The Royal College of Pathologists
Pathology: the science behind the cure

Tissue pathways for cardiovascular pathology

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Foreword

The tissue pathways published by The Royal College of Pathologists (RCPath) are guidelines that enable pathologists to deal with routine surgical specimens in a consistent manner and report to a high standard. This ensures that accurate and relevant diagnostic and prognostic information is available to clinicians for optimal patient care and ensures appropriate management for specific clinical circumstances. This guideline has been developed to cover most common circumstances. However, we recognise that guidelines cannot anticipate every pathological specimen type and clinical scenario. Occasional variation from the practice recommended in this guideline may therefore be required to report a specimen in a way that maximises benefit to the patient. The guidelines themselves constitute the tools for implementation and dissemination of good practice.

The stakeholders consulted for this document were the Cardiac Pathology Network (www.ukcpn.org.uk). This group of pathologists are available for specialist advice and referrals.

The information used to develop this tissue pathway was collected from electronic searches of the medical literature, previous recommendations from the RCPath and local guidelines in the UK. Published evidence was evaluated using modified SIGN guidance. The level of evidence was either grade C or D, or met the good practice point (GPP) criteria (see Appendix A). Consensus of evidence in the tissue pathways was achieved by expert review.

No major organisational changes or cost implications have been identified that would hinder the implementation of the tissue pathways.

A formal revision cycle for all tissue pathways takes place on a five-yearly basis. However, each year, the College will ask the authors of the tissue pathways, in conjunction with the relevant subspecialty advisor to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken whereby a short note of the proposed changes will be placed on the College website for two weeks for members' attention. If members do not object to the changes, the short notice of change will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the publications page of the College.

The pathway has been reviewed by the Clinical Effectiveness department, Working Group on Cancer Services and Lay Governance Group. It was on the College website for consultation with the membership from 26 October to 23 November 2017. All comments received were addressed by the authors to the satisfaction of the Working Group Tissue Pathway Coordinator and the Director of Clinical Effectiveness.

This pathway was developed without external funding to the writing group. The College requires the authors of tissue pathways to provide a list of potential conflicts of interest; these are monitored by the Clinical Effectiveness department and are available on request. The authors of this document have declared that there are no conflicts of interest.

1 Introduction

General background supportive information for the cardiovascular pathology in all the sections can be found in the following textbooks:

- Sheppard MN. *Practical Cardiovascular Pathology (2nd edition).* London, UK: Hodder Arnold, 2011
- Suvarna SK (ed.). *Cardiac Pathology: A Guide to Current Practice*. London, UK: Springer, 2012

- Suvarna SK (ed.). *An Atlas of Adult Autopsy: A Guide to Modern Practice*. London, UK: Springer, 2016.

1.1 Target users of the guideline

The target primary users of the tissue pathway are consultant and trainee pathologists exposed to the dissection, sample taking and histological assessment of surgical and autopsy cardiovascular samples. Secondary users would be surgical and medical practitioners involved in the care of patients with cardiovascular disease.

2 Endomyocardial biopsies (EMBs) for the assessment of pathology of the native heart

2.1 Specimen submission

EMB contributes significantly to sensitivity and specificity in unexplained cardiomyopathy.[1,2] The chief diagnostic limitation with EMBs is sampling error.[2–4] To reduce this problem, it is recommended that at least four to five 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 consensus.[5] For 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. The samples are taken for formalin fixation but, ideally, an additional piece will be placed in glutaraldehyde for electron microscopy (EM) and one piece may be frozen for immunofluorescence, molecular biological and/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 optimal cutting temperature (OCT) embedding medium for storage at -70^{o}C. Alternatively, fresh material can be placed in RNAase for subsequent molecular analysis.[2,4]

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)[6] into which to place one biopsy fragement. If an arrangement is set up to collect frozen tissue, the designated person could also place one biopsy fragment into EM fixative. If the specimen arrives in the laboratory in formalin, then one biopsy fragment 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 and NHS number), along with the name of the responsible consultant, date of the procedure and a relevant clinical summary. The specimen container should be labelled with matching patient details. The clinical summary should include: reference to all medications, including non-prescription and/or illicit drugs; co-existent medical conditions, especially multisystem diseases such as collagen vascular diseases, thalassaemia, porphyria, amyloidosis and sarcoidosis; echocardiographic and other cardiac imaging findings. It is helpful if previous histology, which is pertinent to the current specimen, is identified and reviewed.

