Hardeep Singh Mudhar

The partnership between an ophthalmologist and an ophthalmic pathologist is critical to ensure that the final histopathology report is accurate and intelligible and contains as much relevant information to ensure safe patient management. At all stages of this process, clear verbal and written communication is key; every link in the chain must be robust to ensure the patient comes to no harm. To quote Professor Christopher Fletcher, Director of Surgical Pathology at Harvard Medical School, ‘the reality is that the histology report is the principal determinant of diagnosis, likely clinical course and therapy in any patient found to have a mass....’ [1].

Ocular pathology specimens travel through three phases: pre-analytical (pre-surgery communication, surgery, request form, specimen handling prior to fixation, fixation and transportation), analytical (processing of specimen, stains, ancillary investigations and histological interpretation by pathologist) and post-analytical (histopathology report, post-report discussion at multidisciplinary meeting, supplementary reporting).

This chapter will cover the following topics:

- Pre-analytical phase
 - Pre-surgical planning.
 - The ophthalmic pathology request form.
 - Handling specimens in theatre before fixing.
 - Fixation.
 - Transportation of specimens to the histopathology laboratory.
- Post-analytical phase
 - Basic histopathology terminology and understanding the histopathology report.
 - The use of ancillary investigations in ophthalmic pathology.
 - Ocular tumour prognostic factors.

As this chapter is meant to be a practical guide for clinicians, it will not cover the analytical aspects of how the specimen is grossed/cut up, its processing or staining in the ocular pathology laboratory. Many texts are available that cover these steps in detail and interested readers should consult these.

Pre-analytical Phase

Discussion of Cases Prior to Surgery

Some cases (complex margins), specimens requiring frozen sections or those requiring special requirements (e.g. certain fixatives), are best discussed in advance with the ocular pathology laboratory. This will ensure the pathology

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laboratory can meet the needs of the surgical procedure and ensure adequate handling without compromising the specimen.

How to Complete the Ophthalmic Pathology Request Form [2]

A pathology request form is a referral letter and a primary source of communication between the ophthalmologist and pathologist. It is also a medico-legal document. For all specimens submitted to the laboratory, a fully completed request form should accompany each case. Request forms are designed to provide:

- Unique identification of the patient.
- A destination for the report and any charging information.
- The laboratory with the clinician contact details if discussion of the case is required.
- Date and time of specimen collection/removal and investigations required (e.g. histology/cytology).
- Type of specimen and anatomical site of origin. This is especially important for multiple map biopsies taken from the conjunctiva to establish the extent of ocular surface squamous, sebaceous and melanocytic malignancies. It is critical that the request form include a diagram indicating the precise origin of each biopsy.
- Clinical information so that the pathologist may handle the specimen appropriately and interpret microscopic findings in the proper context.
- An awareness of any health and safety issues with a given specimen.
- Complete information must be supplied on the request form. Each specimen container, no matter how small, should also be labelled with the appropriate patient identification data (minimum of three identifiers, e.g. first and

last name, date of birth/age and gender and preferably patient's hospital no).

- The information must be consistent with the request form, to prevent errors in specimen and patient identification.

See Fig. 15.1a–e showing examples of well-filled out and suboptimally filled out pathology request forms.

Specimen Handling in Operation Theatre Prior to Fixation

Multiple Specimens from a Surgical Procedure

As a general principle, specimens from different locations should be placed in separate fixation pots. If multiple pieces of tissue from different sites are placed in the same fixation pot and one of the pieces of tissue turns out to be malignant, it is impossible to ascertain where the tissue has been sampled from.

Orientation of Specimens

Orientation of specimens is required, to ensure the pathologist can report the margins of excision accurately. This is best done by placing sutures at particular margins and then specifying the sutured margin on the pathology request form in writing or as a diagram (Fig. 15.2a).

Conjunctival Mapping Biopsies

Conjunctival biopsies comprise thin strips of epithelium and stroma and are prone to curling if placed straight into fixative. They should be attached to sponge or card before placing into the fixative, so that they arrive in the ocular pathology laboratory in a flattened state. The latter facilitates orientation during embedding

a

IF YES, THEN PLEASE ATTACH STICKER HERE

RELEVANT OPHTHALMIC, GENERAL MEDICAL AND DRUG HISTORY Please write clearly
Use ophthalmic diagrams overleaf if required

P.O.Hx

- Right amblyopia
- Right squint surgery 1947
- Left squint surgery 1978
- Right complicated cataract surgery
- + vitreous loss + 2° glaucoma
- + 2° corneal decompensation
- + 2° Aphakia
- Recurrent right corneal ulcer
- + 2° corneal scarring
- Currently painful right eye
- Left eye's heterochromic cyclitis

G.Mx

- Perforated large bowel - adenocarcinoma - Hartmann's procedure
- Hypertension
- Previous DVT
- Hypothyroid

Drx

- L-Thyroxine
- Amlodipine
- Furosemide
- Lasix

Requesting Clinician (BLOCK CAPITALS)

b

70 - TURNAROUND INITIATIVE

and patient has (see point 2 below)

ANT OPHTHALMIC, GENERAL MEDICAL AND DRUG HISTORY Please write clearly
Use ophthalmic diagrams overleaf if required

H/o Forward Protrusion of R/E.
for 1 year.

