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ANATOMIC PATHOLOGY

Inking a specimen without the mess

Shinde, V; Phelan, C; Gater, W; Thomas, J

Some specimens—neck dissections, rectal tumours or salivary gland tumours—require inking not for orientation but purely for assessment of the surgical margin. Surgical margins are assessed traditionally by painting with India ink or other coloured ink; however, ink obscures the view of the trimming pathologist, spreads onto the cut surface and stains the trimming board and trimming tools. Authors describe a technique of “inking” a specimen with plain gelatine, which is a translucent, colourless substance. This enables the pathologist to have a better view and at the same time prevents the mess.

Method

The gelatine solution is prepared by adding approximately 6 ml distilled water to 4 g gelatine powder (VWR International, Poole, UK). This solution is then heated in a microwave (800 Watt) for 10 seconds at 10% power and stirred thoroughly to produce a viscous solution.

The specimen is dried with a soft paper towel. The gelatine solution is then applied thinly on the specimen with a paintbrush. Industrial methylated spirit is squirted on the inked surface to fix the gelatine. Trimming can then start immediately. Any unused gelatine is stored at approximately 50°C in a dry-block heater to maintain its viscosity until required.

As gelatine is made of proteins, it takes up eosin during the staining process and appears as bright homogenous pink under the microscope.

Discussion

India ink and other coloured ink are used widely in painting margins of surgical specimens. There are disadvantages of using ink: it obscures the view of the trimming pathologist; and as coloured inks are pigments suspended in an aqueous medium, they spread onto the cut surface of the specimen and stain the trimming board. Use of coloured gelatine has been described in guidelines for examination of colorectal cancer resection specimens. This again conceals the tissue underneath. In our technique however, we use plain gelatine without mixing it with a coloured dye. This technique is not practised widely and no articles describing it were found in a Medline search. There are several advantages of painting specimens with plain gelatine: it is a colourless, translucent substance through which structures such as lymph nodes can be identified during trimming; its viscosity prevents its spread onto the cut surface of the specimen; it does not stain the trimming bench and tools; and as it is made up of proteins, it takes up eosin during the staining process and appears bright pink, making it easy to see the surgical margin under the microscope.

Authors conclude that use of plain gelatine to paint surgical margins of specimens is superior to the use of coloured ink or coloured gelatine.

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A new grossing knife with two parallel blades for preparing uniform thickness gross tissue sections

Yang, J; Chen, X; Su, B; Zhao, S; Qiang, L

Grossing, a term that refers to examination and dissection of surgical specimens, along with preparation of sections from those tissues requiring processing, is the initial step in surgical pathology dissection. According to the textbooks and manuals of surgical pathology dissection, the thickness of gross tissue section is 2–4 mm. Every pathologist is familiar with the frustrations of trying to submit uniform thickness tissue sections. Through grossing is a simple job, the process is typically a time-consuming, hands-on process and is all too often not carried out appropriately.

The hollow structure and cystic specimens (eg, oesophagus, stomach, bowel, appendix and gallbladder) are the more frequently encountered specimens in the surgical pathology laboratory. Although the specimens are all anatomically simple hollow or saccular structures, they consist of several layers of different structures (outer surface and inner surface, and muscular wall or soft tissues wall), which often slide over each other during sampling. In practice, it is difficult to obtain representative full-thickness tissue sections of specimens (such as stomach mucosa, wall and serosa) through the full thickness of the lesion and the underlying wall by using a traditional cutter.

In order to resolve this problem, the authors have designed a new grossing knife (or dissection knife) for grossing, which allows standardisation and greatly facilitates easy and rapid preparation of optimal uniform thickness tissue sections. The grossing knife is composed of a handle and a head with two parallel slots for supporting two essentially parallel blades. The gap between the two parallel blades is predetermined at 3 mm to form a tissue-receiving gap.

The sampling steps are as follows:

- According to surgical pathological dissection principles, after the specimen has been opened, the lesion areas to be sampled are detected.
- While sliding the grossing knife along the surface through the lesion areas from one end of the specimen to the other, gross desired representative uniform thickness tissue sections (3 mm thickness) are produced through the tissue-receiving gap.
- The last step is to hand-cut the representative slice sample to fit into the embedding mould; maximum area will be about 22 mm.