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

2.2 Processing and embedding

The fragments submitted for 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 may be 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 glycogen.[6]

2.3 Sectioning and staining

Ideally, five spaced histological sections (levels) are obtained in the first instance. The exact interval between levels will vary between laboratories but will typically be 20–25 microns. These may have at least two to three (and up to five) serial sections mounted on each slide at each level.[1,2,4]

At least three to five different levels are stained with haematoxylin and eosin (H&E).[2–4]

At least one section per biopsy is stained with:

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

In the presence of acute inflammatory cell infiltration or granulomas, special stains for infective organisms (Ziehl-Neelsen [ZN], modified ZN, Grocott, PAS and Gram) are performed.

2.4 Further investigations

2.4.1 Immunofluorescence/immunoperoxidase

In suspected Duchenne and Becker muscular dystrophies, immunohistochemical staining for dystrophin may be useful. Moreover, dystrophin components[7] should be investigated in all young male patients. Amyloid should be immunohistochemically typed. This can be performed on paraffin-embedded or frozen tissue, and tissue can be sent to the National Amyloidosis Centre at the Royal Free Hospital for complete amyloid work-up. Subtyping the inflammatory infiltrate may be useful.[2,4] There is some evidence that lamin A/C immunohistochemistry may also be helpful.

2.4.2 Electron microscopy

Small samples of myocardium may be submitted for EM.[2–4] If a diagnosis is not reached by LM, then EM is performed on the glutaraldehyde sample, or if necessary, reprocessed paraffin tissue. Reprocessing may involve some substance being partially removed by routine processing yielding EM unhelpful (e.g. Fabry's disease). A number of entities can only 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 paclitaxel.[2–4]

2.4.3 Infectious agents

Special stains (e.g. Grocott, PAS or ZN) are used in cases of granulomatous inflammation. PAS is also performed if foamy macrophages are present, to look for Whipple's disease.

2.4.4 Molecular biology

Polymerase chain reaction (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.[2,4] 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 often 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. in situ hybridisation or PCR or 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 likely will become increasingly useful.

2.5 Report content

The report[3] should refer specifically to the following:

- the myocytes: with comment on any hypertrophy, atrophy, necrosis, vacuolation, inclusions, iron deposition and/or disarray
- the interstitium: with comment on fibrosis, inflammation, adipose tissue infiltration and amyloid deposition
- the endocardial aspect: with comment on fibrosis/fibroelastosis
- interstitial vessels: with comment on thrombi, thickening and dysplastic changes
- the endocardium: with focus on an assessment of fibrosis/elastosis and inflammation. Be aware that tangential cutting mimics increased fibrosis, particularly if the samples are not perpendicular to the plane of section.

Specifically with regard to the myocardium, the presence or absence of the following are noted:

- 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".[3] However, it is now becoming recognised that these criteria may need to be refined and it is recommended that the nature and degree of infiltrate is clearly indicated.[2]
- 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 (RV) this is a normal finding, particularly at the junction of the septum and free wall. In left ventricular biopsy specimens, the mycocyte 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, and this is an indication for EM. The presence or absence of iron and amyloid, and the extent and pattern of fibrosis are noted.
- blood vessels: evidence of inflammation, endothelial swelling, thrombosis or abnormal wall characteristics, such as hypertrophy, intimal fibrosis and small vessel medial vasculopathy

- 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.

- it is recommended that the degree of diagnostic certainty (e.g. certain/definite, probable, possible and non-specific) together with the adequacy of the sample (e.g. optimal or suboptimal) be included in the report.[2]

2.6 EMB artefacts and sampling errors

2.6.1 Sampling error

This is 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.

2.6.2 Contraction bands

These 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 sarcotubular elements).

2.6.3 Oedema

Oedema is difficult to diagnose as variations in fixation and processing alter the degree of separation of myocytes, mimicking or masking oedema.

[Level of evidence – D.]

