



Tissue Pathways for Cardiovascular Pathology

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Forenote: General background supportive information for the cardiovascular pathology in all the sections can be found in the following textbook: Silver MD, Gotlieb AI and Schoen FJ (eds) Cardiovascular pathology. 3rd edition, Churchill Livingstone, London, 2001. Section-specific peer-reviewed citations are given for each section below.

SECTION A TISSUE PATHWAY: ENDOMYOCARDIAL BIOPSIES FOR THE ASSESSMENT OF PATHOLOGY OF THE NATIVE HEART

A1 Specimen submission

Endomyocardial biopsy (EMB) contributes significantly to sensitivity and specificity in unexplained cardiomyopathy¹. The chief diagnostic limitation with endomyocardial biopsies (EMBs) is sampling error². To reduce this, it is recommended that at least 4–5 biopsies be taken. Although the morphometric evidence for this recommendation is based on International Society of Heart and Lung Transplantation (ISHLT) criteria in the transplantation scenario (for reasons of study size), it is generalised to native biopsies by consensus³. For more focal processes, more biopsy specimens might be recommended. Note that biopsies cannot detect abnormalities that are only present in the conduction system or an accessory pathway as these are avoided when selecting the site for biopsy. Ideally, an additional piece will be placed in glutaraldehyde for electron microscopy (EM) and one piece frozen for immunofluorescence, molecular biological or enzyme analysis, if required.

It is recognised that most centres do not have a procedure set up for the collection of fresh material to freeze. When practical, this should be instituted with the aid of the clinicians and will require an arrangement where the laboratory is informed of the time of the biopsy and a designated person goes to the procedure room to immediately freeze a biopsy in OCT embedding medium for storage at -70°C .

For EM, ideally, a biopsy will be immediately placed in glutaraldehyde. This can be arranged by giving the clinicians small vials containing fresh EM fixative (at room temperature when used to minimise contraction band artefact)⁴ or alternatively, if an arrangement is set up to collect frozen tissue, the designated person could also place one biopsy into EM fixative. If the specimen arrives in the laboratory in formalin, then one biopsy is taken out and put into EM fixative immediately.

The specimen must be accompanied by a request card with identifying patient details (full name, gender, date of birth, NHS number), name of the responsible consultant, date of the procedure and a relevant clinical summary. The specimen container is labelled with matching patient details. The clinical summary should include reference to all medications, including non-prescription and illicit drugs; co-existent medical conditions, especially multisystem diseases such as collagen vascular diseases, thalassaemia, porphyria, amyloidosis and sarcoidosis; and echocardiographic findings. It is helpful if previous histology, which is pertinent to the current specimen, is identified.

For the assessment of suspected adriamycin/doxyrubicin toxicity, the majority of pieces should be submitted for electron microscopy. It is useful to submit at least one for light microscopy in case of unexpected findings.

A2 Processing and embedding

The fragments submitted for light microscopy (LM) are processed in a cell safe cassette or other method to avoid the loss of tiny specimens. All the biopsy specimens are embedded in one paraffin block. The fragments are arranged in a line or as a small group to facilitate analysis, if practicable.

EM processing should not involve en bloc staining with uranyl acetate as this extracts glycogen⁴.

A3 Sectioning and staining

A minimum of five spaced histological sections (levels) are obtained (in the first instance). These may have at least two to three (and up to five) serial sections mounted on each slide at each level¹.

At least two to five different levels are stained with haematoxylin and eosin (H&E)².

At least one section per biopsy is stained with:

- a connective tissue stain (e.g. elastic van Gieson and/or trichrome)
- Congo red or other stain for amyloid (section thickness 10µm)
- Perls stain (or other stain for iron)
- PAS ± Diastase to assess glycogen, interstitium, and intramyocardial small vessels
- Gomori modified trichrome for mitochondrial cardiomyopathy

In the presence of an acute inflammatory cell infiltration or granulomas, special stains for infective organisms (ZN, modified ZN, Grocott, PAS, Gram) are performed.

A4 Further investigations

A4.1 Immunofluorescence / Immunoperoxidase

In suspected Duchenne and Becker muscular dystrophies, immunohistochemical staining for dystrophin is useful. Moreover, dystrophin components⁵ should be investigated on all young male patients. Amyloid may be immunohistochemically typed on paraffin embedded tissue.

A4.2 Electron microscopy

Small samples of myocardium may be submitted for EM². If a diagnosis is not reached by LM then EM is performed on the glutaraldehyde sample, or if necessary reprocessed paraffin tissue. There are a number of entities only able to be diagnosed or suggested by EM, including desmin cardiomyopathy, Fabry's disease, other metabolic/storage diseases, and mitochondrial cardiomyopathies. Populations with a higher yield of helpful findings include infants and children, those with neuromuscular disease such as myopathy (e.g. myotonic dystrophy), familial cases (e.g. Fabry's disease) and those with a history of exposure to toxins or drugs such as prescription medications, including anthracycline, chloroquine and Paclitaxel².

A4.3 Infectious agents

Special stains, e.g. Grocott, PAS, ZN for granulomatous inflammation. PAS is also performed if foamy macrophages are present, to look for Whipple's Disease.

A4.4 Molecular biology

PCR analysis for viral genomes may be performed. Molecular biology is becoming more important in the diagnosis of cardiomyopathies as more exact mutations become known. These are not regarded as routine at present, but there should be a route for referral to a specialist genetic service for relevant cases. For most molecular biology purposes, snap-frozen tissue is optimal. EMBs are hard-won tissues and samples will be limited. The residual deep-frozen tissue left after cryostat sections are retained for *diagnostic* use. Some molecular biology methods may be applicable to paraffin-embedded tissue (e.g. ISH, or PCR using primer sets of about 1 kB or closer). These are not currently routine methods, but their prospect should at least inform handling because they will become useful.

A5 Report content

The report² should refer specifically to:

- myocytes: hypertrophy, atrophy, necrosis, vacuolation, inclusions, iron deposition and disarray

- interstitium: fibrosis, inflammation, adipose tissue infiltration and amyloid deposition
- the endocardial aspect: fibrosis/fibroelastosis
- interstitial vessels: thrombi, thickening and dysplastic changes.

The endocardium is usually not well orientated but an assessment of fibrosis/elastosis and inflammation is made. Be aware that tangential cutting mimics increased fibrosis.

With regard to the myocardium, the presence or absence of the following are noted.

i. Inflammation: if present, the composition is described, i.e. eosinophilic, neutrophilic, mononuclear, giant cell or granulomatous. Associated muscle damage/necrosis must be documented. For a diagnosis of myocarditis, the Dallas Criteria are used: “an inflammatory infiltrate of the myocardium with necrosis and/or muscle degeneration of myocytes not typical of ischaemic damage associated with coronary artery disease”².

ii. Myocyte hypertrophy: assess by increased nuclear size and hyperchromasia as myocyte attenuation occurs with dilation of the chamber and atrophy. Myocyte disarray is assessed, but in biopsies from the right ventricle this is a normal finding, particularly at the junction of the septum and free wall. In left ventricular biopsy specimens the myocyte disarray of hypertrophic cardiomyopathy is deep and usually missed by EMB; there is also normally disarray at the junction of the septum and free wall. Vacuolation of myocytes is noted if present and may suggest the presence of a storage disease; this is an indication for EM. The presence or absence of iron and amyloid, and the extent and pattern of fibrosis are noted.

iii. Blood vessels: evidence of inflammation, endothelial swelling, thrombosis or abnormal wall characteristics, such as hypertrophy, intimal fibrosis, small vessel medial vasculopathy.

iv. Evidence of epicardium: indicated by the presence of mesothelial cells and perforation is then strongly suggested. Fat is not indicative of epicardium as it can be present in the endocardium and vascular septa of the myocardium, especially on the right side.

Endomyocardial biopsy artefacts and sampling errors

1. Sampling error: a particular problem for focal lesions, e.g. myocarditis, haemochromatosis. These may be completely missed in small biopsy sets and a negative result should not be interpreted as proof of absence.
2. Contraction bands: can be minimised by using fixative at room temperature and allowing the muscle to ‘relax’ for a few minutes on a saline swab prior to putting into fixative. The attenuated cytoplasm between the contraction bands resembles but should not be mistaken for myocytolysis (dilation of sarco-tubular elements).
3. Oedema: difficult to diagnose as variations in fixation and processing alter the degree of separation of myocytes mimicking or masking oedema.