The knife has been used for a long time for sampling sundry specimens. By using it, gross desired uniform thickness tissue sections (3 mm in thickness) are produced through the tissue-receiving gap easily and quickly. The knife can not only be used for sampling uniform thickness tissue sections from hollow structures and cystic specimens, but also from solid organs, by changing different length of blades.

In summary, the grossing knife is an accurate, reliable, user-friendly instrument for sampling uniform-thickness slices of tissue, especially for full-thickness tissue sections from hollow or cystic structural specimens. It allows standardisation and greatly facilitates grossing by providing tissue sections consistently uniform in thickness, and can be used in all surgical pathology laboratories by staff pathologists, pathologists' assistants, histotechnologists, residents, etc.

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Technical pitfalls potentially affecting diagnoses in immunohistochemistry

Bussolati, G, Leonardo, E

Result of the immunohistochemical reactions routinely used in diagnostic surgical pathology should be properly interpreted, since false results, related to technical and interpretative pitfalls may lead to incorrect diagnosis. The main sources of such pitfalls are reviewed, analytically described and related to different steps (fixation, tissue processing and embedding, decalcification, antigen retrieval) which may affect the accuracy of immunohistochemistry. In addition, the presence of endogenous enzyme activity, improper binding of avidin to endogenous biotin, incorrect use of antibodies, chromogen and detection systems, as well as incorrect interpretation may produce unreliable data. The high frequency and extension of such pitfalls make mandatory the use of internal and external controls and adoption of cross-validation programmes. The present study, supported by an extensive review of the related literature, is intended as a guideline leading to proper interpretation of immunohistochemical data, an essential component of the diagnostic process. Experience on the antigen retrieval procedures for different antigens is also presented.

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A practical approach to the understanding and diagnosis of lymphoma: an assessment of the WHO classification based on immunoarchitecture and immuno-ontogenic principles

Tan LH.

This review aims to interrelate the major lymphoma types in the current World Health Organization (WHO) classification to construct a framework for understanding and diagnostic application. Multiple morphological, phenotypical and molecular genotypical data are assessed in order to categorise lymphomas into germinal centre (GC) and extracentric (EC) subgroups. GC entities [lymphocyte-predominant Hodgkin, follicular, Burkitt's, angioimmunoblastic T-cell and diffuse large B-cell lymphoma (DLBCL) with GC profile] express bcl-6, CD10 and/or the GC-homing chemokine CXCL13, and harbour ongoing somatic hypermutations (SHM), but not Epstein-Barr virus (EBV) in its higher latency states. Post-GC entities [classical Hodgkin, marginal zone and lymphoplasmacytic lymphomas, half of chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma (SLL), DLBCL with 'activated' or post-GC profile, primary effusion lymphoma, plasmacytoma and myeloma] express, instead, MUM.1 and/or CD138, harbour static rather than ongoing SHM, and may harbour EBV in higher latency states. The remainder of CLL/SLL and the majority of mantle cell lymphoma without SHM constitute the pre-GC ('naive') category, with coexpression of IgD and CD5. Lymphomas can be categorised across lineage (B- or T-cell) and relationship against host immune response (Hodgkin or non-Hodgkin) into GC and EC groups, affording leverage in their differential diagnosis.

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Patterns of liver infiltration in lymphoproliferative disease

D Baumhoer, A Tzankov, S Dirnhofer, L Tornillo & L M Terracciano

Aims: Liver involvement is a common finding in patients suffering from lymphoproliferative disease, and histopathological patterns of infiltration vary according to lymphoma subtype. Data correlating the form of liver involvement with distinct lymphoma subtypes is, however, scarce. The aim was to review 89 liver biopsies diagnosed with lymphoma infiltration and evaluate the infiltration patterns.

Methods and results: In equivocal cases, additional immunohistochemical and molecular pathology analyses were performed to differentiate between neoplastic and reactive cell infiltrates and to classify the lymphoma subtypes. Diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukaemia (CLL), Hodgkin's lymphoma (HL) and Burkitt lymphoma (BL) were the most prevalent subtypes in our series, which included 14 different lymphoma entities in total. Whereas DLBCL and BL predominantly demonstrated tumour nodules deranging the

normal hepatic architecture, CLL and HL mostly showed infiltration of the portal fields. Interestingly, distinct lymphoma entities, particularly marginal zone B-cell lymphomas (MZL) and HL, commonly revealed lympho-epithelial lesions of bile ducts, which were observed in 10% of all investigated cases. Four cases, initially interpreted as T-cell lymphomas, proved to be reactive T-cell lesions.