3 EMBs for the assessment of cardiac allograft rejection

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

The ISHLT guidelines are used.[8–10]

3.1 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.

3.2 Specimen submission

Owing 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, but 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 now available.

The specimen must be accompanied by a request card with identifying patient details (full name, gender, date of birth and 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.

3.3 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.

3.4 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's trichrome and EVG 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.

3.5 Further investigations

The ISHLT has changed the recommendations for immunohistochemical testing for antibody-mediated rejection from when there are histological and/or clinical grounds for suspicion to a mandatory minimum number of biopsies at different times post-transplantation.[8]

Immunohistochemistry (immunoperoxidase or immunofluorescence) should be performed on a minimum of two biopsies during the first two months post-transplantation and then follow a similar schedule to the centre's serological testing for donor-specific antibodies. The mandatory panel of immunostains for immunoperoxidase is C4d and CD68, and for immunofluorescence is C4d, C3d and anti-HLA-DR. Additional optional immunostains that can be performed for immunoperoxidase include CD3, CD20, C3d, CD31, CD34 and complement regulatory proteins. The optional panel for immunofluorescence include fibrin, IgG and IgM. If a positive stain is obtained, all biopsies subsequent to this should be stained until they are negative.

A positive C4d is considered to be staining of >50% of capillaries; however, lesser degrees of staining warrant discussion with the clinicians and testing for a donor-specific antibody, particularly when the C4d is performed on formalin-fixed tissues. A positive CD68 is considered when intravascular macrophages are present in >10% of capillaries.

Any clinical or histological concern for antibody-mediated rejection should prompt testing of more biopsies than the minimum and many centres have adopted routine staining of all biopsies.

3.6 Report content

3.6.1 Adequacy

The biopsy is assessed for adequacy to exclude rejection and the report refers accordingly. To be adequate, a minimum of three pieces containing at least 50% myocardium free from previous biopsy site changes, scars or fat is required. If this criterion is not fulfilled, the biopsy is designated inadequate.

3.6.2 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 should be informed if an epicardial surface lined by mesothelial cells is included in the specimen – indicating potential wall breach.

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.

3.6.3 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 current 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 post-transplant lymphoproliferative disorder (PTLD).

Acute humoral rejection

The slides are examined for:

- the histological features of antibody-mediated rejection: intravascular activated mononuclear cells, endothelial swelling, interstitial oedema and haemorrhage
- immunohistochemical features of antibody-mediated rejection: C4d attached to capillary endothelial cells and intravascular macrophages.

A pathological grading system for antibody-mediated rejection is used and is based on whether histological and/or immunohistochemical features are present and the severity of the histological features.[10]

3.6.4 Pathology of immunosuppression

Involvement by PTLD or infection (e.g. *Toxoplasma gondii* or cytomegalovirus) may be encountered in these biopsy specimens. While 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.

3.6.5 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.

[Level of evidence – C.]

4 Cardiac valve specimens

This pathway applies to cardiac valve specimens resected at ante-mortem valve repair and has been developed from evidence-based peer-reviewed guidelines.[11,12]

4.1 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 unfixed, but if sent fresh, a protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary. Those with suspected infective endocarditis are best sampled in theatre prior to transfer of valve tissue for histology. This can also be done in histopathology facilities if there is a laminar flow hood to supply sterile downdraft (Class II microbiological safety cabinet). The specimen should be 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 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 should be labelled with matching patient details.

4.2 Specimen dissection

The key features of valve disease are typically macroscopic, not microscopic. Macroscopic photographs may be 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 [MV]). 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 (i.e. at least one perpendicular section, although more are possible. Note: calcified valves should be decalcified before section) (Image courtesy of Dr Patrick Gallagher).

4.3 Sectioning and staining

Valves have one (minimum) representative sample from each leaflet, plus additional blocks of focal lesions. All sections are stained with H&E, although additional stains may be of use.

4.4 Further investigations and comments

- Microbiology sampling, if fresh tissue.
- Other stains include alcian blue-diastase-PAS (ABDPAS) for connective tissue and fungi, Gram stain for bacteria and EVG for connective tissues. Alizarin red staining (for calcium) is optional; it may help with interpretation and is more practicable than von Kossa.
- IgG, IgA, IgM, C3 and basic leukocyte subsets (e.g. CD3, CD68 and CD79a) are useful in considering various inflammatory processes (e.g. suspected rheumatic or autoimmune endocarditis).

