

BIOPSY TECHNIQUES IN THE DIAGNOSIS AND TREATMENT OF MOUTH DISEASES AND TUMOURS

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BIOPSY is the removal of tissue from a living patient for microscopic examination and diagnosis. Professor Ruge in 1879 is credited with the introduction of the biopsy. The biopsy is our most accurate and reliable check on our clinical diagnosis. It is our greatest aid to diagnosis.

INDICATIONS

The aim of doing a biopsy is to establish an accurate diagnosis as early as possible in order that correct treatment may be initiated with the least possible delay. A biopsy is indicated whenever a careful clinical examination along with the history fails to lead to a correct diagnosis. Any ulcer, excluding well-known entities such as periadenitis mucosa necrotica recurrens (PMNR), which with treatment does not show evidence of healing in one week should be subjected to microscopic examination. All oral precancerous conditions should be submitted to histopathological studies to determine the premalignancy index scores. A biopsy should be carried out on any growth or swelling suspected of being a neoplasm. In cases of patients with oral squamous cell carcinoma a biopsy should be done not only to confirm the clinical diagnosis but also to assess the histological features which influence the prognosis. Any tissue surgically excised or any tissue spontaneously expelled from a body orifice should be examined under the microscope. It is also desirable to do a microscopic examination of material from a persistent draining sinus, the source of which cannot be readily identified, and along with some of the lining of the sinus. A biopsy should be done on any intraosseous jaw lesion which cannot be positively diagnosed by radiographs. A biopsy is indicated when lesions fail to respond to recognized therapy within a reasonable period of time or when there is doubt about the clinical diagnosis.

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TYPES OF BIOPSY

Incisional biopsy is the removal of a portion of the lesion along with some normal tissue for identification. It is best to remove the tissue as a small wedge of pyramid with the point in the connective tissue and the triangular base at the surface. Incisional biopsy is contraindicated in cases of suspected haemangioma. *Excisional biopsy* is the complete removal of the lesion in question by circumscribing it in such a way that it is totally excised. *Aspiration biopsy* is done by inserting a large-gauge needle into the softer or semifluid in consistency areas of a mass. Strong negative pressure is created which draws fluid and cells into the needle. This technique as a preliminary procedure to exclude intraosseous haemangiomas is mandatory. Aspiration and examination of fluid contents in cases of odontogenic cysts are also useful. A soluble protein level of below 4.8 gm per 100 ml of cystic fluid is strong evidence that the lesion is a primordial cyst. Further-more microscopic examination of aspirate consisting of cheesy-like material in primordial cysts will demonstrate the presence of parakeratin or keratin cells.

In skilled hands, *needle biopsy* of bone tumours has a definite use, particularly in cases where the site of the lesion, or the treatment to be adopted make open biopsy difficult or undesirable. Material can be obtained for imprint preparations, paraffin sections of for histochemical studies. Specimens for biopsy can be obtained from the mouth by the use of a suitably modified Ellis *biopsy drill*, which fits into a straight dental handpiece. This instrument can be used to obtain specimens 1.2 cm long and 0.14 cm in diameter. It is particularly useful for obtaining biopsy material from central fibroosseous lesions in the jaws, being easy, safe, and suitable for use under local anaesthesia on out-patients. However, the specimen obtained may be so small that it may not be truly representative, whilst the heat which may be generated during the procedure, unless great care is exercised, may cause distortion. A negative drill biopsy must never be accepted, for it is very easy to miss the lesion when inserting the drill.

Curetage is the removal of small bits of tissue

with a sharp instrument. Curettement is used to remove tissue from a bony cavity such as a dental socket, a sinus tract, or a body space such as the maxillary antrum. *Tissue imprints* provide an excellent means of studying the cells of tumours of the lymphoreticular system. Imprints are made of the tissue biopsy by gently touching the freshly cut surface on clean glass slides. The imprints may then be air dried, fixed in methyl alcohol and stained with one of the standard haematological Romanovsky methods. Not all lymphomas give equally satisfactory preparations by this technique. If the tumour has an abundant reticulin or fibrous tissue stroma, few cells may come off onto the imprint, or they may be distorted or disrupted. Most lymphomas, however, give satisfactory preparations and the cells of Burkitt's lymphoma biopsies usually separate easily from each other, making excellent imprint preparations.