A6 References

1. Ardehali H, Qasim A, Cappola T, Howard D, Hruban R, Hare JM, Baughman KL, Kasper EK. Endomyocardial biopsy plays a role in diagnosing patients with unexplained cardiomyopathy. *Am. Heart J* 2004;147:919–923.
2. Cunningham KS, Veinot JP, Butany J. An approach to endomyocardial biopsy interpretation. *J Clin Pathol* 2006;59:121–129.
3. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ Jr *et al.* Myocarditis. A histopathologic definition and classification. *Am J Cardiovasc Pathol* 1987;1:3–14.
4. Virmani R, Burke A, Farb A, Atkinson J.B. The endomyocardial biopsy: techniques and role in diagnosis of heart diseases. In: *Cardiovascular Pathology* (2nd edition). W.B. Saunders; 2001.
5. Arbustini E, Diegoli M, Morbini P, Dal Bello B, Banchieri N, Pilotto A *et al.* Prevalence and characteristics of dystrophin defects in adult male patients with dilated cardiomyopathy. *J Am Coll Cardiol* 2000;35:1760–1768.

SECTION B TISSUE PATHWAY: ENDOMYOCARDIAL BIOPSIES FOR THE ASSESSMENT OF CARDIAC ALLOGRAFT REJECTION

The following recommendations apply to endomyocardial biopsy (EMB) specimens taken for the assessment of cardiac allograft rejection, both cellular and humoral.

The International Society of Heart and Lung Transplantation (ISHLT) guidelines are used.¹⁻³

B1 Staffing and workload

Two or more pathologists in a unit should be competent in the reporting of cardiac transplant biopsies, in order to provide cover for periods of leave. If an out-of-hours, on-call service is offered, this is staffed by pathologists who contribute to the routine cardiac transplant pathology service. All pathologists reporting cardiac transplant biopsies should follow the revised 2004 ISHLT grading system.

B2 Specimen submission

Due to the potential for sampling error in diagnosing acute rejection, multiple myocardial biopsy samples are obtained from different right ventricular sites. Samples are not divided once procured in order to obtain the required number of pieces as this practice results in less representative sampling. Although the original ISHLT grading system required at least four pieces of myocardium, the trend has been to accept three evaluable samples as the absolute minimum for interpretation. Therefore a minimum of three, and preferably more, evaluable pieces of myocardium are now recommended for the grading of acute cellular rejection. An evaluable piece of myocardium contains at least 50% myocardium, excluding previous biopsy site, scar, adipose tissue or blood clot, which may comprise the remainder of the piece.

The biopsy specimens are fixed in 10% buffered formalin. If immunofluorescence is used by the laboratory, one or more additional biopsies are snap frozen if required to assess antibody mediated rejection. Antibodies to C3d and C4d which work well on paraffin embedded sections are available.

The specimen must be accompanied by a request card with identifying patient details (full name, gender, date of birth, NHS number), name of the responsible consultant, date of the procedure and relevant clinical summary. The specimen container is labelled with matching patient details.

The clinical summary should include reference to the date of transplantation, the underlying cardiac disease leading to transplantation, immunosuppressive and other drug treatment, previous biopsy result, cardiac function and clinical state of the patient. It is helpful if previous pertinent histology is identified.

B3 Processing and embedding

The fragments submitted are processed in a cell safe cassette or other method to avoid the loss of tiny specimens. All the biopsy fragments are embedded in one paraffin block. The pieces are arranged in a line or as a small group to facilitate screening.

B4 Sectioning and staining

Sections are cut from a minimum of three levels through the block, with at least three sections at each level. Several serial sections/slides are recommended. Additional spare slides may be saved unstained, in case additional studies are needed.

Special stains are not routinely required, but Masson trichrome and elastic van Gieson stains may be of value in showing myocyte damage in the early post-transplant period and fibrosis. Stains for organisms are also required on occasions, as suggested either by the clinical findings or histological changes of necrosis, granulomas, or unusual patterns of inflammation.

B5 Further investigations

Acute antibody-mediated rejection is associated with worse graft survival. It is observed in allosensitised patients, including those with previous transplantation, transfusion or pregnancy and previous use of ventricular assist devices. Pathologically, it can be recognised by myocardial capillary injury with endothelial cell swelling and intravascular macrophage accumulation. Interstitial oedema and haemorrhage can be present together with neutrophils in and around capillaries. Intravascular thrombi and myocyte necrosis may also be identified.

When these features are seen in the presence of unexplained cardiac dysfunction, it is proposed that immunostaining be performed by immunofluorescence or immunohistochemistry as follows.

- Immunoglobulin (IgG, IgM and IgA) plus complement deposition (C3d, C4d and/or C1q) in capillaries by immunofluorescence on frozen sections; and/or
- CD68 staining of macrophages within capillaries and C4d (+/- C3d) staining of capillaries by paraffin immunohistochemistry.

It is recommended that patients with haemodynamic compromise undergo assessment for circulating antibodies.

Immunofluorescence or immunoperoxidase staining for C4d, CD31 and CD68 is performed if there is a previous history of antibody mediated rejection or features suspicious for antibody mediated rejection found on the H&E stained sections (endothelial swelling, inflammatory cells within vessels, neutrophils around vessels, interstitial oedema or haemorrhage). This does not preclude doing C4d if there are any other grounds for suspicion.

B6 Report content

B6.1 Adequacy

The biopsy is assessed for adequacy to exclude rejection and the report refers accordingly. A minimum of three pieces containing at least 50% myocardium free from previous biopsy sites, scars or fat. If this criterion is not fulfilled, the biopsy is called inadequate.

B6.2 Broader pathology

The endocardial aspect of the biopsy fragments is assessed with regards to fibrosis, endocardial infiltrates (Quilty lesions) and previous biopsy site. Any evidence of peritransplant injury, myocyte hypertrophy, myocardial calcification, ischaemic/infarct damage or myocardial vascular change is noted. If epicardial adipose tissue is included with the sample there may be evidence of epicardial inflammation, epicardial vascular change or epicardial lipogranulomata. The clinician is informed if an epicardial surface lined by mesothelial cells is included in the specimen.

An attempt to differentiate a tangentially cut Quilty lesion involving underlying myocardium from cellular rejection is undertaken by cutting further levels and/or performing immunohistochemistry for CD3, CD20, CD31, CD34 and CD68.

B6.3 Rejection

The biopsy material is assessed for evidence of cellular and humoral/antibody mediated rejection.

Acute cellular rejection

An adequate biopsy is assessed for cellular rejection and graded according to the 2005 ISHLT criteria. Acute cellular rejection is characterised by an inflammatory infiltrate predominantly composed of lymphocytes, as well as macrophages and occasional eosinophils. For the grading of rejection, it is recommended that the revised 2004 ISHLT heart biopsy grading scale is used.

Note that the presence of neutrophils (except in the most severe form of rejection) should raise the question of an alternative process, such as healing ischaemic injury, antibody mediated (humoral) rejection or infection. Plasma cells are also not typically present in acute cellular rejection and suggest a Quilty lesion, healed ischaemic injury or a PTLD (plasmacytoid lymphocytes).

Acute humoral rejection

The slides are examined for features suggestive of antibody mediated rejection and if present the diagnosis of antibody mediated rejection confirmed by immunohistochemistry. If C4d staining of interstitial capillaries is positive and/or macrophages are confirmed within vessels, then it is suggested in the report that blood be sent to look for donor specific antibodies. The presence or absence of features suspicious of acute humoral rejection is coded according to the 2005 ISHLT criteria.

B6.4 Pathology of immunosuppression

Involvement by post-transplant lymphoproliferative disorder (PTLD) or infection (e.g. *Toxoplasma gondii*, CMV) may be encountered in these biopsy specimens. Whilst uncommon in EMBs, infections and PTLDs should be considered and ruled out if the histological or clinical findings are suspicious. The biopsy is examined specifically for evidence of these and their presence or absence commented upon.