[Level of evidence – GPP.]

5 Non-malignant and malignant masses removed from the heart and great vessels

5.1 Specimen submission

These guidelines have been adapted from evidence-based, peer-reviewed material.[13] Histological examination of all excised cardiac masses is mandatory.[14] 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 it is, 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 should be 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 erythrocyte sedimentation rate (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 should be labelled with matching patient details.

5.2 Specimen dissection

The specimen is described in detail, including all three maximum dimensions, surface (glistening, gelatinous, papillary, fronded) 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 the margin(s). The base (surgical margin; often inked) 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.

In malignant lesions (e.g. sarcomas), soft tissue sampling protocols should be followed.

5.3 Sectioning and staining

Sections are stained with H&E, EVG and ABDPAS in the first instance. Additional levels and sampling are carried out as necessary.

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.

5.4 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).

5.5 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, by contrast, rare. A comment on any required or intended additional investigations or referrals is made.

5.6 Referral

If a specific benign entity cannot be diagnosed with reasonable certainty, referral to a specialist cardiopathologist and/or soft tissue tumour pathologist is advised. The case then may enter the cancer tissue pathway.

[Level of evidence – GPP.]

6 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.[15]

6.1 Specimen submission

The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. If sent fresh, a protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary.

The specimen should be 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 should be labelled with matching patient details.

6.2 Specimen dissection

Macroscopic photographs are of value in the interpretation of the histology and for case review purposes. The specimen should be described carefully, with particular note made of calcification, atherosclerosis and haematoma. If there is haematoma, the size and location of the dissection flap is recorded, along with the approximate location of the dissection within the media (inner 1/3, 1/2 way through, outer 2/3). Peri-aortic adventitial haematoma is noted. The Stanford type may not be assessable from a limited resection specimen alone, and would be more obvious on imaging.[15] Useful pathological examination concentrates on possible predisposing factors.

6.3 Sectioning and staining

In general, vessels are embedded on end. All sections are primarily stained with H&E, ABDPAS and EVG or alcian blue-EVG (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 of minimal value, as all vessels are somewhat alcianophilic. ABDPAS tells predominantly acid mucopolysaccharides from neutral mucopolysaccharides. However, deposition of mucopolysaccharides without tissue destruction is usually insignificant. Thus, ABDPAS plus EVG, or a single ABEVG, needs to be examined.

6.4 Description

6.4.1 General systematic description

The vessel type and size are described. Systematic description of the endothelium, intima (normal or abnormal), media and then adventitia will avoid omissions. The recent consensus statements of the pathology of the aorta are applicable to other large vessels[15,16] and all reports should follow these guidelines.

6.4.2 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),[17,18] and the formal class of lesion severity/stage (I–VI). 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.

6.4.3 Medial degeneration 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 'myxoid degeneration' (previously called cystic medionecrosis) are evidenced by 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 may be seen in a variety of degenerative conditions, but large foci are seen in Marfan's syndrome and related connective disorders (e.g. osteogenesis imperfecta).

6.4.4 Aortitis/vasculitis

Description of vasculitis should conform to the recent consensus.[15] 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, and T and B lymphocytes. Appropriate immunostains should be available.

6.5 Further investigations and comments

Knowledge of the pathology often guides the diagnosis to possible inherited conditions. This may be pertinent to the patient and indeed their families. Mutations are often highly heterogeneous. Patients with suspected inherited disorders should be referred to a clinical genetics service.

[Level of evidence – D.]

7 Temporal artery specimens

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

7.1 Specimen submission

The specimen is ideally received in 10% formalin, which should be of adequate volume to ensure proper fixation. 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.

The specimen should be 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. If the patient is already on corticosteroid therapy then the dose and start date should be given. 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 should be labelled with matching patient details.