Conclusions: Distinct lymphoma subtypes show characteristic patterns of liver infiltration. Additional molecular analyses can support diagnosis by verification of clonality or detection of characteristic genetic aberrations.

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CYTOPATHOLOGY

The gross appearances of fine needle aspiration cytology samples

Mayall, F G; Cormack, A; McAnulty, K; Darlington, A

Aims: This study set out to photograph and describe the gross appearances of fine needle aspiration (FNA) cytology samples of commonly encountered lesions.

Methods: During a 2 year period, a cytopathologist photographed the gross appearances of near patient FNA samples, concentrating on commonly encountered lesions.

Results: The gross appearances are described, accompanied by photographic illustrations.

Conclusions: This paper describes and illustrates the gross appearances of FNA cytology samples of some commonly encountered lesions.

Pathologists and other clinicians performing fine needle aspirations (FNAs) are aware that it is often possible to assess the diagnostic utility of the material obtained from its gross appearances. In addition in some cases it is possible to suggest a specific diagnosis from the gross appearances. Some of these specific diagnoses are straightforward to recognise. Others require more experience. There is very little in the literature about the gross appearances FNA samples. This is partly a consequence of the practical difficulties of photographing these samples before they are altered by processing and staining.

In this study we set out to photograph and describe the gross characteristics of FNA samples from some commonly encountered lesions.

METHODS

The study took place in a tertiary hospital in rural New Zealand. Most FNAs were performed by an experienced cytopathologist in a dedicated FNA outpatients' suite. Image-guided FNAs were performed by a radiologist, with an experienced cytopathologist in attendance. It is our practice to give an FNA gross material grade to samples according to their gross appearance: grade 1, probably inadequate diagnostic material; grade 2, possibly contains diagnostic material; grade 3, probably contains diagnostic material; and grade 4, material suggesting a specific diagnosis (eg, "grade 4, suggests a pleomorphic adenoma"). An alternative system for the macroscopic grading of (pulmonary) FNA samples has been described, using five grades, and this has been reported to give a good indication of likely cytological diagnostic yield.

Our study concentrated mainly on "grade 3" and "grade 4" samples. Those considered to be of particular interest were digitally photographed using a Nikon Coolpix 950 in the outpatients' FNA suite or the radiology department prior to staining and preliminary near patient microscopic examination. This camera is able focus on objects as close as 20 mm from its objective lens.

RESULTS

FNA gross material grade 3 (probably contains diagnostic material)

The sample is often thick and pale with a consistency similar to cream. On smearing it has a glistening granular appearance, the granules being confluent at the thick end of the smear and more dispersed at the thin end of the smear. When necrotic material is obtained the granules are less evident. Cellular material from lymphoid lesions is described below.

Colloid nodule or cyst (thyroid)

Colloid cysts of the thyroid yield material that ranges from being watery straw-coloured fluid to being thick golden fluid. The latter produces "cracked pavement" colloid on microscopic examination. The former gives rise to fine thread-like colloid on microscopic examination. When the thick fluid is dried it has an appearance similar to a film of matt varnish. Often the golden fluid is seen in the hub of the needle at the start of the aspiration but becomes blood stained as the FNA progresses. For reasons that are not clear this blood appears to have a particularly bright "arterial" colour. Colloid cysts often contain historic haemorrhage (the associated sudden enlargement and tenderness being the reason for presentation). The appearance of this haemorrhagic colloid depends on the interval between the haemorrhage and the FNA. Initially the material has an appearance indistinguishable from haemorrhage introduced by the FNA procedure, except that there is haemorrhage evenly throughout the aspirate. As the time interval between haemorrhage and aspirate increases so the haemorrhage discolours, becoming darker and eventually chocolate-like. It is often easier to appreciate the degenerate nature of the blood

by gross examination than by microscopic examination. It should be remembered that an encysted papillary carcinoma could yield material with a similar appearance to a haemorrhagic colloid cyst.

Epidermoid cyst

This has a bright white flaky appearance and a strong distinctive odour akin to old socks. This is perhaps the only occasion in FNA cytology in which smell contributes to the diagnosis. It is notable that squamous carcinoma does not have this odour.