B6.5 Recurrence

Recurrence of the original cardiac disease may also be encountered in these biopsy specimens. The likelihood of this depends on the original disease. An index of suspicion is maintained and correlation with the clinical history and explant findings is important.

B7 References

1. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J *et al*. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005;24:1710–1720.
2. Stewart S, Cary NRB, Goddard MJ, Billingham ME. *Atlas of Biopsy Pathology for Heart and Lung Transplantation*. London: Arnold, 2000.
3. Billingham ME *et al*. A Working Formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. *J Heart Lung Transplant* 1990;9:587–593.

SECTION C TISSUE PATHWAY: CARDIAC VALVE SPECIMENS

This pathway applies to cardiac valve specimens resected at valve repair and are developed from evidence-based peer-reviewed guidelines.^{1,2}

C1 Specimen submission

The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. The specimen does **not** need to be sent to histopathology fresh, but if sent fresh a protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary.

Unlike many other pathology specimens, proper microbiological evaluation is essential, particularly in cases of suspected infective endocarditis. Screening of specimens without prior probability of pathogenic infection is of no real value. Thus, while usually the surgeon refrains from interfering with the specimen, it is helpful if suspected endocarditis lesions are promptly diverted in an unfixed state to microbiology. This is better done in the operating theatre or under microbiological conditions than in open histopathology 'cut up'. It may be done in histopathology facilities if there is a laminar flow hood to supply sterile downdraft (Class II microbiological safety cabinet). The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary. It is helpful if previous histology that is pertinent to the current specimen is identified. The patient details required may vary from laboratory to laboratory but must include as a minimum the patient's full name, gender, date of birth and NHS number. The specimen container is labelled with matching patient details.

C2 Specimen dissection

The key features of valve disease are typically macroscopic, not microscopic. Macroscopic photographs are important for a permanent record and should be available at the time of writing of the microscopic report and final sign out. The specimen is described carefully with particular note of calcification, fusion along the commissural lines, nodules, presence or absence of focal lesions, thrombus, vegetations and defects. The thickness and translucency are commented upon as these are key features of myxoid change (e.g. floppy mitral valve). The possibility of dual pathologies should be borne in mind. These are more likely than chance since abnormal valves are predisposed to secondary lesions, e.g. endocarditis and calcification. An example of appropriate blocking is shown below (Image courtesy of Dr Patrick Gallagher):



C3 Sectioning and staining

Valves have one representative sample from each leaflet, plus additional blocks of focal lesions. All sections are stained with H&E, alcian-blue-diastase-periodic-acid-Schiff (ABDPAS) and elastic van Gieson. Alizarin red staining is optional, may help interpretation and is more practicable than von Kossa.

C4 Further investigations and comments

Culture – see above; Gram and DPAS on vegetations – see above. Martius Scarlet Blue adds little real information but is useful for demonstration and clinicopathological conference purposes.

IgG, IgA, IgM, C3 and basic leukocyte subsets (e.g. CD3, CD68, CD79a) are useful in suspected rheumatic or autoimmune endocarditis.

C5 References

1. Dare AJ, Harrity PJ, Tazelaar HD, Edwards WD, Mullany CJ. Evaluation of surgically excised mitral valves: revised recommendations based on changing operative procedures in the 1990s. *Hum Pathol* 1993;24:1286–1293.
2. Thiene G, Basso C. Pathology and pathogenesis of infective endocarditis in native heart valves. *Cardiovasc Pathol* 2006;15:256–263.

SECTION D TISSUE PATHWAY: NON-MALIGNANT MASSES REMOVED FROM THE HEART AND GREAT VESSELS

D1 Specimen submission

These guidelines are adapted from evidence-based, peer-reviewed material.¹ Histological examination of all excised cardiac masses is mandatory.² The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. The specimen does **not** require to be sent fresh, but if sent fresh a protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary. The surgeon should refrain from interfering with the specimen once it has been removed from the body, i.e. no opening, slicing, etc. The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary including ESR. It is helpful if previous histology that is pertinent to the current specimen is identified. The patient details required may vary from laboratory to laboratory but must include as a minimum the patient's full name, gender, date of birth and NHS number. The specimen container is labelled with matching patient details.

D2 Specimen dissection

The specimen is described in detail, including all three maximum dimensions, surface (glistening, gelatinous, papillary, frondose) and colour (tan, brown, grey, etc). Any potential stalk or cardiac attachment is identified, and preferably inked unless the pathologist is very confident of blocking and there is very good communication. The base of cardiac myxomas, and cardiac attachments of other masses are identified and blocked in their entirety. Liberal blocking of the lesion (possibly up to entirety) is appropriate. The mass comprises what the surgeon resects; a lower limit of adequacy is not recommended.

D3 Sectioning and staining

Sections are stained with H&E, elastic van Gieson (EVG) and alcian-blue-periodic-acid-Schiff (ABDPAS) in the first instance. Additional levels and sampling are carried out as necessary.

D4 Further investigations and comments

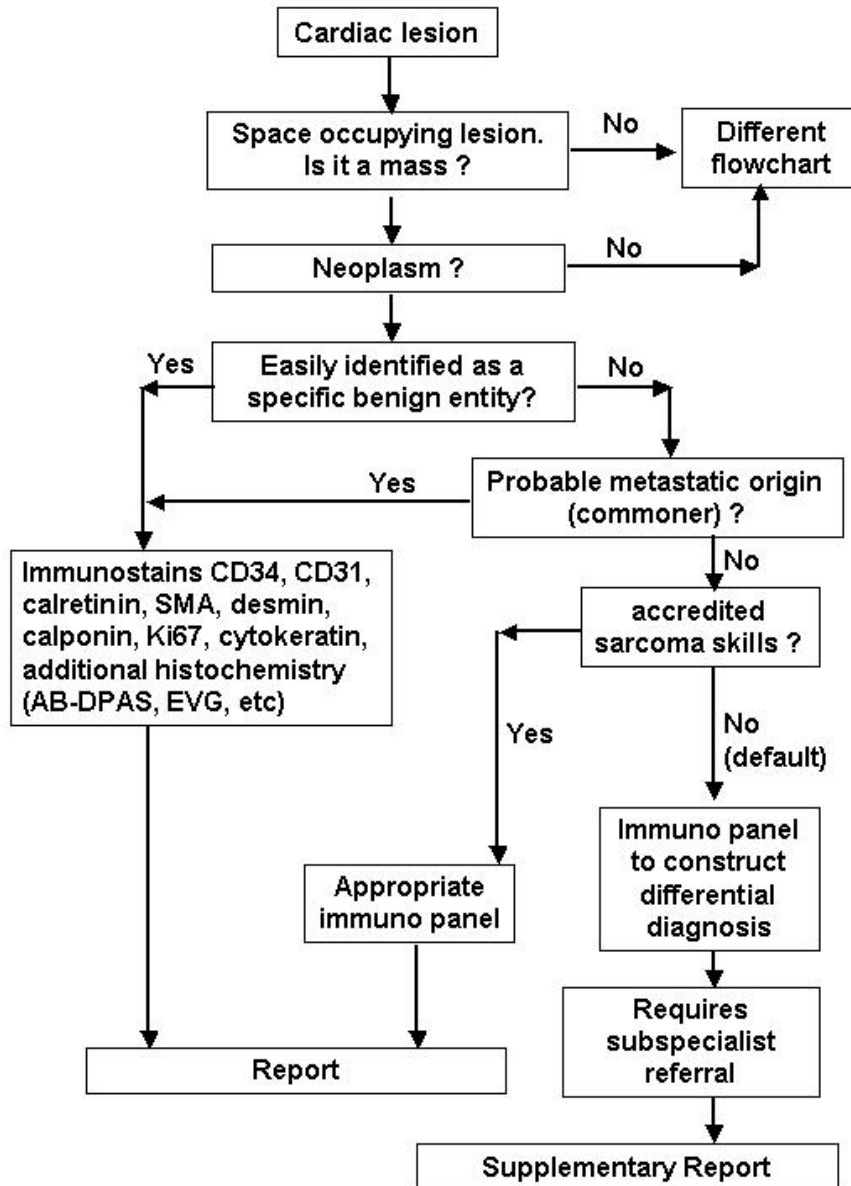
Immunostaining may be helpful to confirm a benign entity and exclude malignancy (CD34, CD31, calretinin, smooth muscle alpha-actin, desmin, Ki67, cytokeratin, melan-A).