7.2 Specimen dissection

There is evidence that the size of the biopsy and extent of sampling are critical to diagnostic sensitivity.[19–21] Sensitivity is much higher in biopsies over 5–10 mm 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 3–5 mm 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 laboratory staff. All the tissue must be submitted for histology, as active/prior arteritis may be focal.

7.3 Sectioning and staining

The key point is that the disease is inherently patchy. A single H&E-stained section is only adequate for examination if it is positive. Enhanced sensitivity has been described when using serial sections of the entire tissue at 50 µm intervals,[19,21] although this must balance with local laboratory cost/work realities. As a minimum, the artery biopsy is examined through at least three histological 'levels'. If negative, additional levels (three or more) might be requested until one is satisfied the tissue has been adequately examined. Some consider that the block should be exhausted before definitively calling a biopsy negative, although many laboratories cope with a defined set of levels. If there is tangential sectioning of initial levels, then deeper levels may be necessary.

7.4 Further investigations and comments

Identification of a destructive infiltrate of macrophages and giant cells on H&E is diagnostic of GCA. Of note, the diagnosis may be made in the presence of a destructive infiltrate of macrophages without fully formed giant cells. Since the disease is focal, serial sections or multiple levels may be required to identify diagnostic features. Speedy communication is essential and this should be recorded if the case is being submitted for early diagnosis. However, many cases have had steroid therapy for up to one month prior to sampling, and urgency is not so much of an issue then. Even with early/acute cases, the patient will normally have been commenced on steroids before 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 and composition of infiltrate – mapped against the clinical context.[22,23]

Intimal thickening itself is non-diagnostic. Special histochemical stains and immunohistochemistry are not required. However, 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 and age-related changes)
- identify breaks in the internal elastic lamina, possibly indicating prior (healed) vasculitis.

Intimal elastosis is often seen in hypertension and progressive age and is not diagnostic of 'healed arteritis'. 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.

[Level of evidence – D.]

8 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.[24–27]

8.1 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 intraoperative 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.

8.2 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.

8.3 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.

8.4 Sectioning and staining

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

8.5 Further investigations

Although extremely rare, primary pulmonary artery (PA) 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.[24–27] 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 PA thrombosis. Possible infective cause for the inflammation should be excluded with the appropriate stains (Gram, Grocott, ZN or modified ZN) depending on the nature of the inflammatory process.[24]

8.6 Report content

Histological examination may reveal intimal thickening due to thromboembolic material of varying ages.[24] 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 PA 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 should be described.

The presence or absence of malignancy should be stated.

[Level of evidence – GPP.]

9 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 guidelines.[28–31]

9.1 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 clots 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 should be 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 (e.g. valves) or research should be indicated.

9.2 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. The following set of assessments will suffice for most hearts: check whether the total weight was recorded prior to their excision; weigh the fixed 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 (TVs) from the atria and record any abnormality; and examine the aortic and pulmonary valves (PVs) 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 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 down onto and along the length of the stented segment and remove the stent. If long-standing stents are present, cut across the vessel above and below the stent to examine the vessel patency. The stent can also be examined in some centres with resin embedding and diamond sectioning with the stent in situ.

Obtain a 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 (LV) and wall thickness and cavity diameter for the RV. After the rest of the examination is complete, isolated ventricular weights can be done using the Fulton technique (mandatory for assessing right ventricular hypertrophy) – but only in cases where no further macroscopic review is needed.

9.3 Sectioning and staining

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

A connective tissue stain (EVG and/or Masson's trichrome), as well as H&E, are done as standard on blocks of myocardium.

9.4 Further investigations and comments

Staining with Congo red (thick section, for amyloid), Perls' (Prussian blue stain for iron) and ABDPAS (for storage disorders), and immunohistochemistry for CD3, CD20 and CD68, etc. (myocarditis) are done as required.

Photographs of the whole heart or a transverse slice may be useful; digital images offer ease of storage, annotation and retrieval for future reference. Sectioning along the flow of blood or in echocardiography planes may be appropriate in particular cases, depending on the clinical scenario.

[Level of evidence – GPP.]