U/E (2) Axial proptosis

CT scan shows circumscribed orbital mass at orbital apex.

Orbital lesion is excised & sent for histopathology.

Requesting Clinician (BLOCK CAPITALS)

Fig. 15.1 (a–c) These are examples of well-filled out pathology request forms that detail all the relevant information. (c) The surgeon has drawn a very useful diagram, to indicate the orientation of the specimen. (d, e)

Suboptimally filled out pathology request forms that merely state the procedure carried out with no indication of the disease process, no differential diagnosis and no further history

STICKER HERE

CLINICAL DETAILS

Ophthalmic History:
PLL BCC biopsy
prone (by ourselves)
3mm margin taken,
2 suture marker

Medical History if relevant: on lid
margin
laterally.

Drug history if relevant:
Only anterior lamella

Consented for molecular testing? Y/N takes

Of which type

WARNINGS

c

d

CLINICAL DETAILS

Left evisceration
sample.

(left eye
contents)

e

SAMPLE TYPE
x if fasting
conjunctival + lower
lid tissue

CLINICAL INFORMATION
Suture marks superior
edge of lesion

Fig. 15.1 (continued)

and greatly facilitates the histological assessment for the presence or absence of invasion by tumour. If multiple mapping biopsies are performed, the pathology request form must state the precise location of the biopsies. Figure 15.2b shows a very helpful diagram drawn by the surgeon that shows the extent of this patient’s conjunctival lesion (shaded areas) and the precise location of the mapping biopsies.

Sampling Fresh Tissue Prior to Fixation

Some tumours require to be sampled fresh for special genetic/ molecular investigations (diagnostic and prognostic testing) or for research.

- In the case of an enucleation, sample the optic nerve first and place in a separate pot of formalin. This is to prevent fresh tumour

Fig. 15.2 (a) shows an eyelid skin ellipse excision orientated by the surgeon using two sets of sutures. (b) A conjunctival mapping biopsy diagram sketched by the surgeon in theatre. The hash shading denotes the distribution of the lesion and the letters denote the locations of the biopsies that correspond to the formalin pots into which the biopsies have been placed/fixed

contaminating the optic nerve resection margin.

- The globe is transilluminated and using a scalpel, a sclera-choroidal flap is created beneath the tumour, leaving the scleral-choroidal flap hinged along one side. Fresh tumour is harvested with scissors and forceps.
- Fresh tumour is placed in an appropriate medium and vessel.

- Some use a corneal trephine to cut a window into the sclera and choroid.
- After harvesting fresh tumour, the specimen is fixed.
- An alternative strategy is to submit the intact specimen unfixed to the ocular pathology laboratory, where the above steps can be carried out.

Fixation of Ocular Pathology Specimens

Routine Surgical Specimens

Standard 10% phosphate-buffered formalin in a leak-proof container is the fixation of choice (Table 15.1) [3–5]. This will allow routine haematoxylin and eosin staining (H&E), other special tinctorial stains and immunohistochemistry. In addition, numerous molecular studies for diagnosis and prognosis can be performed on formalin-fixed tissue (Fig. 15.3a).

- If certain molecular biological diagnostic techniques or research is to be done on unfixed tumour samples, the clinician or assistant must remove fresh tumour from the specimen prior to fixing in formalin.
- The formalin should ideally be 10x the volume of the specimen to allow timely penetration of the tissues at room temperature. To ensure this, an adequate sized plastic container is required. Most well-stocked pathology labs have a variety of sizes of plastic container to facilitate this. Many containers come pre-filled with formalin (Fig. 15.3a).
- Specimens should be placed into the container that has been pre-filled with formalin. If formalin is poured into a dry container that has the specimen in it, there is a chance that the specimen will be ejected by the formalin and will end up on the bench or floor.
- Specimen containers must then be sealed tightly with the lids to avoid spillage of hazardous formalin.
- Smaller containers are then usually placed in a plastic bag which also accommodates a side compartment for the request form. Follow

Table 15.1 Various surgical specimen types and fixations used

Type of specimen	Type of fixative
Eyelid tumour incisional biopsy	10% buffered formalin
Eyelid tumour excision	10% buffered formalin
Conjunctival tumour biopsy	10% buffered formalin
Conjunctival tumour excision	10% buffered formalin
Intraocular solid tissue biopsy (not FNA)	10% buffered formalin
Localised resection of intraocular tumour	10% buffered formalin
Tumour enucleation	10% buffered formalin—remember to sample any fresh tumour prior to fixation if indicated
Tumour exenteration	10% buffered formalin—remember to sample any fresh tumour prior to fixation if indicated
Orbital tumour biopsy	10% buffered formalin
Orbital tumour excision	10% buffered formalin—remember to sample any fresh tumour prior to fixation if indicated
Lacrimal drainage system/sac biopsy or excision	10% buffered formalin
Aqueous tap for cytology	Equal volume of CytoLyt or similar methanol-based fixative
Aqueous tap for flow cytometry	RPMI
Vitreous tap or pars plana vitrectomy specimen for cytology	Equal volume of CytoLyt or similar methanol-based fixative
Vitreous tap or pars plana vitrectomy specimen for flow cytometry	RPMI
FNA intraocular of solid tumour (e.g. retinal, subretinal uveal tumour and orbital)	CytoLyt or similar methanol-based fixative