Squamous carcinoma

Less well-differentiated squamous carcinoma has a less characteristic appearance. The fluid is clear or straw coloured with a mucoid quality that allows it to cling to the needle as the needle is drawn from it. Often “snow flakes” of white keratin can be seen suspended in the fluid. Novices at performing FNAs tend to misinterpret the mucoid quality of the material being indicative of mucinous lesion.

Pleomorphic adenoma

This material has a grey or slightly purple mucoid appearance that does not cling to the needle to form “strings” and produces a coarsely granular smear. Often the differential diagnosis is an enlarged lymph node and it is usually easy to distinguish between the gross appearances of these two types of tissue.

Lymphoid tissue

FNA material from a cellular lymphoid lesion is similar to cellular material from a carcinoma except that when the material is spread on the slide it yields a smooth film rather than a granular film, perhaps a manifestation of the lack of cohesion between the cells. However, this is a subtle distinction. There is no consistent difference between a benign node and a lymphoma, except that an overtly enlarged suspicious node that yields no material despite a determined FNA attempt is suggestive of nodular sclerosing Hodgkin lymphoma or some other fibrotic process such as a mediastinal large B cell lymphoma, and a reactive node often gives rise to rather watery material. The latter may be a result of oedema.

Pus

Pus hardly needs describing, as its appearances are well known. It may be watery or thick and creamy, with a yellow or greenish colour. Sometimes it is haemorrhagic.

Mucinous carcinoma and low-grade mucoepidermoid carcinoma

The material may range from thin “stringy” mucus to thicker more solid material that may have a “lumpy” appearance and may crumble into granular material on smearing. The authors’ experience of mucoepidermoid carcinoma is limited. One assumes that high-grade mucoepidermoid carcinoma yields less overtly mucinous material.

Tuberculosis

Usually the amount of material obtained is small. The material is snow white and thick. When smeared it is granular.

Lipoma

Lipomas give rise to a sample of fatty globules, sometimes mixed with a small amount of blood. The typical appearance of a lipoma with altered fat due to traumatic fat necrosis. The latter has a creamy, rather than clear appearance, as the degenerate fat becomes emulsified into minute droplets. With FNA samples from lipomas and other fatty specimens, the globules of fat are often best seen in the fresh specimen, and may float away during fixation and staining of the slide. The fat is denser than methanol and sinks to the bottom of the jar. In aqueous stains the fat residue floats on the surface. Novice FNA practitioners frequently become confused by fatty specimens as they are impossible to “dry” with a hairdryer for Diff Quik staining despite the most determined effort.

Take-home messages

- It is often possible to determine the cellularity of a fine needle aspiration (FNA) specimen from its gross appearance.
- Some lesions yield FNA samples with a characteristic gross appearance that can be recognised by experienced FNA practitioners.

Suture debris

Experienced FNA practitioners will appreciate that it is often possible to recognise a suture granuloma by tactile sensation during the FNA procedure. The needle is felt to catch on the suture material and there may even be an associated “clicking” sound. In addition suture debris may be seen microscopically. It may also be seen macroscopically on rare occasions.

Pigmented material

Novice FNA practitioners may be eager to attribute significance to dark pigment when it is seen grossly in samples. In particular there is a temptation to make a link with melanoma. In the experience of the authors most (more than two thirds) FNA samples of metastatic melanoma are not obviously pigmented on gross examination. It should be remembered that dark pigment, when seen, is usually from another source, particularly haemosiderin.

Warthin's tumour

The authors' experience of this lesion is rather limited, but the lesion typically yields watery fluid that is discoloured brown due to degenerate haemorrhage. These appearances are not typical of other salivary tumours and so suggest the diagnosis, particularly in an elderly patient.

Ganglion cyst

These lesions usually yield a scanty amount of thick crystal clear mucinous material. Although mucoid material is obtained from other types of lesion (as above) the crystal clear appearances together with clinical context, particularly its position on a limb or digit, gives away the diagnosis.

DISCUSSION

Previous photographic illustrations of the gross appearances of FNA samples are hard to find. However, in the authors' experience, FNA practitioners are already aware of the significance of many of the features described in this paper. It is often possible to assess the likely cellularity of a sample without examining it microscopically and sometimes to suggest a likely diagnosis. We plan to photograph the gross appearances of FNA samples of other lesions and to publish these, as they may be valuable to pathologists and other clinicians that perform FNAs.

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