10 Autopsy cardiac dissection

10.1 Introduction

This document complements another RCPath document, *The Royal College of Pathologists: Guidelines on autopsy practice: Sudden death with likely cardiac pathology*.[32]

A European-wide draft covering autopsy cardiac examination exists and there are several books that deal with this type of specimen. Unless otherwise specified, a basis for these recommendations may be found in references 18, 30, 32, 33–36. The UK Cardiovascular Pathology Network (UKCPN) has now been established (www.ukcpn.org.uk), which may also allow contact with specialists who will be able to offer advice and points for specimen referral. Patient groups are supportive of this network. The network will also facilitate improvements in the understanding and diagnosis of sudden adult death syndrome (SADS); particularly by crystallising its molecular genetic definition(s). The network also aims to provide a platform for education across the UK.

All autopsy practitioners should be able to perform a sound review of the heart and its vasculature (not least to avoid criticism and/or litigation). Knowledge of normal cardiac architecture and basic embryology is therefore required. 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 and with photography, later case analysis is possible.

10.1.1 Case preparation

Consideration of consent or 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. If clinical history data is not provided, then it should be sought before dissection of the body. The possible requirement for samples for special investigation, including EM, microbiology and DNA extraction, should be considered before starting the dissection in order to optimise sampling.

10.1.2 Photography

Photography may be required and digital recording facilities should be available in any mortuary. Stepwise photographs of the dissection, through the chambers and a digital image of a mid-ventricular transverse section (with ruler) are very helpful as a record, and for referral. Although optimally carried out in all complex cases (particularly those with congenital heart disease or post-surgery realities), photography is not essential for all routine cases.

10.1.3 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. However, some cases may be better placed in the hands of specialist cardiac pathologists. This will help to maintain diagnosis standards and optimal care of relatives. The threshold for referral will differ according to the diagnosis, the complexity of the case and confidence 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.

10.2 Macroscopic assessment

The dissection of the heart is described in detail elsewhere with explanatory diagrams and photographs.[35] The heart is initially examined in situ, having exposed the pericardium and lung tissues by removal of the anterior chest wall.

Begin the macroscopic 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, the following should be examined:

- connections of the major arteries
- architecture of the heart and the pericardium prior to removal of the cardiac tissue
- arterial bypass grafts e.g. saphenous vein grafts (SVGs) and left internal mammary artery (LIMA). Electrical pacemaker connections are ideally identified early and preserved intact with the cardiac tissue (i.e. not disrupted) if there is suspicion of pacemaker-related pathology.

10.2.1 Great vessels

- Open the PA about 10 mm above the valve, with fingertip palpation of the inner 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), thereby preserving the sinoatrial 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.
- 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, triangular auricle and rectangular appendages. The normal RV will be palpably much thinner than the normal left. Both ventricles are more precisely and objectively measured as described below.

10.2.2 Coronary arteries

- Determine the course and pattern of the coronary arteries, looking for abnormal pathways and connections.
- Serially transversely slice the coronary arteries at no more than 3–5 mm intervals. See diagram in reference 35. It is now generally agreed that cutting coronary arteries longitudinally can destroy thrombi/emboli and make assessment of stenosis impossible.[34,35] A sharp scalpel blade is essential. Blunt blades are ineffective and dangerous. This procedure will be more difficult with heavily calcified coronaries.

  Solutions are:

  1) on occasion, scissors are required to transect heavily calcified arteries, leading to plaque formation/distortion
  2) 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 serial sectioning
  3) severely calcified coronaries can be dissected from the heart en bloc and then serially sectioned at 5 mm 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.
  4) some pathologists advocate assessing the coronary vessels after perfusion-fixation, where this is practicable.

- Coronary artery inspection

In practice, inspection and cutting are nearly simultaneous. A standard system will reduce errors:

1) start in the middle of the left anterior descending (LAD), sweeping downwards towards the apex, then upwards towards the left main stem orifice
2) identify the circumflex, and local branches (diagonal/obtuse marginals OM1/OM2)
3) identify the right coronary artery in the sulcus between the atrial appendage and RV
4) similar examination should run around the right side of the heart towards the anterior marginal 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 ideally 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 medico-legal autopsies.