Fig. 15.3 (a) shows a globe specimen (containing a uveal melanoma) in a standard plastic pot, containing 10% buffered formalin for fixing the specimen. (b) This is a universal tube that contains green, methanol-based cytofixative for intraocular tumour aspiration cytology. The colour of the fluid varies according to the manufacturer. (c) The arrows point to black specs of an aspirated melanotic tumour, fixed in cytofixative in a universal tube. (d)

This is a vitrectomy cassette containing vitreous washings from a patient with suspected intraocular lymphoma, fixed in green cytofixative of a volume equal to the volume of the vitreous washings. (e) This shows an impression cytology plastic ring, fixing in 10% buffered formalin. (f) A small plastic tube containing buffered glutaraldehyde for the fixation of specimens for transmission electron microscopy

Fig. 15.3 (continued)

your institutional standard operation protocols for additional packaging.

- The appropriate institutional health and safety protocols must be followed when handling formalin.

Which Specimens Should Not Be Fixed?

- Tissue destined for frozen sections (to assess margins of excision for eyelid, adnexal and orbital tumours).
- Tissue for research or tissue banking.
- Some tumours requiring prognostic information (e.g. sarcomas, paediatric small round

blue cells tumours, retinoblastoma) may require special studies on fresh tissue.

- Biopsies destined for flow cytometry (e.g. vitreous and fine-needle aspiration specimens for assessment of lymphoma). In this situation, many practitioners send the cell sample in Roswell Park Medical Institution medium (RPMI) to enhance the survival of the cells.
- Some labs still do Oil Red O staining for suspected cases of sebaceous carcinoma. However, this is being superseded by haematoxylin and eosin-stained (H&E) sections and immunohistochemistry.

Fixation of Cytology Specimens

- Fine-needle aspiration specimens (FNA) are preferably fixed in CytoLyt or Shandon cytofixative (or equivalents) which are methanol-based mild fixatives (Table 15.1). They preserve the cyto-morphology well and permit good-quality DNA to be extracted for molecular diagnostic tests. CytoLyt preserved specimens can be used to make thin-prep slides as well. FNA samples are usually squirted into pre-filled universal tubes that contain the fixative. The syringe used in the collection of the sample may be submitted with the fluid inside. Needles must be removed and the syringe capped. If the specimen is to be fixed, an equal volume of cytology fixative may be drawn up into the same syringe, capped and then agitated gently to mix the sample with the fixative (Fig. 15.3b, c).
- Vitrectomy specimens: It is preferable to receive a pars plana vitrectomy specimen in a vitreous cassette or bag. If samples are required for microbiology, virology, PCR, etc., please remove these from the cassette/bag before fixation, although, ideally, these should have been sent from the initial core vitreous tap. Vitrectomy samples need to be preserved/fixed in an equal or excess volume of RPMI or cytology fixative. It is important to seal the bag or cassette to prevent leaks in transit (Fig. 15.3d).
- Impression cytology plastic rings can be dropped into a small pot of formalin for fixation (Fig. 15.3e).

Fixation of Specimens Requiring Transmission Electron Microscopy

- Buffered 2.5–3% glutaraldehyde is used to fix fresh specimens for transmission electron microscopy. This would be done for cases that are intended for research or in which the original interpretation of an initial tumour biopsy was equivocal and clarification is sought from ultrastructure. It is best to place

only part of the fresh tissue into buffered glutaraldehyde, as whilst it is an excellent fixative for ultrastructure, it adversely affects antigen retrieval for immunohistochemistry and extraction of DNA for molecular studies (Fig. 15.3f).

Transporting Specimens to the Pathology Laboratory

High Risk/Danger of Infection Specimens

- It is the ophthalmologist's responsibility to indicate on the request form and the specimen pot if the patient is known or suspected to be a 'High Risk/Danger of Infection' category (e.g. HIV, TB, hepatitis B, hepatitis C), to facilitate appropriate handling in the laboratory.
- It is the ophthalmologist's responsibility to ensure that all specimens are submitted to the laboratory in suitable and approved containers.
- Approved specimen containers have leak-proof lids and the appropriate hazard warning signs for the fixative, e.g. formalin.
- Specimen containers should be closed securely and placed inside a sealed specimen bag.
- Specimens received leaking or damaged are a danger to all those who come into contact with them, including theatre staff, porters and laboratory staff and can also compromise the diagnosis.

Mailed or Couriered Specimens

- Specimens mailed or couriered should be packaged in approved containers in accordance with the requirements of the delivery service. To confirm receipt of specimen(s) in the department, it is good practice that a 'confirmation of receipt fax-back' form, providing the senders' confidential fax number, is enclosed with the specimen(s).

Post-analytical Phase

Understanding the Ophthalmic Histopathology Report

The ophthalmic histopathology report contains a lot of specialised pathology terminology that can seem rather bewildering. To understand histopathologic terminology related to tumours, it is best to return to basics. Table 15.2 outlines the tissue of origin and their related tumours that are commonly encountered [6] (Fig. 15.4a–j).

