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TISSUE PATHWAYS FOR INFLAMMATORY AND NON-NEOPLASTIC DERMATOSES AND NON-NEOPLASTIC LESIONS

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1 Staffing and workload

Many cases of inflammatory dermatoses require detailed clinicopathological correlation to establish an accurate diagnosis. Although some clinical information is usually provided on specimen request forms, this may prove insufficient to establish a definitive diagnosis or differential diagnoses. To ensure adequate clinicopathological correlation, these cases should therefore be reported in situations where there is easy access to the submitting clinician, patient's records and ideally the possibility to examine the patient, on either an outpatient or inpatient basis, in consultation with clinical colleagues. Clinicopathological correlation can be assisted significantly by information being conveyed on either body diagrams or by clinical photographs, the latter feasibly also being transmitted electronically. For similar reasons, cases should be reported in an environment that permits discussion at regular clinicopathological meetings. The latter may be of teleconferencing type.

It is useful have a lead sub-specialist or consultant with a special interest in inflammatory dermatoses to whom queries over non-neoplastic dermatoses can be directed. The requirement for adequate clinicopathological correlation should be reflected in direct clinical care programmed activities in job plans.

Some larger centres offer an 'out of normal hours' service to provide urgent diagnostic opinions on frozen sections.

As well as participation in general histopathology external quality assessment (EQA) schemes containing dermatopathology, consultant histopathologists within the United Kingdom and Republic of Ireland can participate in the National Specialist Dermatopathology EQA (www.nsdeqa.co.uk). Performance monitoring in the scheme is set at the level of knowledge, skills and competence of a specialist dermatopathologist serving NICE Specialist Skin Cancer MDTs and the equivalent level in inflammatory dermatoses.

Histopathologists with a special interest in dermatopathology can obtain qualifications in dermatopathology offered by the Royal College of Pathologists and/or European Union of Medical Societies. They are also encouraged to join relevant national bodies such as the British Society for Dermatopathology, the Melanoma Study Group and the UK Cutaneous Lymphoma Group.

2 Laboratory facilities

As well as standard paraffin-embedding and immunohistology service, those reporting inflammatory and non-neoplastic dermatoses should also have access to the following facilities, as necessary on an individual patient basis. This may be on a local, network, regional or supraregional basis.

2.1 Frozen sections

These have a useful role in providing rapid opinions on severe and life-threatening blistering disorders.

2.2 Direct immunofluorescence

This has an important diagnostic role in blistering disorders, lupus erythematosus and vasculitis.

Network centralisation can be cost-effective, improve quality and facilitated by the use of a transport medium.

2.3 Molecular biology

Molecular biology is important in the distinction between reactive and neoplastic cutaneous lymphoproliferative disorders and difficult infective disorders including mycobacterial, leishmania and viral infections. It is also important in some bullous disorders and genodermatoses.

2.4 Transmission electron microscopy

Transmission electron microscopy is the most sensitive and specific method to diagnose cutaneous amyloidosis. In general, it exceeds both histochemistry and immunohistology. Transmission electron microscopy also has a significant role in the investigation of infections, matrix disorders (collagen and elastic tissue), hyperkeratinisation disorders, some blistering disorders and histiocytoses. Scanning electron microscopy is important for some hair shaft structural abnormalities.

X-ray microanalysis may be necessary to diagnose diseases with particulate elemental deposition.

2.5 Secondary and tertiary referral

Difficult and uncertain cutaneous haemoproliferative/lymphoproliferative disorders must be referred to the Cancer Network Specialist Skin Cancer MDT for central histopathological review and discussion according to NICE and Department of Health Cancer Peer Review Measures.

Some non-neoplastic dermatoses warrant referral to tertiary/supraregional specialised centres. In particular, this relates to blistering disorders such as epidermolysis bullosa, matrix disorders and genodermatoses.

2.6 Biochemical investigations

These may be indicated in Ehlers-Danlos disease, ichthyosis and Fabry's disease, with referral to tertiary (supraregional) centres.

3 Specimen submission and dissection

It is important to share with clinical colleagues the dermatopathological information contained in The Royal College of Pathologist's *Histopathology and Cytopathology of Limited or No Clinical Value*.[2]

Under 'Skin biopsies', this states:

i. In secondary care, plastic surgeons in many units triage specimens, which they send for histopathology. This applies in particular to small (3 mm or less) multiple skin tags. This can be supported.

ii. In primary care, there is a widespread good practice clinical consensus that general practitioners undertaking minor surgery and general practitioners with a specialist interest in dermatology should submit all tissue removed for histopathological examination. This requirement is often part of local protocols to accredit service provision, as endorsed by NICE (*Improving Outcomes in Patients with Skin Tumours*), to ensure that any case of skin pre-cancer or cancer is not missed. In view of the low risk, however, it would appear reasonable that small (3 mm or less) multiple skin tags are submitted in one specimen container.