- Coronary stents are increasingly common, but still a contentious area. Coronary metal stents cannot be dissected with scalpel or scissors, without crushing the metal device and distorting/damaging the tissues. 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/downstream artery analysis usually suffices, with the proviso that gentle injection of water along the stented vessel may 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. There is a method described for dissolution of the metal stent, although this is not commonly practiced. Likewise, radiology may be employed, but rarely is used.

10.2.3 Myocardium

Three transverse slices of ventricular tissues (approximately 10–15 mm thick) are taken starting at the apex up to the mid-transverse level, making sure that the atrioventricular valvular tissue is not damaged (see diagram in reference 35).

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 atrial septum and PIVD. This posterior approach allows direct inspection of the complete TV, atrial/auricular and ventricular tissues. Note: no further slices are required into the auricle. The cut is continued onto the front of the RV and upwards through the RVOT and 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 atrial septum and 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 LV and runs along the edge of the anterior free wall, parallel to the LAD, until just under the anterior leaflet of the 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 MV as later consideration/demonstration of pathology is more difficult. To assess hypertrophy objectively, it is often helpful to measure the thickness (mm) of the left ventricular free wall 10 mm below the AV.[34] As an adjunct, it may be helpful to weigh the LV and RV independently by cutting off the atria and cutting the RV free from the left (Fulton

weights).[34] This is a destructive procedure and should be used only where genuinely informative (i.e. chronic lung disease) and where the heart requires no further examination.

10.3 Histological sampling

Tissue blocks are taken, or not, from areas of myocardial tissue of relevance according to the consent and medico-legal requirements. Frank cardiac disease with overt pathology (e.g. myocardial infarction and tamponade) does not automatically need histology. However, histology may yet provide information beyond the macroscopy – such as the pattern and dating of infarction.

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 should sampled.

10.3.1 Cases with no identified macroscopic cardiac pathology

It is emphasised that deaths 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 also SADS (10.3.3).

10.3.2 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 RV and LV can be of assistance in assessing the degree of cardiac failure, although dissected Fulton weights (RV/LV and septum) are also useful when considered against ante-mortem data. Valvular heart disease is recorded and may need histology (e.g. atypical fibrosis/calcification or suspected 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 one to four blocks).

Cardiac involvement by systemic disease often requires tissue sampling. In general, one to two 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 one block, but consideration/exclusion of granulomatous myocarditis (e.g. cases of sarcoid) might need at least four to six 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 before histology.

10.3.3 Sudden death and no pathology seen (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 most times should include (at least):

- atria (often high; right next to the SAN)
- interventricular septum

- RVOT
- anterior LV
- lateral LV
- apical LV
- posterior LV.

Complex cardiac cases may also require further blocks including full transverse section (jumbo blocks) of the RV, septum and LV. Ideally, one should retain the heart for a period of time, in case further blocks are needed, but this will have to fit with the needs of relatives.

10.3.4 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 macrophotograph in reference 35).

The SAN is at the 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 sinoatrial artery.

The atrioventricular 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 TV
- posteriorly; the sinus septum containing the tendon of Todaro
- inferiorly; the orifice of the coronary sinus
- superiorly (apex of the triangle); the membranous septum (bounded by the superior limb of the coronary sinus, membranous septum and superior edge of the TV leaflet).
  - Remove as a tissue block including the membranous septum.
  - Transversely section across the specimen to capture the AVN, the His bundle and the bundle branches.
  - Take a transverse section of the septum immediately below this block to identify the radiating bundle branches.

10.4 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). However, knowledge of the details of the cardiac intervention, the indication for intervention and where any complications have occurred is vital if the maximum information is to be secured from the autopsy. Assessment of the surgical intervention with its complications/successes provides feedback for clinicians, relatives and the coroner.

10.4.1 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/or sepsis around the site of introduction may require microbiological sampling and/or histology.

10.4.2 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, in cases where pathology involving the device is suspected, 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:

- defibrillator pacemakers (implantable cardioverter defibrillator) must be switched off before autopsy manipulation or removal, to avoid the risk of discharge during the autopsy procedure. These devices are designed to generate high voltage shocks in response to fluctuating currents. The clinical notes will contain the pacemaker label with device type. The local pacemaker clinic can switch them off.
- the pacemaker box should ideally be returned to the local ECG/cardiac pacemaker department. This can confirm functionality by testing, removal of the patient from national registers and further investigations if device failure is under consideration.
- the pacemaker should never be left in the body in case of cremation, which will pose a risk of explosion.