Knowledge of this basic table does go a long way to understanding the various tumours encountered in ocular oncology practice and their behaviour and prognosis. It also allows for a conversation with your local pathologist, who is often equally keen to learn specialised ophthalmology terminology to enhance the quality and clarity of the professional interaction.

Examples of Histopathology Reports with Explanations of Specialised Terminology

Set out below are examples of ophthalmic histopathology reports that may be encountered in a busy ocular oncology practice with specialised terms defined and figures provided for clarity [6].

Eyelid Tumour Histopathology Report Example

This full thickness wedge excision contains an *invasive neoplasm*. It is arising from the Meibomian gland and is composed of *pleomorphic* cells, with *vacuolated* cytoplasm, indenting the nuclei. Numerous mitotic figures are present. The individual tumour cells exhibit *hyperchromatic chromatin* and nucleoli are prominent. Foci of *intraepithelial pagetoid* spread are seen over the invasive tumour, involving the tarsal conjunctival epithelium and the epidermis of the skin. The invasive tumour shows focal *comedo-like necrosis* and shows *vascular and perineural invasion*.

Immunohistochemistry shows that the invasive tumour is positive for adipophilin, EMA and shows nuclear androgen receptor positivity.

The features are those of an invasive sebaceous *carcinoma*, derived from the meibomian gland. The excision of the invasive component is complete (nearest margin is 3 mm). However, the pagetoid spread contaminates the tarsal conjunctival margin and is therefore incompletely excised.

Sebaceous neoplasms can be associated with the Muir-Torre syndrome. Is there a history of internal malignancies in this patient or is there a family history of malignancies?

Table 15.2 Tissues of origin and related tumours

Tissue of origin	Benign	In situ	Invasive malignancy
Squamous Epithelium	Squamous cell papilloma	In situ squamous carcinoma	Invasive squamous cell carcinoma
Sebaceous gland epithelium	Sebaceous adenoma	In situ sebaceous carcinoma	Invasive sebaceous carcinoma
Melanocytes	Nevus	In situ melanoma	Invasive malignant melanoma
Blood cells	Reactive lymphoid hyperplasia		Lymphoma or leukaemia
Soft tissue	Lipoma		Liposarcoma
	Haemangioma		Angiosarcoma
	Rhabdomyoma		Rhabdomyosarcoma

a **b**

Fig. 15.4 (a) Haematoxylin & eosin (H&E)-stained histology image of a typical viral squamous papilloma of the conjunctiva. Note the typical cauliflower-like appearance caused by the fibrovascular cores covered in thickened epithelium. (b) H&E-stained section showing conjunctival in situ squamous carcinoma. The epithelium is thicker than usual and packed full of atypical nuclei, with no maturation at all. (c) H&E section showing conjunctival invasive squamous cell carcinoma with keratin formation. The latter is the orange material in the centre of the irregular invasive islands of tumour. (d) H&E of a cystic conjunctival nevus. The asterisk is the cystic space and the arrow points to the bland, monotonous nevus cells. (e) H&E of conjunctival in situ melanoma. The arrow points to the melanoma cells confined to the epithelium. (f) H&E of invasive conjunctival melanoma. The asterisk indicates an invasive nodule of melanoma in the substantia propria. (g) H&E of reactive lymphoid hyperplasia. The thicker arrow shows the paler germinal centre lymphocytes of the well-organised nodular lymphoid tissue. The thinner arrow indicates the darker staining mantle lymphocytes, surrounding the germinal centre lymphocytes. (h) H&E of a conjunctival extranodal marginal zone lymphoma. Note the sheet-like population of monotonous lymphoid cells. (i) H&E of an alveolar variant rhabdomyosarcoma of the orbit. (j) H&E of a higher power of Fig. 15i showing small round blue cells with occasional larger pink (eosinophilic cells) present. (k) H&E of a squamous cell carcinoma of the conjunctiva that illustrates nuclear size, shape and colour variation-called pleomorphism and is a feature of malignant cells. (l) H&E of the same tumour in plate K showing a typical tumour mitotic figure (arrow). (m) H&E showing cytoplasmic vacuolation (clear spaces) of sebaceous tumour cells (arrow). (n) H&E of a sebaceous carcinoma with the arrow pointing to a hyperchromatic nucleus (darker in shade compared to other nuclei in image). (o) H&E of a sebaceous carcinoma showing intraepithelial 'Pagetoid' spread (arrows). (p) H&E of a