Exfoliative cytologic smears have been useful in the diagnosis of lesions of herpes simplex infection, herpes zoster, pemphigus vulgaris, benign familial pemphigus, keratosis follicularis, hereditary benign intraepithelial dyskeratosis, white sponge nevus and pernicious and sickle cell anaemias. *Tissue smears* from the mouth and stained with periodic acid Schiff (PAS) will readily help in the diagnosis of oral candidosis. *Frozen sections* are seldom used for oral diagnosis, but where histochemical methods are envisaged, sections of unfixed tissue cut in a cryostat may be required.

BASIC PRINCIPLES

The scalpel is the instrument of choice, since it cleanly removes the tissue and does not dehydrate it as the cautery or the high-frequency cutting knife may. This latter instrument is of great value, however, in dealing with vascular lesions, where it controls bleeding at the biopsy site. Always use a sharp scalpel to avoid tearing tissue. Do not paint the surface of the area to be biopsied with gentian violet, iodine or a highly coloured antiseptic. Infact, there is no need to apply antiseptics prior to biopsy. If using infiltration anaesthesia do not inject anaesthetic solution directly into the lesion as this procedure tends to distort the tissue. It would be best to inject around the periphery of the lesion.

It is desirable to take an adequate amount of tissue from the most clinically suspicious areas to help in the prompt histopathological diagnosis. The site for biopsy should be chosen with care otherwise the percentage of value one obtains from the histopathological diagnosis will decrease accordingly. Moreover one must select a site that has not been subjected to trauma or

to irritation from previous treatment. In the majority of cases the margin of the normal and abnormal is suitable for it helps in the orientation of the specimen and avoids necrotic areas in the centre. Also the pathologist must assess microscopically the manner in which the disease process interacts with the surrounding tissue with respect to such features as invasiveness, encapsulation, and the like, thus necessitating a small border of normal tissue. If the lesion is small it would be better to do an excision biopsy by removing it along with a margin of normal tissue. No good purpose is served by submitting a patient to two operations if one will suffice.

An adequate amount of tissue must be removed for purposes of biopsy. It must always be remembered that excised tissue shrinks and the excised specimen should be at least $1.0 \times 0.5 \times 0.5$ cm. in size. Biopsies that appear to give large specimens in the mouth often produce disappointingly small specimens in the bottle of 10% formal saline and even smaller sections on the slide. Thin, deep wedge sections taken from the substance of the lesion across the border into the normal tissue are more desirable than large shallow specimens taken from the surface. Surface specimens often provide only necrotic tissue or crust and do not represent the deeper, more typical character of a lesion. Moreover the frequency of infection in oral lesions often lead to difficulties in interpretation. Removal of bits of necrotic tissue or the cutting of superficial specimens usually results in a negative or an inconclusive pathology report.

Biopsy specimens should not be ruined by being crushed by the use of toothed forceps or haemostats. This complication can be avoided by transfixing the lesion with one or more sutures, which can then be used to move and control the specimen and to tense the soft tissues during its removal. When forceps are used to grasp the specimen be careful not to mutilate it. Similarly, the use of electrocautery to take a biopsy is usually contraindicated as it is deleterious to the interpretation of the biopsied tissue. Although it is desirable for surgery because it aids haemostasis, the effect of the cautery is to boil tissue fluid and precipitate protein. Microscopically, such tissue shows a coagulated and torn appearance and makes histologic evaluation impossible in the cauterized areas. Fix the tissue immediately upon removal in 10% formal saline. If the specimen is thin, place it upon a piece of blotting paper and drop it into the fixative. This will prevent curling of tissue. If the suspected malignant tumour is related to bone care should be taken not to penetrate the periosteum when a biopsy specimen is removed. When a

biopsy specimen is being taken from the buccal mucosa in cases of suspected lichen planus, sites other than the occlusal line must be used. Frictional keratosis is often found in this area and it may modify the microscopic picture.

