy (BT): In the slide, BT leprosy in subsiding phase. The epithelioid-tuberculoid infiltrate is separated from the epidermis by a free zone. The epithelioid cells show vacuolization and degeneration. A single Langhans giant cell is present. Bacilli were not found.
Borderline Tuberculoid leprosy (BT): In the slide, BT leprosy after a reversal reaction in subsiding phase. The infiltrate has become largely lymphocytic, with only a few, degenerated epithelioid cells, and a Langerhans giant cell.

Borderline Tuberculoid leprosy (BT): In the slide, BT leprosy in a nerve. The nerve is surrounded by an epithelioid-tuberculoid infiltrate. The nerve sheath is heavily infiltrated but the nerve fibres are not completely destroyed: a few bacilli were found in the nerve.
Borderline leprosy (BB): The infiltrate consists largely of undifferentiated histiocytes and lymphocytes. Lymphocytes increase especially during reactive states. Small number of epithelioid cells may be present but epithelioid-tubercules as seen in TT and BT leprosy are usually absent.

The subepidermal zone is free of infiltrate. Acid-fast bacilli can be easily found. Some of the histiocytes contain large number of bacilli but globi (big bundles of bacilli) are absent.

Borderline leprosy (BB): The infiltrates, separated from the epidermis by a free zone, consist of undifferentiated histiocytes and scattered lymphocytes. Fairly large number of bacilli are found, suggesting a development towards borderline-lepromatous leprosy.
Borderline leprosy (BB): BB leprosy in a nerve. The nerve is surrounded by a dense infiltrate consisting of undifferentiated histiocytes and large numbers of lymphocytes. The nerve sheath is infiltrated, but there is only mild endoneural lymphocytic infiltration. Several bacilli were found in nerves and in cellular infiltrates.

Borderline lepromatous leprosy (BL): The infiltrates are round or band shaped containing slightly vacuolated macrophages and varying numbers of lymphocytes. The sub-epidermal zone is free from infiltrate.

Large number of bacilli are present but globi are usually absent or few in numbers and small. Even with large number of bacilli in the nerve branches, there is relatively little destruction of nerve fibres, except in a very late stage.
Borderline lepromatous leprosy (BL): The infiltrate has increased numbers of lymphocytes. The numbers of bacilli are high. A few small globi are present.

Borderline lepromatous leprosy (BL): A higher magnification of the previous slide showing undifferentiated histiocytes with scattered lymphocytes and bunches of bacilli, without globi.
Lepromatous leprosy (LL): The infiltrates consist of vacuolated macrophages, containing abundant bacilli. Globi are present. In more advanced stages, giant vacuoles and very large globi are seen. Lymphocytes are few or absent. Foreign body giant cells may be present occasionally in advanced cases.

The subepidermal zone is free from infiltrate. The nerve sheaths are laminated (onion peel appearance). As compared with large number of bacilli in nerve branches, there is relatively little cellular infiltrate and relatively little destruction of nerve fibres, at least in early stages.

Lepromatous leprosy (LL): In this advanced case, the slide shows abundant bacilli including globi. Only a few lymphocytes are present.
Lepromatous leprosy (LL): In this advanced case, the slide shows massive infiltration of foamy macrophages, filled with bacilli and few lymphocytes.

Lepromatous leprosy (LL): In this case, the slide shows infiltrate with foamy macrophages, containing granular bacilli only. The number of lymphocytes is rather high, as a result of recent ENL reaction that is subsiding.
Lepromatous leprosy (LL): LL leprosy in a nerve. The nerve shows the presence of large number of bacilli but with little cellular reaction. Lymphocytes are virtually absent. The nerve sheath is infiltrated and laminated.

Lepromatous leprosy (LL): LL leprosy in a nerve. The nerve shows the typical lamination (onion peel appearance). Abundant bacilli are present in the surrounding foamy cell infiltrate, in the nerve sheath and intraneurally. Very few lymphocytes are seen. In this very advanced stage of the disease the nerve fibres are not yet completely destroyed.
Histoid variety of Lepromatous leprosy (LL): Histoid leprosy usually occurs after a relapse. The infiltrate consists of a rounded mass of elongated macrophages which grow in whorls as in a fibroma. The macrophages contain large numbers of bacilli, mainly in vacuoles in the polar ends of the cells.

Bacilli may form bunches but globi are absent. Varying numbers of lymphocytes are present, but not very many. The connective tissue around nodus is compressed and may form a pseudo-capsule with sometimes necrosis in centre of nodus.

Histoid variety of Lepromatous leprosy (LL): A higher magnification of the section shows spindle shaped macrophages, containing large number of bacilli, which are largely located at the polar ends of the cells. The great majority of the bacilli are morphologically similar. In this case they are intact or only slightly fragmented.
Thank you for completing the online course on histopathology of leprosy.

Your comments and suggestions about improving the course will be greatly appreciated. They will also help us to improve this course. Please send an email to <sunil.Deepak@aifo.it>