Not all masses are neoplastic and the possibility of an inflammatory pseudotumour should be considered in the differential diagnosis. The composition of any inflammatory infiltrate is defined and described, if necessary with the help of appropriate immunostaining.

D5 Report content

The report includes the macroscopic description, the name of the preferred benign entity, and comment on the completeness of excision based on evaluation of the stalk / base / attached normal cardiac tissue. The commonest diagnoses will be left atrial myxoma and papillary fibroelastoma. Other diagnoses will be extremely rare. A comment on any required or intended additional investigations or referrals is made (see Figure 1).

Figure 1



D6 Referral

If a specific benign entity cannot be diagnosed with reasonable certainty, referral to a soft tissue tumour pathologist is mandatory. The case then enters the cancer tissue pathway.

D7 References

1. Burke A, Jeudy J Jr, Virmani R. Cardiac tumours: an update: Cardiac tumours. *Heart* 2008;94:117–123.
2. Sheppard M, Davies MJ. Tumours of the Heart. In: Sheppard M, Davies MJ (eds). *Practical Cardiovascular Pathology (1st edition)*. London: Arnold, 1998.

SECTION E TISSUE PATHWAY: SPECIMENS OF AORTA AND OTHER LARGE OR MEDIUM-SIZED VESSELS

This pathway applies to specimens of the aorta resected typically for thoracic aneurysm repair, but is intended to be generally applicable to other vessel specimens. Practical aspects are described.¹

E1 Specimen submission

The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. The specimen does **not** require to be sent to histopathology fresh, but if sent fresh, a protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary.

The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary. It is helpful if previous histology that is pertinent to the current specimen is identified. The patient details required may vary from laboratory to laboratory but must include as a minimum the patient's full name, gender, date of birth and NHS number. The specimen container is labelled with matching patient details.

E2 Specimen dissection

Macroscopic photographs are important for a permanent record, and are made available at the time of writing of the microscopic report and final sign out. The specimen is described carefully, with particular note of calcification, atherosclerosis and haematoma. If there is haematoma, the size and location of the dissection flap is recorded, and the approximate location of the dissection within the media (inner 1/3, 1/2 way through, outer 2/3). Periaortic adventitial haematoma is noted. The Stanford type may not be assessable from the limited resection specimen alone, and would be more obvious on imaging.¹ Useful pathological examination concentrates on possible predisposing factors.

E3 Sectioning and staining

In general, vessels are embedded on end. All sections are stained with H&E, alcian-blue-diastase-periodic-acid-Schiff (ABDPAS) and elastic van Gieson (EVG) or alcian-blue-elastic van Gieson (ABEVG). Alizarin Red staining is optional, may help interpretation, and is more practicable as a calcium stain than Von Kossa. Alcian blue on its own is useless as all vessels are somewhat alcianophilic. ABDPAS tells predominantly acid mucopolysaccharides from neutral mucopolysaccharides²⁻³. However, deposition of mucopolysaccharides without tissue destruction is usually insignificant. Thus one needs to examine either ABDPAS plus EVG; or a single ABEVG.

E4 Description

E4.1 General systematic description

The vessel type and size are described. Systematic description of the endothelium, intima (normal or abnormal), media, then adventitia will avoid omissions.

E4.2 Atherosclerosis

In describing atherosclerosis (a predominantly intimal process), it is helpful to give both text description of the lesion state, following the general outline of the descriptions used in the American Heart Association (AHA) consensus classification (Stary classification), and the formal class of lesion severity/stage (I–VI)⁴⁻⁶. Features to note include: intimal thickening, foam cells only, lipid necrotic core, cholesterol clefts, amount of fibrosis, fibrous cap, mononuclear cell infiltration, medial erosion, ulceration, mural thrombosis, calcification,

neovascularisation, recanalisation, or a predominantly fibrous plaque. Medial erosion by advanced plaques is commented upon. These may be aetiological in aortic widening if there is an advanced plaque overlying an aneurysmal aorta.

E4.3 Cystic medionecrosis and genetic diseases

The completeness of the internal elastic lamina is commented upon and the medial architecture is described. The architecture will normally be of parallel laminae of elastic fibres sandwiching vascular smooth muscle cells. Small foci of Erdheim's myxoid degeneration (cystic medionecrosis) are loss of vascular smooth muscle cells and replacement by myxoid tissue (positive with alcian blue). These may be associated with neovascularisation. Small foci of this change are **not** indicative *per se* of Marfan's change^{2,3}. They are usually seen in common thoracic aneurysms, in which they may be aetiological^{2,3}. Marfan's or a related connective disorder (e.g. osteogenesis imperfecta related aneurysms) is strongly suggested by widespread change of this type. The difference is quantitative not qualitative²⁻⁶.

E4.4 Aortitis/vasculitis

Description of vasculitis should conform to the Chapel Hill consensus⁷⁻⁹. This aids communication and maintains a systematic approach. However, since vasculitis syndromes may overlap or be unclassifiable, some flexibility is also important. This includes integrating autoimmune serology and clinical information and requires a precise description of the severity and type of tissue damage, and the composition of the inflammatory infiltrate. This includes descriptions of fibrinoid necrosis, adventitial versus intimal involvement, thrombosis, eosinophils, neutrophils, macrophages, T and B lymphocytes. Appropriate immunostains should be available to confirm.

E5 Further investigations and comments

Beta fibrillin genotyping is not currently performed on the aortic specimens themselves. These aneurysms are not usually fatal, so if the clinical team is alerted appropriately, the patient will be able to give blood for germline analysis. In fact, the mutations are highly heterogeneous. Patients with suspected inherited disorders should be referred to a clinical genetics service.

E6 References

1. Sheppard M, Davies MJ. Diseases of the aorta. In: *Practical Cardiovascular Pathology*. Sheppard M, Davies MJ. Arnold. London 1998.
2. Sariola H, Viljanen T, Luosto R. Histological pattern and changes in extracellular matrix in aortic dissections. *J Clin Pathol* 1986;39:1074–1081.
3. Nakashima Y, Kurozumi T, Sueishi K, Tanaka K. Dissecting aneurysm: a clinicopathologic and histopathologic study of 111 autopsied cases. *Hum Pathol* 1990;21:291–296.
4. Sary HC. Natural history and histological classification of atherosclerotic lesions: an update. *Arterioscler Thromb Vasc Biol* 2000;20:1177–1178.
5. Sary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W *et al*. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1995;92:1355–1374.
6. Sary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME *et al*. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994;89:2462–2478.
7. Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL *et al*. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187–192.
8. Falk RJ, Nachman PH, Hogan SL, Jennette JC. ANCA glomerulonephritis and vasculitis: a Chapel Hill perspective. *Semin Nephrol* 2000;20:233–243.
9. Jennette JC, Falk RJ. Nosology of primary vasculitis. *Curr Opin Rheumatol* 2007;19:10–16.

SECTION F TISSUE PATHWAY: TEMPORAL ARTERY SPECIMENS

This pathway applies to temporal artery biopsies taken to establish the diagnosis of giant cell arteritis.

F1 Specimen submission

The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. The specimen does **not** need to be sent fresh, but if sent fresh a protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary. The surgeon should refrain from interfering with the specimen once it has been removed from the body, i.e. no opening, slicing, etc. The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary including ESR. It is helpful if previous histology that is pertinent to the current specimen is identified. The patient details required may vary from laboratory to laboratory but must include as a minimum the patient's full name, gender, date of birth and NHS number. The specimen container is labelled with matching patient details.

F2 Specimen dissection

There is evidence that the size of the biopsy and extent of sampling are critical to diagnostic sensitivity¹⁻³. Sensitivity is much higher in biopsies over 0.5–1 cm formaldehyde-fixed length. The extended length and greatest diameter of the biopsy are measured in centimetres or millimetres. The presence of any tortuosity is commented upon. A series of transverse sections are made, producing segments 0.5 cm long which may be placed in the same or separate cassettes. The segments are sectioned transversely and embedded on end after appropriate communication with the embedding biomedical scientist. ALL tissue is submitted for histology, as active arteritis may be focal.