retinoblastoma. The asterisk indicates pink irregular necrotic tumour. (q) H&E of an adenoid cystic carcinoma of the orbit showing tumour around a nerve (arrow). This is perineural invasion. (r) Melanoma cells (stained red) are within the lumen of a lymphatic channel (stained brown). This is intra-lymphatic spread of tumour. (s) H&E of conjunctival squamous dysplasia (mid- to right aspect of image). The latter epithelium is thicker compared to the epithelium on the left which is much thinner. (t) H&E of a spindle-rich uveal melanoma. (u) H&E of an epithelioid rich uveal melanoma. The cells are much rounder and larger compared to the spindle cells in plate T. This is because they possess larger nuclei and more cytoplasm. (v) This is a Periodic Acid Schiff's (PAS) stain of a uveal melanoma showing rounded clones of tumour cells surrounded by pink/purple basement membrane boundaries, so-called loops. (w) This is a fluorescence in situ hybridisation (FISH) image showing different chromogenic-labelled DNA probes (green, red, blue, yellow) hybridising with various gene loci. (x) This is an orbital lymphoma stained with a B-cell marker antibody called CD20, by the technique of immunohistochemistry. The positive cells stain brown (different coloured chromogens can be employed such as red, brown, blue, etc.). Note how it is the cell membranes that stain, imparting a chicken-wire appearance. (y) This is an orbital lymphoma stained with Ki67 antibody by immunohistochemistry. The antibody stains the nuclei brown. The proliferation fraction of the tumour can be estimated by counting the number of stained nuclei out of 100 cells. (za) This trace shows a typical polyclonal population of B-cells when assessed for B-cell IgH rearrangement by PCR. Note the spread of varying sized peaks in a Gaussian dumbbell curve configuration. The histology showed a reactive lymphocytic population in the vitreous with a final diagnosis of chronic vitritis. (zb) This trace shows a typical monoclonal population of B-cells when assessed for B-cell IgH rearrangement by PCR. The final diagnosis was vitreo-retinal high-grade B-cell lymphoma

Fig. 15.4 (continued)

Fig. 15.4 (continued)

Fig. 15.4 (continued)

Terminology Explained

Invasive Malignant tumours invade through basement membranes into the surrounding tissue and can metastasise by invading into blood vessels and lymphatics.

Neoplasm Literally means ‘new growth’ (derived from Greek).

Pleomorphic Variation in size and shape of the nucleus and cytoplasm of the individual tumour cells. This is a feature of malignant cells that have lost their normal constraining signals (Fig. 15.4k).

Mitotic Figures Prophase, metaphase, anaphase, telophase and cytokinesis are the five phases of mitosis (refer to any intermediate biology textbook).

Mitotic figures are seen in normal and repairing tissues. An example of a normal tissue containing mitotic figures is the proliferative phase of endometrial glands and stroma. When injured tissue is regenerating, it is common to see many mitotic figures, e.g. regenerating epithelium in skin after trauma. Benign and malignant tumours contain mitotic figures. Malignant tumours tend to have more mitotic figures than benign tumours. Of the malignant tumours, poorly differentiated tumours tend to have higher mitotic figure counts compared to better differentiated tumours. In malignant tumours, the mitotic figures can have an abnormal morphology that is not seen in benign, normal or regenerating tissues. These include tripolar or multipolar forms (Fig. 15.4l).

Vacuolated A bubbly appearance to the cytoplasm due to the presence of various substances. In this case, it is lipid as the diagnosis is sebaceous carcinoma. It could be mucin (mucin forming carcinomas) or glycogen (e.g. renal cell carcinoma). The products made by tumour cells can be very helpful for classification (Fig. 15.4m).

Hyperchromatic Chromatin Simply means dark staining DNA in the nucleus. This is a feature of malignant cells (Fig. 15.4n).

Intraepithelial Within the confines of the epithelium.

Pagetoid Horizontal and vertical spread of malignant tumour cells as individuals or groups within epithelium. Named after Sir James Paget, an English surgeon and pathologist who described Paget's disease that shows this pattern of spread. Tumours that show this pattern of spread include malignant melanoma and sebaceous carcinoma (Fig. 15.4o).

Comedo Literally means like a 'zit'. Malignant tumour cells forming sheets are hypoxic in the middle and undergo necrosis. The appearance is that of a necrotic centre surrounded by a viable rim of malignant tumour cells. If the tissue is squeezed gently during handling, the necrotic

tumour centre oozes out of the cut surface like zits being squeezed.

Necrosis Cell death with loss of cell contents to the exterior. Malignant tumours that outstrip their blood supply often become necrotic (Fig. 15.4p).

Vascular and Perineural Invasion Malignant tumours can invade into vessels and metastasise to distant locations. They also follow the paths of least resistance and spread along natural tissue scaffolds such as nerves (Fig. 15.4q, r).

Immunohistochemistry A common ancillary technique employed in histopathology to detect signature proteins using antibodies in various disease processes. In malignant tumours, it can help to type the tumour (e.g. carcinoma, lymphoma, melanoma, sarcoma) and can also reveal vital prognostic and therapeutic information.

Carcinoma A malignant epithelial neoplasm.

Further Comments

The last comment in the report relates to the surgical margins. In this case, the contamination of the conjunctival margin may necessitate mapping biopsies to ascertain the extent of the pagetoid spread that would inform subsequent therapy choices.

Conjunctival Tumour Histopathology Report Example

The bulbar conjunctiva contains a focus of in situ squamous carcinoma, arising in a background of various degrees of *dysplasia*. No invasion is identified in the planes examined. The surgical margins are clear. The background stromal tissue shows solar elastosis.