Further to this publication, it is noted that there has been medicolegal endorsement for the necessity of general practitioners to submit all tissue removed at minor surgery for histopathological examination.

Clinical colleagues may need to be reminded of the minimum requirement for clinical details on request forms. This should include the duration of the disease process, number, size, shape, colour and distribution of lesions, with any clinical symptomatology such as pruritis or blistering. This information can be facilitated by clinical body diagrams or clinical photographs. References for previous biopsies and relevant past medical history should be provided together with current favoured differential diagnoses.

The majority of specimens are submitted in formalin fixative.

Fresh unfixed material is sent for direct immunofluorescence investigation. The sending clinician must ensure that the specimen is free of Category 3 risk. Prior liaison is made with the laboratory if the specimen for direct immunofluorescence is to be used for split skin investigation on a blistering

disorder. Specific transportation media are available to permit direct immunofluorescence specimens to be sent through the normal postal service.

Ideally, specimens for transmission electron microscopy are taken separately and fixed in an appropriate fixative such as glutaraldehyde. Reasonable transmission electron microscopy analysis can, however, be achieved from a formalin fixed specimen. In the least ideal situation, transmission electron microscopy can be attempted from paraffin embedded material.

The investigation of hair loss can be facilitated by examining both vertical and horizontal sections. In particular, horizontal sections are the only accurate way to provide information relating to hair numbers and loss per unit area. On that basis, clinical colleagues are encouraged to submit two biopsies in cases of this type. If only a single specimen is received, those of sufficient size can be bisected and processed separately for horizontal and vertical sections.

There is an increasing clinical tendency for the use of small punch biopsies to investigate inflammatory and non-neoplastic dermatoses. Clinicians must be advised, however, on aspects of specimen adequacy in relation to specific diseases. In some instances, this may necessitate broader and/or deeper biopsies to achieve a successful diagnosis, e.g. in cutaneous lymphoproliferative disorders, panniculitis and medium-sized vessel vasculitis. It may be necessary to biopsy the active edge of the lesion (e.g. porokeratosis) or to provide a biopsy of control unaffected skin (e.g. morphoea).

Inflammatory dermatoses

Specimens in the category of inflammatory dermatoses should, if necessary, be cut in their long axis. Ideally, to avoid tangential cutting problems, no more than two pieces of tissue are contained per block. Inking can be used to ensure correct orientation, for example, in blocks for horizontal sections in hair loss.

Differential diagnoses offered by the clinician are important but their value must be judged in the context of the training and experience of the person submitting the specimen. This can be particularly relevant to some specimens received from primary care and especially for those relating to inflammatory dermatoses. In the latter instance, it may be appropriate to recommend, in the report, clinical referral to secondary care, to achieve improved clinicopathological correlation at the specialist level.

Non-neoplastic lesions

The number of blocks necessary for routine common non-neoplastic lesions (such as cysts, lipomas, seborrhoeic warts, etc.) to a degree depends on specimen size and any perceived atypical macroscopic features but often one block will suffice. There should, however, be a low threshold when indicated for increasing block numbers and embedding the whole specimen. This is particularly so for specimens that are suspicious of neoplasia and for possible melanocytic lesions with a suggestion of macroscopic atypia. In general, blocks are taken in the transverse axis providing the best assessment of the perceived nearest margins.

4 Embedding options

If identifiable, the epidermis for inflammatory dermatoses is embedded parallel to the long axis of the slides.

Laboratory systems must be in place to ensure that specimens for horizontal sectioning for hair loss are correctly embedded.

5 Sectioning

Most inflammatory and some non-neoplastic skin biopsies will need to be examined by more than one section. This is particularly important for small pieces of tissue. Usually this will be up to three to six haematoxylin and eosin (H&E) sections. This can be considerably more for horizontal sections in cases of hair-loss, to ensure that the hair-follicles are seen in their entirety. Depending on local practice, this can be by serial sections, 50–100 µm levels, step sections on microtome settings or a locally preferred combination.