F3 Sectioning and staining

The key point is the disease is inherently focal. A single H&E-stained section is adequate for examination only if it is positive. Sensitivity is demonstrably higher when using serial sections of the entire tissue at 50 µm intervals³. This is linked to the issue of biopsy length¹⁻³. The biopsy is examined through at least three histological 'levels' in the first instance. If negative, additional levels are requested until it is clear that the tissue has been completely examined. That is, the block should be exhausted before definitively calling a biopsy-negative. If there is tangential sectioning, deeper levels may be necessary.

F4 Further investigations and comments

Identification of a destructive infiltrate of macrophages and giant cells on H&E is diagnostic (see Figure 2). Of note, the diagnosis may be made in the presence of a destructive infiltrate of macrophages without fully formed giant cells. The process is inherently focal. Serial sections or multiple levels may be required to identify diagnostic features. Speedy communication is essential and this should be recorded. The patient will normally have been commenced on steroids before receipt of the biopsy. However, a decision to discontinue steroids may be taken, taking the biopsy into account, to reduce serious side effects. As with other vasculitides, reporting should be in keeping with the Chapel Hill consensus and take account of site, composition of infiltrate and clinical context^{4,5}.

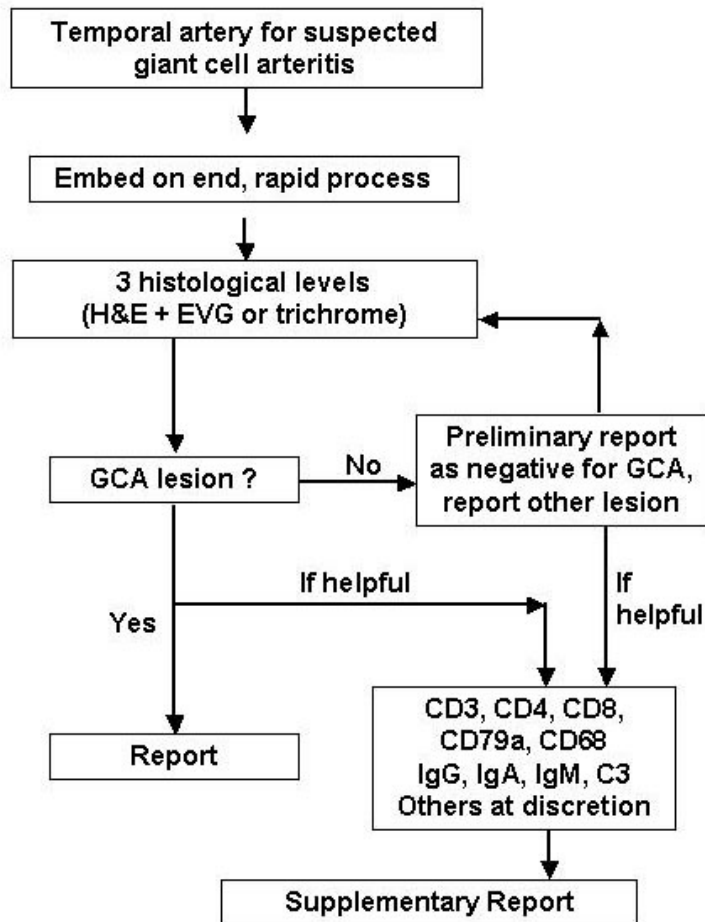
Intimal thickening itself is non-diagnostic. Special histochemical stains and immunohistochemistry are not required. However, elastic van Gieson (EVG) may help to:

- identify a strong single internal elastic lamina with a thick parallel-oriented media (identifying the specimen as an artery)
- determine intimal reduplication of the internal elastic lamina (indicating arteriosclerosis)

- identify breaks in the internal elastic lamina.

Intimal elastosis is often seen in hypertension and age and is not diagnostic of 'healed arteritis'. Alcian-blue-diastase-periodic-acid-Schiff (ABDPAS) is of limited use – it may help to define recent intimal thickening which would be non-diagnostic of active arteritis. Immunohistochemistry for CD68 and CD3 is confirmatory only and is not a requirement.

Figure 2



F5 References

1. Gonzalez-Gay MA, Garcia-Porrúa C, Miranda-Filloo JA. Giant cell arteritis: diagnosis and therapeutic management. *Curr Rheumatol Rep* 2006;8:299–302.
2. Taylor-Gjevre R, Vo M, Shukla D, Resch L. Temporal artery biopsy for giant cell arteritis. *J Rheumatol* 2005;32:1279–1282.
3. Nordborg E, Nordborg C. The influence of sectional interval on the reliability of temporal arterial biopsies in polymyalgia rheumatica. *Clin Rheumatol* 1995;14:330–334.
4. Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL *et al*. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187–192.
5. Jennette JC, Falk RJ. Nosology of primary vasculitis. *Curr Opin Rheumatol* 2007;19:10–16.

SECTION G TISSUE PATHWAY: PULMONARY THROMBOENDARTERECTOMY (PTE) SPECIMENS

This pathway applies to specimens received from a thromboendarterectomy procedure performed to treat chronic thromboembolic pulmonary hypertension with obstruction of main, lobar and segmental pulmonary arteries. They are based on peer-reviewed evidence ¹⁻⁴.

G1 Fixation

The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. The specimen does **not** need to be sent fresh, unless a frozen section is required due to unexpected intra-operative findings. If the tissue is sent fresh, a protocol is required to ensure rapid transport to the laboratory. The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary. The specimen container should be labelled with matching patient details and the side and site of origin of the tissue.

G2 Specimen dissection

The specimen usually consists of irregular vascular casts and fragments of variable thickened fibrointimal tissue. The range of the maximum dimension of the fragments is measured and the presence and amount of any fresh thrombus noted.

Representative sections are selected. If any focal, unusual appearances are noted, these areas are sampled more extensively.

G3 Embedding

Multiple fragments may be embedded in one block. The fragments should, if possible, be embedded on edge to allow assessment of the intimal changes and adjacent inner media, if present.

G4 Sectioning and staining

One H&E stained section and an elastic van Gieson (EVG) stained section from each block are sufficient in the first instance. Further levels may be cut if indicated.

G5 Further investigations

Although extremely rare, primary pulmonary artery sarcomas or tumour emboli from other primaries including lung, breast, kidney, liver, pancreas and the GI tract, may clinically present as pulmonary thromboembolism and may therefore be encountered in PTE specimens ¹⁻⁴. In these situations, abnormal tissue is often recognised at the time of the PTE procedure leading to a request for a frozen section. If tumour is present, relevant immunohistochemical stains should be performed on the paraffin embedded tissue to determine the tumour type.

Infrequently, an underlying vasculitis (including giant cell and Takayasu's arteritis) may predispose to pulmonary artery thrombosis. Possible infective cause for the inflammation should be excluded with the appropriate stains (Gram, Grocott, ZN, modified ZN) depending on the nature of the inflammatory process ¹.

G6 Report content

Histological examination may reveal intimal thickening due to thromboembolic material of varying ages¹. The presence of haemosiderin, focal calcification and atherosclerotic change may be noted. A variable, usually small, amount of intima is often included in the specimen. If there is a significant amount of medial tissue, this should be pointed out in the report and the clinician informed, as there is a significant risk that perforation or significant weakening of the pulmonary artery wall may have occurred during the procedure.

The presence, degree and type of any significant inflammatory infiltrate and any associated architectural disruption of the inner vascular wall are commented upon.

The presence or absence of malignancy should be stated.

G7 References

1. Blauwet LA, Edwards WD, Tazelaar HD, McGregor CG. Surgical pathology of pulmonary thromboendarterectomy: a study of 54 cases from 1990 to 2001. *Hum Pathol* 2003;34:1290–1298.
2. Miura S, Meirmanov S, Nakashima M, Hayashi T, Abe K, Tamaru N *et al.* Intimal sarcoma of the pulmonary artery: report of an autopsy case. *Pathol Res Pract* 2005;201:469–474.
3. Wilson MK, Granger EK, Preda VA. Pulmonary hypertension due to isolated metastatic squamous cell carcinoma thromboemboli. *Heart Lung Circul* 2006;15:143–145.
4. Brister SJ, Wilson-Yang K, Lobo FV, Yang H, Skala R. Pulmonary thromboendarterectomy in a patient with giant cell arteritis. *Ann Thorac Surg* 2002;73:1977–1079.