Terminology Explained

Dysplasia Derived from Greek meaning 'disordered growth'. Dysplasia is a term that is applied to epithelium. Histologically, the individual epithelial cells show pleomorphism (defined above), can have hyperchromatic nuclei and have a disor-

ganised distribution within the epithelium. Mitotic figures may be seen. Pathologists can grade epithelial dysplasia according to how much of the epithelial thickness is occupied by the dysplastic cells. For example, common classifications of epithelial dysplasia are mild, moderate and severe or low-grade and high-grade. When the full thickness of the epithelium is consumed by dysplastic cells and the basement membrane has not been breached, it is called carcinoma in situ and is said to be pre-invasive. Invasion is not present. Therapeutic intervention at this stage of the disease is potentially curative (Fig. 15.4s).

Intraocular Tumour Histopathology Report Example

This enucleation contains a malignant melanoma of uveal type. It is located in the ciliary body and choroid and has a diffuse component. It is composed of *spindle and epithelioid cells* and has up to 15 mitotic figures *per 10 hpf*. *Closed loops and networks matrix patterns are seen*. Focal tumour necrosis is present. The tumour does not breach Bruch's membrane. There is no scleral invasion. The vortex veins are tumour-free. *FISH* analysis on the tumour has shown monosomy 3 and a gain of iso-chromosome 8q in 90% of the cells.

Summary: A *pathological stage pT3b* uveal melanoma of the ciliary body and choroid. The presence of monosomy 3 and iso-chromosome 8q indicates a poor prognosis.

Terminology Explained

Spindle and Epithelioid Cells This refers to the shape of the cells found in uveal melanoma, referred to as the Callender classification. This is of prognostic significance; more epithelioid cells carry a worse prognosis [7] (Fig. 15.4t, u).

Per 10hpf This is a mitotic count performed in 10 'high-power fields' (each with an area of 0.152 mm² and usually corresponding to a ×40 lens on a high-quality light microscope).

Closed Loops and Networks' Matrix Patterns This refers to the presence of specific

basement membrane patterns stained with a periodic acid-Schiff (PAS) stain, present around expanding nests of melanoma cells. These matrix patterns are related to a poorer prognosis in uveal melanoma [8] (Fig. 15.4v).

FISH: Fluorescence In Situ Hybridisation

FISH has been used in this case to gain genetic data on the status of chromosomes 3 and 8 in this uveal melanoma. Uveal melanoma genetics data is a powerful predictor of prognosis [9–13] (Fig. 15.4w).

Pathological Stage pT3b This refers to the TNM pathological classification of the stage of the uveal melanoma [14].

Further Comments

Ophthalmic pathologists will use the TNM¹⁴ or AJCC [15] cancer staging manuals to include all of the relevant tumour prognostic information required for prognosis and treatment planning for uveal melanoma.

Also note how the report has combined histopathological and genetic data. This is increasingly common and is called integrated reporting, in the context of individualised oncology practice.

Orbital Tumour Histopathology Report Example

This orbital fat biopsy contains numerous *reactive lymphoid aggregates*. On this background, there is a larger expansile nodule composed of small lymphocytes, with a *monocytoid appearance*, associated with cells exhibiting lymphoplasmacytic *differentiation*. These monocytoid cells are positive for *B-cell markers* using immunohistochemistry (CD20 and CD79a positive) and are negative for CD5, CD10, Cd23 and Cyclin D1. *The Ki67 fraction is 5%. PCR for IgH rearrangement* shows clonal peaks in FR1, FR2 and FR3 confirming an unequivocal *clonal population of B lymphocytes*.

The appearances are those of an indolent B-cell lymphoma, sub-classification: extranodal marginal zone lymphoma of MALT type

(MALToma-WHO classification). This patient requires referral to your haemato-oncology service for further management.

Terminology Explained

Reactive Lymphoid Aggregates These are non-lymphomatous collections of lymphocytes comprising a germinal centre of larger lymphocytes undergoing apoptosis surrounded by a mantle of smaller lymphocytes. They indicate chronic antigenic stimulation and contain polyclonal groups of B lymphocytes.

Monocytoid Appearance It is common for a pathologist to describe the appearance of certain tumour cells with peculiar adjectives. Monocytoid means a lymphocyte nucleus within a clear cytoplasm, resembling a fried egg.

B-cell Markers This refers usually to immunohistochemistry markers for staining B-cells. Common markers include CD20 and CD79a. It is vitally important that any lymphoma should be differentiated into B or T cell type for diagnostic, prognostic and treatment purposes (Fig. 15.4x).

The Ki67 Fraction This refers to the % of cells that express Ki67. This protein is expressed in cells in all non-G0 phases of the cell cycle. It gives an indication of how fast tumour cells are proliferating. A higher Ki67 fraction tends to predict aggressive tumour behaviour (Fig. 15.4y).

PCR for IgH Rearrangement PCR, polymerase chain reaction. IgH-heavy chain of the immunoglobulin molecule. This is a common molecular diagnostic test performed in cases of suspected lymphoma.

Clonal Population of B Lymphocytes This means a population of B-cells all expressing the same heavy chain of immunoglobulin. Monoclonality usually means lymphoma but not always. Reactive lymphoid populations can contain monoclonal lymphocyte populations. The pathologist will always interpret the results of ancillary diagnostic/supportive molecular tests in the context of the clinical and histopathological findings (Fig. 15.4za, zb).