In speckled leukoplakias it is better to take the biopsy from the atrophic red areas which are more ominous. Since a prickle cell can either form keratin or divide and it cannot do both at the same time the part which is likely to show the most advanced premalignant change is any area that resembles granulation tissue or red atrophic areas. Small patches are better removed in their entirety. A high percentage of speckled leukoplakias have a superimposed candidial infection. It is best to treat the candidosis before surgical excision for with such treatment the speckled leukoplakias often shrink in size thus reducing in size the area to be excised. In multiple or large leukoplakias or erythroplakias biopsies should be taken from several places because they may show varying premalignancy index scores (PMI scores). Erythroplakias very often on microscopic examination prove to be carcinoma-in-situ. A biopsy specimen must be taken from the most highly suspicious site and should be sufficiently deep enough. Oral tissue biopsies for diagnosing leukaemias are useless and only add to the patient's distress. Generally speaking, when a choice exists, areas to be avoided are those nearest to bone, cartilage or teeth, near large blood vessels, where the tumour bed is shallowest and sites of necrosis.

DISCUSSION

All excised surgical specimens should be sent for pathological examination, even when the doctor is certain that his clinical diagnosis is correct and that the lesion is benign. In the vast majority of instances the histological examination merely serves to confirm the clinical diagnosis but even experienced clinicians are occasionally surprised by the findings of the pathologist. The omission of this essential procedure could result in the patient's life being jeopardized. Furthermore, it is a valuable self-teaching diagnostic aid. The repeated microscopic verification or correction of clinical impressions improve diagnostic acumen. A positive biopsy report is the only acceptable diagnosis of malignant tumours and this is important when the clinician has to tell either the patient or his relatives the nature of the condition. It is also essential for the purposes of treatment, records and research. A histological diagnosis forms a permanent record and is invaluable on those occasions when it becomes necessary to confirm that an excised lesion was benign. Thus a patient who is found to have a

cancer may give a history of having had a tumour removed say from the tongue some time before. It will then become important to determine whether the malignant tumour is either a primary growth, a recurrence or a metastatic deposit related to the earlier tongue tumour, before the prognosis of patient can be assessed or a treatment plan prepared. In malignant tumours for example squamous cell carcinoma the histological appearances of the lesion may be of great importance in determining the prognosis of the patient and may provide some indications of the likely response of the tumour to radiotherapy. The histopathology examination will also tell whether the entire cancer has been removed or whether additional surgery is required.

On the rare occasions upon which the histological diagnosis and the clinical diagnosis do not agree, a full discussion between the surgeon and the oral pathologist will usually either resolve the problem or indicate further investigations which may clarify the picture. Ideally the oral pathologist should form part of the team for the management of tumours so that his expertise can be utilised to determine the site and extent of biopsy. When a lesion appears to be malignant on clinical grounds but the biopsy fails to confirm the suspicions of the surgeon, it means only that there are no features to suggest the expected diagnosis in that particular piece of tissue which was removed at that particular time. A repeat biopsy should always be performed when there is any doubt about the adequacy or representative nature of the original specimen. It is worth bearing in mind that the clinician is able to study the patient first hand and must always maintain a high index of cancer suspicion. Whereas the pathologist must depend on the clinical data submitted by the surgeon and diagnose only what he sees on a microscopic slide. He cannot diagnose the cancer that is left behind on the patient.

The diagnosis of bony lesions should be established by a combination of clinical, radiological and pathological investigation, supplemented when necessary by biochemical and haematological studies. The levels of calcium, phosphorus, alkaline phosphatase, and acid phosphatase in the serum are important in some conditions e.g. hyperparathyroidism, Paget's disease, metastatic carcinoma and myeloma. Plasma and urinary proteins, urinary catecholamines, and bone marrow cytology are important in myeloma and metastatic neuroblastoma respectively, and haematological investigation is essential in malignant lymphomas and leukaemic involvement of bone. Radiological investigation is always of the utmost importance, and it must be emphasized that the

relevant radiological information should always be available to the pathologist when he is asked to make a histological diagnosis of a bony lesion. Open surgical biopsy is the technique usually adopted. A generous sample of tissue, preferably from the growing margin of the tumour, should be obtained, care being taken to obtain a representative sample of the lesion and to avoid restricting the specimen to necrotic tissue or to reactive tissue outside the margin of the tumour.

SUMMARY

The biopsy is the most accurate and reliable

check on one's clinical diagnosis. It is the greatest aid to oral diagnosis. The indications for oral biopsy, types of biopsy techniques applicable to diagnosis and treatment of oral diseases and tumours and principles that will enhance the value of a biopsy and make it meaningful to the doctor and patient are emphasized.

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