SECTION H TISSUE PATHWAY: HEART DISSECTION – EXPLANTS POST CARDIAC TRANSPLANTATION

This pathway applies to explanted hearts received after cardiac transplantation and is in line with more general accepted general guidelines¹⁻⁴.

H1 Fixation

The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. If received fresh, it is washed out to remove blood clot and suspended or nested in paper towel to allow fixation without distortion due to flattening under its own weight. Paper towels soaked in formalin may also be placed within the chambers to aid fixation without distortion. The specimen is accompanied by a request card with identifying details of the patient, name of the responsible clinical consultant, date of procedure, type of specimen and a brief, relevant clinical summary (a copy of the transplant referral letter is preferred). Any removal of tissues for graft harvest or research should be notified to the pathologist.

H2 Specimen dissection

The explanted heart may have had pieces removed for research purposes and one or more valves may have been harvested for use as homografts. Check whether the total weight was recorded prior to their excision. Weigh the specimen received. Describe the completeness of the chambers, attached great vessels and the epicardial surface. Detail any wires or catheters from implantable defibrillator, pacemaker, or ventricular assist devices, etc. The atria are usually partially absent, apart from the atrial appendages. Check the atrial appendages for thrombus. Examine the mitral and tricuspid valves from the atria and record any abnormality. Examine the aortic and pulmonary valves from above.

Check coronary arterial ostia and major branches such as the diagonals, obtuse marginals and the posterior descending branch of the right coronary artery. Ideally, summarise the findings on the American Heart Association (AHA) diagram of coronary artery anatomy, indicating the minimum luminal diameter of stenosed segments. If recently inserted stents are present, open the vessel by incising it longitudinally along the length of the stented segment and remove the stent. If long-standing stents are present, cut across the vessel with sharp scissors to check patency.

Do complete transverse (short-axis) slices of the ventricles at 1 cm intervals from apex to mid-ventricular level and assess carefully for symmetry, focal lesions and mural thrombus. Examine all valves from below.

Measure septal and posterior left ventricular wall thickness and cavity diameter (mean of two planes) for the left ventricle and wall thickness and cavity diameter for the right ventricle. After the rest of the examination is complete, isolated ventricular weights can be done using the Fulton technique (mandatory for assessing right ventricular hypertrophy).

H3 Sectioning and staining

Take representative blocks from each chamber (minimum five blocks, i.e. anterior and posterior mid interventricular septum, anterior, lateral and posterior free wall from the left ventricle and free wall of right ventricle) and from abnormal valves. As a minimum, take mapped blocks of anterior, lateral and posterior left ventricle and septum from a representative transverse slice, right ventricular outflow tract and both atria. Take representative sections from areas of greatest narrowing in each coronary vessel. These may require decalcification.

A connective tissue stain (Elastica van Gieson [EVG] or Masson's trichrome), as well as H&E, are done as standard on blocks of myocardium.

H4 Further investigations and comments

Congo red (thick section, amyloid), Perl's Prussian blue (iron) and PAS/AB/Diastase (storage disorders), immunohistochemistry for CD3, CD20, CD68 etc (myocarditis) are done as required.

Digital photographs of the whole heart or a transverse slice may be useful. Sectioning along the flow of blood or in echocardiography planes may be appropriate in particular cases, depending on the clinical scenario.

H5 References

1. Basso C, Burke MM, Fornes P, Gallagher PJ, Rosa Henriques de Gouveia, Sheppard M, Thiene G, van der Wal A. Guidelines for autopsy investigation of sudden cardiac death. *Virchows Archives* 2008; 45: 11-18.
2. Davies MJ, Mann JM. How to examine the heart and cardiac biopsies. In: W St C Symmers (ed). *The Cardiovascular System. Part B: Acquired diseases of the heart. Systemic Pathology (3rd ed), Volume 10*. Edinburgh: Churchill Livingstone, 1995.
3. Sheppard M, Davies MJ. Cardiac examination and normal cardiac anatomy. In: *Practical Cardiovascular Pathology*. London: Arnold, 1998.
4. The Royal College of Pathologists: *Guidelines on Autopsy Practice: Scenario 1: Sudden death with likely cardiac pathology*. www.rcpath.org/publications

SECTION I TISSUE PATHWAY: AUTOPSY CARDIAC DISSECTION

I1 Introduction

This document compliments the other R.C.Path. documents Tissue Pathway: Heart Dissection – Explants Post Cardiac Transplantation, and The Royal College of Pathologists: *Guidelines on autopsy practice: Scenario 1: Sudden death with likely cardiac pathology*. www.rcpath.org/publications. A European wide draft covering autopsy cardiac examination is in preparation and, once published, may take precedence over the UK R.C.Path. model. Unless otherwise specified, a basis for these recommendations may be found in ¹⁻⁷. A UK Cardiovascular Pathology Network (UKCPN) has now been established. This will facilitate referral pathways. Patient groups are supportive of this network. The Network will also facilitate improvements in the understanding and diagnosis of sudden adult death syndrome; particularly by crystallising its molecular genetic definition(s).

All autopsy practitioners should be able to perform a sound review of the heart and its vasculature (not least to avoid successful litigation). Knowledge of normal cardiac architecture is therefore mandatory. The majority of cases are recognised to be routine and can be assessed and signed off without recording every detail of the dissection as set out below. However, by following the dissection guide, later case analysis is possible.

For explanatory diagrams, see reference 4.

I1.1 Case preparation

Consideration of consent and coronial instructions is essential before the autopsy, and critical if considering retaining tissues. Knowledge of the patient's medical history and interventions may be directly relevant to the dissection and interpretation and, if not provided, should be sought before dissection of the body. The possible requirement for samples for special investigation, including electron microscopy, microbiology, and DNA extraction, should be considered before starting the dissection, in order to optimise sampling.

I1.2 Photography

Photography may be required and facilities should be available in any mortuary. A digital image of a midventricular transverse section (with ruler) is very helpful as a record, and for referral. Although optimally carried out in all cases, it is not essential for routine cases.

I1.3 Referral pathways

Complex cardiac disease, such as primary congenital heart disease, operated congenital heart disease (also known as grown up congenital heart disease/ GUCH) or those cases with complex medical interventions (electrophysiological surgery, mechanical devices etc.) can be performed by general pathologists, but may be better placed in the hands of specialist cardiac pathologists. This will help to ensure the safety and optimal care of relatives. The threshold for referral will differ according to the diagnosis, the complexity of the case and skill of the individual pathologist.

It is emphasised that sudden death, particularly in young individuals requires very careful consideration, tissue retention and a wide range of investigations in order to maximise the chance of obtaining a diagnosis. Discussion of the case with the family is beneficial, particularly if there is a positive family history. Liaison with regional cardiac pathologists is advisable. There is now a network to facilitate such referrals. As with other referral practices, depending on the Trusts concerned, there may be cost-implications to be considered. Note that this is a purely clinical series of recommendations.

I2 Macroscopic assessment

The dissection of the heart is described in detail elsewhere; with explanatory diagrams and photographs⁴. The heart is initially examined in situ, having exposed the pericardium and lung tissues by removal of the anterior chest wall.

Begin the macro assessment by examining the arrangement of the great veins, atrial chambers, ventricular chambers, and great arteries to be sure that they are connected in normal fashion. This is to exclude major congenital heart malformations.

At this point, examine:

- Connections of the major arteries.
- Architecture of the heart and the pericardium prior to removal of the cardiac tissue.
- Grafts (e.g. left internal mammary artery (LIMA)) and electrical pacemaker connections identified and preserved intact with the cardiac tissue (i.e. not disrupted).

I2.1 Great vessels

- Open the pulmonary artery (PA) valve with fingertip palpation of the proximal pulmonary arteries (to avoid missing proximal pulmonary emboli).
- Transect the aorta and PA.
- Transect 10-20 mm above the interface between the atrium and superior vena cava (SVC), preserving the sino-atrial node (SAN).
- Lift the apex of the heart upwards in a cranial direction, allowing transection of the four pulmonary veins and the inferior vena cava (IVC).
- Examine the heart from anterior and posterior aspects to assess whether the arrangement of the atria and ventricles is normal. The normal right and left atria have, respectively, a triangular auricle and rectangular appendage. The normal right ventricle will be clearly much thinner than the normal left. Both ventricles are more precisely and objectively measured as described below.