Further Comments

Lymphoma diagnosis often requires histopathological, immunohistochemical and molecular diagnostic tests to secure a firm diagnosis. An ophthalmic pathologist will often discuss the case with a specialist lymphoma pathologist and the case reviewed at the local multidisciplinary team meeting to plan further investigations (e.g. staging) and treatment. Again, note how the report contains integrated items of histopathological and molecular data and a suggestion for referral to an appropriate clinical team.

Common Ancillary Investigations Used in Ophthalmic Histopathology

Ancillary investigations are used to assist in making a diagnosis and to give prognostic information. The results of ancillary tests must always be interpreted alongside the light microscopic histological features in order to avoid an over or under diagnosis.

Immunohistochemistry

This is the application of antibodies to antigens in formalin-fixed, paraffin-embedded sections, visualised with various coloured chromogens. The antibodies are directed toward certain proteins that are expressed by specific cell types. Tumour cells express specific proteins allowing the tumour type to be established, which otherwise may be difficult on light microscopic examination alone.

Diagnostic Application

Example 1 Figure 15.5a shows a biopsy of the conjunctiva. The morphology shows atypical cells occupying the full thickness of the epithelium. The morphological differential diagnosis includes in situ melanoma, in situ squamous carcinoma and in situ sebaceous carcinoma. These are tumours with varying behaviour, prognosis and treatments so the diagnosis must be refined. Application of a small panel of antibodies allows discrimination. In this case, the tumour is positive for Melan A antibody

Fig. 15.5 (a) H&E of a conjunctival biopsy showing amelanotic atypical cells occupying the full thickness of the epithelium. (b) Melan A immunohistochemistry shows positivity in the atypical intraepithelial cells (brown signal), indicating in situ melanoma. (c) H&E showing a diffuse sheet of round lymphoid cells. (d) The lymphoid cells in Fig. (c) are positive for CD20. (e) The lymphoid cells in

panel c are positive for nuclear Cyclin D1, indicating a diagnosis of Mantle cell lymphoma. (f) BAP 1 immunohistochemistry on a uveal melanoma showing nuclear positivity. (g) BAP 1 immunohistochemistry on another uveal melanoma showing a lack of nuclear staining. (e, courtesy of Dr. Karen Sisley, Senior Lecturer, Department of Oncology, Sheffield University, Sheffield, UK)

(Fig. 15.5b) and negative for cytokeratin and androgen receptor. Melan A is a protein specific to melanoma cells. Cytokeratins are expressed by epithelial malignancies (in situ squamous and sebaceous carcinoma), and androgen receptor is expressed strongly by in situ sebaceous carcinoma. The final diagnosis is in situ melanoma.

Example 2 This orbital biopsy shows a lymphocytic infiltrate that histologically is a lymphoma (Fig. 15.5c). However, every lymphoma must be precisely sub-typed as each lymphoma has a specific morphology, immunohistochemical phenotype, molecular signature, prognosis and treatment (WHO classification). Application of a panel of lymphoid markers shows that this lymphoma is of B-cell phenotype (positive for CD20, Fig. 15.5d) and furthermore is positive for Cyclin D1 (Fig. 15.5e) which indicates the precise diagnosis of mantle cell lymphoma [16].

Prognostic Application

Figure 15.5f shows a uveal melanoma that shows nuclear positivity for BAP1. Figure 15.5g shows nuclear BAP-1 absence in another uveal melanoma, an indication that the tumour is of probable monosomy 3 genotype, associated with a poor prognosis [17].

Polymerase Chain Reaction (PCR)

Diagnostic Application

Sometimes, it is difficult to ascertain on light microscopy whether a lymphoid infiltrate is reactive or neoplastic. Formalin-fixed, paraffin-embedded extracted DNA can be tested for lymphocyte clonality, to establish whether a population of lymphoid cells are polyclonal (reactive-not lymphoma) or monoclonal (likely lymphoma) (Fig. 15.6a).

Fig. 15.6 (a) This trace shows a single, sharp peak indicating a monoclonal population of B-cells when assessed for B-cell IgH rearrangement by PCR. (b) MLPA study of uveal melanoma showing 'piano key' score. The arrow points to an area of loss of one copy of chromosome 3 (monosomy 3). (c) FISH study of an orbital alveolar rhabdomyosarcoma showing separate green and red hybridisation dots within the nucleus. This indicates rearrangement of the FOXO1 gene on chromosome 13q14. (d) FISH study showing three uveal melanoma nuclei. The red dot indicates one copy of chromosome 3 (monosomy 3), and the extra blue dot in the nucleus on the right indicates a gain of chromosome 8q. (e) Array CGH result from a uveal melanoma. Chromosome deletions in red are on the left of the and gains in blue on the right of the chromo-

somes. This study shows a loss of chromosome 3 (left arrow) and a loss of 8p and gain of 8q (right arrow). Other changes include deletion of chromosome 1 and gains of chromosome 20 and 21. (f) SNP array data from a uveal melanoma showing loss of one copy of chromosome 3 (left arrow) and loss of 8p and gain of 8q (right arrow). (Courtesy of: **b**, Professor Sarah E Coupland-Professor of Ophthalmic Pathology, Liverpool University, Liverpool UK; **c**, Dr Duncan Baker, Cytogeneticist, Sheffield Children's Hospital, Sheffield UK; **d**, Dr Karen Sisley, Senior Lecturer, Department of Oncology, Sheffield University, Sheffield UK; **f**, Dr Natasha van Poppelen and Dr Rob Verdijk, Department of Pathology, Erasmus Medical Centre, Rotterdam, Netherlands)

Fig. 15.6 (continued)

Prognostic Application

The states of chromosomes 1, 3, 6 and 8 allow uveal melanoma to be stratified into good and bad prognostic categories. A technique called MLPA (multi-ligation-dependent probe amplification), which is a variation of PCR, can be used to test for these chromosomal changes [18] (Fig. 15.6b).