Whether these are requested at the time of cut-up or after having examined an index H&E remains a personal choice. Although H&E levels requested at the time of cut up can provide a more timely service, this decision should be balanced against problems that may be encountered at a later stage. In particular, insufficient material may be left in the paraffin block for detailed histochemistry, immunohistology and other investigations (such as molecular biology). This is particularly relevant in laboratories using rapid processing procedures because of the frequent requirement for no more than 2.5 mm thick tissue blocks. In this situation, there is a higher risk for insufficient material being left in the paraffin block for additional work. When requesting H&E levels at the time of cut up, unstained spares can be requested but this does have extra cost implications and the requirement for storage facilities. Decisions in this area must also take into account the technical experience of laboratory staff and their ability to provide H&E levels and still leave sufficient material in the block for further use.

In cases with a clinical query of possible squamous pre-cancer or basal cell carcinoma, it should be routine practice to undertake levels/step sections before the specimen is reported as non-neoplastic.

6 Staining

Appropriate special stains are well described in classic textbooks of dermatopathology.[3] It is generally regarded as professionally prudent to examine H&E sections prior to requesting special stains, although at times there may be a clinical indication to support this at specimen dissection. The investigation of alopecia, for example, can be facilitated at specimen dissection by a request for stains to demonstrate potential scarring, fungi and broken hair shaft remnants.

It is an individual decision whether a fungal stain (such as diastase-periodic acid Schiff) is requested as routine on all inflammatory dermatoses. This could be regarded as inappropriate, however, if a definitive non-infective diagnosis is apparent on the index H&E slide. Fungal stains must, however, be requested in high-risk situations such as a clinical query with regard to fungal infection, clinical lesions that are round and expanding, immunosuppressed patients, neutrophils in the epidermis, oral and genital lesions, and so-called invisible dermatoses.

There is debate as to whether a connective tissue stain (such as elastic-van Gieson) should be requested routinely, although this practice is adopted in some centres.

Stains to use in the context of so-called invisible dermatoses include those for amyloid, mast cells, connective tissue, mucin and iron.

There must be a low threshold for using special stains for the investigation of infective disorders in all immuno-compromised individuals and in those of identified Category 3 risk.

The examination of biopsies for hair loss can be facilitated by connective tissue stains to demonstrate scarring, a Ziehl-Neelsen stain to show hair shaft structures and a fungal stain.

Periodic acid-Schiff (PAS) can facilitate the demonstration of thickened basement membrane in diseases such as lupus erythematosus and lichen sclerosus.

Mucin stains such as PAS, alcian blue at different pHs and toluidine blue can be used in the investigation of dermatoses demonstrating mucin deposition.

The deposition of calcium phosphate in pseudoxanthoma elasticum can be facilitated by the von Kossa stain.

7 Further investigations

Polarised light examination can be useful in identifying exogenous material and in some hair shaft disorders (eg trichothiodystrophy).

Immunohistochemistry can aid the diagnosis of lymphoproliferative disorders, cutaneous infections and some bullous disorders.

8 Report content

In general, reports should contain a descriptive account of the dermatopathological changes present and a discussion resulting in a definitive diagnosis or differential diagnoses. Some centres favour a brief final comment incorporating site, type of biopsy (if known) and concluding diagnosis/differential diagnoses. It is important that any previous related biopsies are reviewed and referenced in the report. Guidance should be given with regard to the necessity for re-biopsy, further clinicopathological correlation, discussion at a meeting, request for more information or an external specialist opinion.

The Royal College of Pathologist's *Histopathology and Cytopathology of Limited or No Clinical Value* makes the following comment on margins in benign lesions:

"Reporting of excision margins on a benign lesion should be limited to those having clinical relevance for potential recurrence and/or as specifically requested by a local clinician or agreed in local protocol."

It should be noted that many clinicians increasingly now request information as to whether benign melanocytic lesions have been excised adequately/completely, in view of the risk of recurrence from shave excisions and the possibility of pseudomelanoma in the recurrence.

It is appropriate, for governance reasons, to mention in the report, any aspects that may have resulted in limited diagnostic interpretation. This could include, for example, insufficient clinical information, tissue size or tissue depth.

9 References

1. NICE. *Improving Outcomes for People with Skin Tumours including Melanoma*. London: NICE, 2006. http://guidance.nice.org.uk/csgstim

2. The Royal College of Pathologists. *Histopathology and Cytopathology of limited or no clinical value (2nd edition)*. London: The Royal College of Pathologists, 2005. www.rcpath.org/resources/pdf/HOLNCV-2ndEdition.pdf

3. McKee PH, Calonje E, Granter SR. *Pathology of the Skin with Clinical Correlations (3rd edition).* Philadelphia: Elsevier Mosby, 2005.