I2.2 Coronary arteries

1. Determine the **course and pattern** of the coronary arteries.

2. **Serially transversely slice** the coronary arteries at no more than 5 mm intervals. See diagram in reference 4. It is now generally agreed that cutting coronary arteries longitudinally can destroy thrombi / emboli and make assessment of stenosis impossible^{3,4}. A sharp scalpel blade is essential - blunt blades are ineffective and dangerous. This procedure will be more difficult with heavily calcified coronaries. Solutions are:

- a) On occasion, scissors are required to transect heavily calcified arteries.
- b) If there is a specific requirement for detailed coronary artery analysis then complete removal of the coronary arteries intact may be required with subsequent decalcification and then sectioning.
- c) Severely calcified coronaries can be dissected from the heart en-bloc and then serially sectioned at 5mm intervals on a cutting board, taking care to note the orientation and sequence of sections. This may be safer for the prosector than attempting to cut very hard arteries in situ.
- d) Some pathologists advocate assessing the coronary vessels after perfusion-fixation, where this is practicable.

3. Coronary artery inspection

1. In practice, inspection and cutting are nearly simultaneous. A standard system will reduce errors.
 - Start in the middle of the left anterior descending (LAD), sweeping downwards towards the apex, then upwards towards the left main stem (LMS) orifice.
 - Identify the circumflex, and local branches (diagonal/obtuse marginals OM1/OM2).

- Identify the right coronary artery (RCA) in the sulcus between the atrial appendage and right ventricle.
- Similar examination should run around the right side of the heart towards the anterior marginal (AM) and posterior interventricular descending (PIVD).

Note: it is possible to remove small segments of artery if the case is to be demonstrated to clinicians or in examination scenarios. However, if there is a possibility that second autopsy examination may follow, then no tissue should be separated from the heart unless it is to be preserved for histology. It is always possible to mark areas of interest with small sutures/clips. This provision mainly applies to coronial autopsies.

4. Coronary stents are a newer and still contentious area. Coronary metal stents are increasingly common, and cannot be dissected with scalpel or scissors. If the stent requires investigation of the lumen then this is removed en-bloc for resin embedding and sectioning using specialist cutting microtomes. Otherwise, up/down-stream artery analysis usually suffices, with the proviso that gentle injection of water along the stented vessel will identify blockage. In the absence of resin embedding and diamond knives, it is reasonably informative and practical to peel the coronary wall off the stent, visually inspect and gently retrieve in-stent material for additional histological evaluation to determine whether it is thrombus or restenotic tissue.

12.3 Myocardium

Three transverse slices of ventricular tissues (approximately 10-15 mm thick) are taken starting at the apex, making sure that the atrio-ventricular valvular tissue is not damaged. See diagram in reference 4.

The cardiac chambers are opened along the posterior aspect of the right atrium and ventricle just next to the septum, approximately 10 mm to the side of the PIVD. This posterior approach allows direct inspection of the complete tricuspid valve (TV), atrial/auricular and ventricular tissues. Note: no further slices are required into the auricle. The cut is continued onto the front of the right ventricle (RV) and upwards through the right ventricular outflow tract (RVOT) and pulmonary valve (PV). The circumferences of the TV, PV and thickness of the RVOT are recorded, along with other comments (e.g. fatty infiltration, vegetation, etc.).

The left side chambers are similarly inspected, with opening of the atrial and ventricle walls, 10 mm to the side of the PIVD. This allows direct inspection of all the left sided chambers and auricle without further cuts. However, the incision is now extended on to the anterior aspect of the left ventricle (LV) and runs along the edge of the anterior free wall, parallel to the LAD, until just under the anterior leaflet of the mitral valve (MV) – without damaging this valve. Thereafter, scissors are usually required to produce a partial right-hand turning cut between the left auricle and immediately behind the left main stem, thereby opening into the left ventricular outflow tract (LVOT) and aorta, through the aortic valve (AV). The MV /AV circumferences and LVOT thickness are recorded. It is advisable to avoid the lateral approach to the left ventricular tissues and/or slicing into the aortic root through the mitral valve as later consideration/demonstration of pathology is more difficult. To assess hypertrophy objectively, it is often helpful to measure the thickness (in mm) of the left ventricular free wall 10mm distal to the mitral valve³. As an adjunct, it may be helpful to weigh the left ventricle and right ventricle independently by cutting off the atria and cutting the right ventricle free from the left (Fulton weights)³. This is a destructive procedure and should be used only where genuinely informative and the heart requires no further examination.

13 Histological sampling

Tissue blocks are taken, **or not**, from areas of myocardial tissue of relevance according to the consent and medico-legal requirements.

The blocks could include simply left (+/- right) ventricular tissues as part of a non-cardiac pathology case but, if cardiac pathology is considered the prime lesion, then macroscopic lesions and relevant areas are sampled.

13.1 Cases with no identified macroscopic cardiac pathology

It is emphasised that cases with no cardiac pathology of significance still require careful examination of the coronary artery system, myocardial tissue, valves and overall architecture. In such cases, it may not be required to sample the tissue for histology or other tests unless an underlying/occult disorder is suspected (e.g. metabolic disorder, myocarditis). See SADS (below).

13.2 Cases with identified macroscopic cardiac pathology

In cases with primary or secondary cardiac disease, most commonly coronary artery disease and myocardial ischaemia/infarction, it may be sufficient to fully examine the tissue macroscopically, record the degree of vascular occlusion/stenosis as a percentage, site/size of infarction and/or areas of patchy fibrosis. In such cases the transverse chamber diameter for the right and left ventricles can be of assistance in assessing the degree of cardiac failure, although dissected Fulton weights (right ventricle/left ventricle and septum) are also particularly useful when considered against ante-mortem imaging data. Valvular heart disease is recorded, and may need histology (e.g. infective endocarditis).

Well defined myocardial ischaemic damage does not automatically require histological sampling as part of the autopsy analysis, unless there is an issue that requires histological assessment (e.g. dating of infarction, and/or exclusion of other myocardial disease). In such cases sampling is directed towards both the pathological and normal tissues (e.g. background coronary artery/maximal area of stenosis (with decalcification) and damaged myocardial tissue – equivalent to 1-4 blocks).

Cardiac involvement by systemic disease often requires tissue sampling. In general, 1-2 blocks suffice, although wider sampling may be governed by the nature of disorder and ante-mortem pathophysiology if clinical correlation is sought. Thus, metastatic disease to the heart could require just 1 block, but consideration of granulomatous myocarditis might need 4-6 blocks, or the specialist investigations as detailed below.

On occasion, examination of the valvular tissue and hinge point histology is required. Ideally, examination involves careful excision and may need decalcification.

13.3 Sudden death and no pathology seen (Sudden Adult Death Syndrome: SADS)

Sudden cardiac deaths (SCDs) without clear macroscopic pathology may require referral of the intact heart for specialist cardiac pathology review. If sampling is undertaken, then blocks should include (at least)

- a) Atria
- b) IV Septum
- c) RVOT
- d) Anterior LV
- e) Lateral LV
- f) Apical LV
- g) Posterior LV
- h) Complex cardiac cases may also require further blocks including full transverse section (jumbo blocks) of the RV, septum and LV.

13.4 Conduction system and special tissue sampling

The cardiac conduction system is not easily discerned macroscopically. Therefore, wide blocking of tissues to “capture” areas of relevance is recommended. See block-taking diagram superimposed on a macro photograph in Reference 4.

- SA node - apex of the crista of the right auricle and SVC interface. This block of tissue is removed in a square piece and longitudinal slices along the line of blood flow will allow identification of the nodal tissue next to the sino-atrial artery.
- AV node (AVN) is at the apex of the triangle of Koch. The borders for the triangle of Koch are:
 - anteriorly; the annular attachment of the septal leaflet of the tricuspid valve
 - posteriorly; the sinus septum containing the tendon of Todaro
 - inferiorly; the orifice of the coronary sinus
 - superiorly (apex of the triangle); the membranous septum have (bounded by the superior limb of the coronary sinus, membranous septum and superior edge of the tricuspid valve leaflet).
 - Remove as a tissue block including the membranous septum.
 - Transversely section across the specimen to identify AVN, the His bundle and bundle branches.
 - Take a transverse section of the septum immediately below this block to identify the radiating bundle branches.