Flow Cytometry

Diagnostic Application

This technique is used in some centres to ascertain whether a neoplastic population of B or T cells are present in vitreous or aqueous biopsies, in limited material. It is a powerful technique for the diagnosis of lymphoma [19]. Multiple

antibodies directed to various lymphoid cell proteins, including kappa and lambda light chains, are applied as a cocktail to an unfixed suspension of lymphoid cells.

Fluorescence In Situ Hybridisation (FISH)

This technique uses fluorescent-tagged DNA or RNA probes to detect the presence or absence of a specific chromosome or the identification of two genes involved in a translocation. It can be used on cytology and paraffin-embedded tissue.

Diagnostic Application

Many sarcomas have distinctive, diagnostic recurrent genetic translocations. The example in Fig. 15.6c shows a FISH analysis of an orbital alveolar rhabdomyosarcoma with the diagnostic 1:13 translocation, which is also important as a prognostic marker in this tumour [20].

Prognostic Application

Uveal melanoma can have its chromosome 3 and 8 status tested by FISH [10, 12, 13] (Fig. 15.6d).

Chromosomal Microarray Analysis (aCGH and SNP)

Techniques such as array comparative genomic hybridisation (aCGH)[21] and single nucleotide polymorphism (SNP) chromosomal arrays [22] are powerful tools to detect genome-wide copy number changes in chromosomes. Furthermore, SNP arrays can detect copy-neutral changes and loss of heterozygosity. These techniques can be applied to fresh and paraffin-embedded tumour tissue successfully.

Diagnostic Applications

Can be used to help differentiate benign nevus from melanoma of the skin.

Prognostic Application

Can be used in uveal melanoma prognostic chromosomal analysis (Fig. 15.6e, f).

Tumour Prognostic Factors

Histopathology reports contain critical bits of information that will reflect the biological behaviour of the tumour; hence, its prognosis will trigger specific clinical management. What follows are brief descriptions of prognostic factors for some ocular tumours impacting on clinical management.

Every prognostic factor for every ocular tumour cannot be reproduced here. The relevant chapters in the TNM classification of Malignant Tumours (8th edition -UICC) [14] and the *AJCC Cancer Staging Manual* (8th edition) [15] have comprehensive lists of prognostic factors for all major ophthalmic tumours. Additionally, the tumour-reporting guidelines/datasets from the College of American Pathologists [23, 24] and the Royal College of Pathologists UK [25, 26] are a very comprehensive guide to macroscopic and microscopic prognostic factors for ocular tumours. Tumour prognostic factors from a pathological perspective can be macroscopic, microscopic and molecular/genetic.

Examples of Tumour Prognostic Factors Encountered in Ophthalmic Histopathology Reports

Eyelid Basal Cell Carcinoma [14, 15, 27]

The growth pattern of basal cell carcinoma will be mentioned in a report. Infiltrating and/or sclerosing/morphoea and/or micronodular growth patterns are high-risk growth patterns. These growth patterns correlate with a significantly increased risk for local recurrence and very occasionally metastasis, hence high risk. The clearance of the tumour in 'mm' from specific margins will be mentioned in the report. Contaminated margins will trigger further treatment to ensure clearance.

Conjunctival Melanoma [14, 15]

For conjunctival melanoma, the site and depth of invasion determine the 'pT' staging of these tumours. Tumours of the palpebral, forniceal and caruncular conjunctiva are higher risk compared

to bulbar conjunctival tumours. The higher risk relates to recurrence and metastatic rates. The deeper the invasive component, the higher the pT stage.

Retinoblastoma [14, 15, 23, 24]

The presence of 'high-risk' histopathological factors on a histopathology report of a retinoblastoma enucleation includes 'massive choroidal invasion', 'extra-scleral tumour extension', 'optic nerve invasion' and 'anterior chamber extension'. These features will lead to post-enucleation chemotherapy.

Uveal Melanoma [14, 15, 25, 26]

Iris melanomas have a much better prognosis than ciliary body and choroidal located tumours. Ciliary body and choroidal uveal melanomas with monosomy 3 and iso-chromosome 8q gain will have a poor prognosis, compared to tumours with balanced genetics.

Orbital Alveolar Rhabdomyosarcoma

Histology reports will make the distinction between the different subtypes of orbital rhabdomyosarcoma as they have different prognoses. An alveolar growth pattern which shows a 1:13 or 2:13 translocation [28] has is more aggressive and of higher risk for recurrence and metastasis compared to the embryonal variant.

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