14 Devices and prosthetic valves

When a patient has had vascular access lines, a pacemaker or prosthetic valve inserted, autopsy examination follows standard protocols (see above) but knowledge of the details of the cardiac intervention, the indication for intervention and where any complications have occurred is vital. Assessment of the surgical intervention with its complications/successes provides feedback for clinicians, relatives and the Coroner.

14.1 Vascular access lines

These lines are inspected externally at the start of the autopsy, cut flush with the skin and left in situ so that their internal positions can be checked during organ dissection. Exclusion of thrombotic change and sepsis around the site of introduction may require microbiological sampling and occasionally histology.

14.2 Pacemakers

Cardiac pacemakers are in common use and the majority are reliable. Exclusion of haemorrhage and sepsis (with respect to the time of implantation / battery change); lead fracture; thrombosis around lines and infective endocarditis at the lead insertion point/s is required. Ideally, the pacemaker box, its lead and electrode are examined in total – preferably in one piece with the thoracic organ block. Microbiology sampling, histology and/or photography may be appropriate.

Important notes:

1. **Defibrillator pacemakers (ICD) MUST be switched off before autopsy manipulation or removal, to avoid the risk of accidental discharge during the autopsy procedure. These devices are *designed* to generate high voltage shocks in response to fluctuating currents. The notes will contain the pacemaker label with device type. The local pacemaker clinic can switch them off.**
2. **The pacemaker box should ideally be returned to the local ECG/cardiac pacemaker department.**
3. **The pacemaker should never be left in the body in case of cremation explosion/risk.**

14.3 Prosthetic valves

Valve replacement surgery broadly involves two types of replacement device: tissue (allograft or xenograft) or prosthetic (usually metal). Rarely, patients with previous valvuloplasty are encountered. Whilst primary valve replacement failure can occur due to technical issues, the primary pathologies include local haemorrhage, infection, malalignment, local leak and tissue overgrowth across the valve (pannus). These are often diagnosed ante-mortem, but cardiac dissection requires exploration of the valve from above/ below to carefully assess any pathology. Thus, opening an atrium along the posterior aspect alongside the septum, then turning 90° to run the incision along/around the atrial base immediately above the atrio-ventricular groove to approximately 50% of the atrial chamber is recommended for atrio-ventricular valve samples. The ventricles are explored by opening the ventricular chambers at the apex of the heart, running a cut adjacent to the ventricular septum posteriorly and then turning 90° towards the lateral aspect of the ventricular chamber, allowing the ventricular chamber to be opened.

14.4 Other devices

Devices are increasingly common in cardiac tissues.

- Septal closure devices which broadly comprise two umbrella mesh platforms that are placed across the septal leak. These are inspected carefully as the chambers are opened with photography, microbiology sampling and removal of the tissues intact with the device if there is a medico-legal consideration to be made.
- Patch repairs, comprising Dacron-style materials, may be seen. Attention to the suture points is particularly important, to exclude infective endocarditis.

15 Other issues

15.1 Surgery for ischaemic heart disease

Surgical and medical intervention for ischaemic heart disease is still common, albeit declining. Historically, vein graft bypass surgery was the norm for coronary artery disease. In recent decades, internal mammary arteries have been used in addition (mainly left (LIMA) but also right (RIMA)).

- Knowledge of the number of grafts and the position to which they were applied is required.
- Exclude or identify general technical problems, sepsis, haemorrhage and infection as the tissues are analysed.
- Primary focus is on the grafted vessels, their attachments/engraftments with consideration of the background vasculature.
- Transversely cut the vessels from the aortic root along the graft, or from the left internal mammary artery.
- Some advocate using scissors to dissect along vessels, providing that the lower part of the graft has been opened to identify any extruded thrombus.
- Evaluate the degree of stenosis. Based on native coronary measurements, stenosis is traditionally measured by a subjective estimate of lumen area / total coronary coronary area. Stable stenosis over 70% correlates reasonably well with ischaemic SCD. Milder stenoses do not reliably predict ischaemic SCD taken in isolation, but may produce local thrombosis and SCD.
- Finally, the native vessel disease and possible obstruction/thrombosis of the anastomoses are assessed. If these are heavily calcified then they are dissected whole, decalcified and assessed histologically - although tissue retention issues are pertinent.

I5.2 Complex cases, probably needing referral

Cases of cardiac transplantation and GUCH are ideally considered by those with appropriate expertise. If appropriate consent and permissions exist, the heart is dissected intact, washed through with formalin, fixed and dispatched for further analysis. Return of tissues to the body will depend on the detail of consent given, the promptness of examination by the referral centre and the time scale of local burial practice.

I5.3 Histology stains

In general terms, routine H&E stained 4µm sections are sufficient. Additional histochemistry should be available to examine connective tissue and intra-cellular components of myocardial parenchyma using PAS, DPAS, EVG +/- Alcian Blue, Masson's trichrome, Congo Red at 10µm, Perl's stain or toluidine blue. Consideration of myocarditis can be enormously enhanced by staining for CD3 and CD68 positive cells (per mm)² using standard immunohistochemistry (6). Although not strictly in accordance with Dallas criteria, these have been questioned as the gold standard (7). Therefore, access to an immunohistochemistry laboratory should be assured.

I5.4 References

1. The Royal College of Pathologists: *Guidelines on autopsy practice: Scenario 1: Sudden death with likely cardiac pathology*. www.rcpath.org/publications.
2. Sheppard MN, Davies MJ. *Practical Cardiovascular Pathology*. 1998.
3. Davies MJ. The investigation of sudden cardiac death. *Histopathology* 1999;34:93-8.
4. Suvarna SK. National guidelines for adult autopsy cardiac dissection and diagnosis - are they achievable? A personal view. *Histopathology* 2008; 53: 97-112.
5. Hangartner JR, Marley NJ, Whitehead A, Thomas AC, Davies MJ. The assessment of cardiac hypertrophy at autopsy. *Histopathology* 1985;9:1295-306.
6. Cioc AM, Nuovo GJ. Histologic and in situ viral findings in the myocardium in cases of sudden, unexpected death. *Mod Pathol* 2002;15:914-22.
7. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. *Circulation* 2007;116:2216-33.

SECTION J TISSUE PATHWAY: CORONARY ENDARTERECTOMY SPECIMENS

Most primary coronary interventions are based on angioplasty and stenting without tissue removal. However, coronary atherectomies are sometimes received in some centres. Best practice is evolving and the evidence base for optimal handling is somewhat thin. In part, this is because the technology for removing them is also evolving.

J1 Macroscopic description

They tend to be small and irregular. They may or may not resemble actual artery. Make a general description, taking note of the overall shape, dimensions, and features of note. Any thrombus, lipid core debris, or calcification should be noted separately. Some may include retrieved stent, which should be noted and removed before processing. Some are simply tiny collections of thrombus and lipid core debris from angioplasty catch devices and should be handled especially carefully to avoid tissue loss.

J2 Processing and staining

They should be processed in entirety in a protective sleeve or grid to obviate loss in the processor. They should be embedded and sectioned. They may need decalcified. H&E and EVG at one level only should be sufficient. EVG staining is useful to define the media.

J3 Microscopy

Regular endarterectomies will have the appearances of atherosclerotic plaque. It is currently normal to classify plaques by the Stary classification¹. This is a very broad classification and the same information could be communicated by descriptions of the major components (calcification, fibrosis, lipid core, diffuse intimal thickening). Particular note should be made of thrombus, hemorrhage or other features that are associated with complication. The presence of tunica media should be commented upon. Note, its presence is common and does not indicate likely complication².

J4 References

1. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W *et al*. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1995;92:1355–1374.
2. Schnitt SJ, Safian RD, Kuntz RE, Schmidt DA, and Baim DS: Histologic findings in specimens obtained by percutaneous directional coronary atherectomy. *Hum Pathol* 1992, 23: 415-420