10.4.3 Prosthetic valves

Valve replacement surgery broadly involves two types of replacement device: tissue (allograft or xenograft) or prosthetic (usually metal). Less common are the endovascularly inserted devices (e.g. transcatheter aortic valve implantation), seen with a pliable leaflet and wire cage structure. On rare occasions, patients with previous valvuloplasty (surgical or prosthesis enhanced) are encountered. While primary valve replacement failure can occur due to technical issues, the primary pathologies include local haemorrhage, infection, malalignment, paravalular leak and tissue overgrowth across the valve (pannus). These may be 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, and then turning 90^{o} to run the incision along/laterally around the atrial base immediately above the atrioventricular groove to approximately 50% of the atrial chamber is recommended for atrioventricular 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^{o} towards the lateral aspect of the ventricular chamber, allowing the ventricular chamber to be opened.

10.4.4 Other devices

Devices are increasingly common in cardiac tissues:

- septal closure devices, which broadly comprise wire closure or umbrella mesh platforms that are placed across the septal leak. These are inspected carefully as the chambers are opened, with thought as to the need for photography, histology or microbiology sampling. Removal of the tissues, intact with the device, should follow 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.

10.5 Other issues

10.5.1 Surgery for ischaemic heart disease

Surgical and medical intervention for ischaemic heart disease is still common, albeit declining. Historically, SVG surgery alone was the norm for coronary artery disease. In recent decades, SVG and LIMA have been used in addition.

- Knowledge of the number of internal mammary artery or vein grafts and the position to which they were applied is particularly of assistance before starting the autopsy dissection.

- Exclude or identify general technical problems, sepsis, haemorrhage and infection as the tissues are analysed.
- The 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 LIMA, akin to the native coronary vessels.
- Some advocate using scissors to dissect along vessels, providing that the lower part of the graft has been opened to confirm patency and/or identify any thrombus pushed along the vessel.
- Evaluate the degree of any stenosis. Based on native coronary measurements, stenosis is traditionally measured by a subjective estimate of lumen area/total 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 may be dissected whole, decalcified and assessed histologically – although tissue retention issues are pertinent.

10.5.2 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.

10.5.3 Histology stains

In general terms, routine paraffin-embedded tissues have H&E-stained 4 μm sections. Additional histochemistry should be available to examine connective tissue and intracellular components of myocardial parenchyma using PAS, DPAS, EVG ± alcian blue, Masson's trichrome, Sirius/Congo red at 10 μm, Perls' 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, although not strictly in accordance with Dallas criteria.

[Level of evidence – GPP.]

11 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.

11.1 Macroscopic description

Specimens tend to be small and irregular. They may or may not resemble an 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.

11.2 Processing and staining

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

11.3 Microscopy

Normal endarterectomy specimens show evidence of atherosclerotic plaque. It is currently normal to classify plaques by the Stary classification.[18] 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, haemorrhage 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 complication.[36]

[Level of evidence – GPP.]

12 Criteria for audit

In keeping with the recommended key performance indicators published by the RCPath (see *Key Performance Indicators – Proposals for implementation,* July 2013*,* www.rcpath.org/clinical-effectiveness/kpi):

- histopathology surgical cases should be reported, confirmed and authorised within seven and ten calendar days of the procedure
  - standard: 80% of cases must be reported within seven calendar days and 90% within ten calendar days
- reports should be made by pathologists validated in these areas by appropriate external quality assessments (EQA). Standard is EQA activity and CPD compliance.

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Appendix A Summary table – explanation of grades of evidence

(modified from Palmer K *et al. BMJ* 2008;337:1832)

Grade (level) of evidence	Nature of evidence
Grade A	At least one high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target cancer type or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Grade B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in A.
Grade C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high-quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in B.
Grade D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group.

Appendix B AGREE tissue pathways monitoring sheet

The tissue pathways of The Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in the table.

AGREE standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	1
2 The health question(s) covered by the guideline is (are) specifically described	1
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword, 1
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	1
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword, 1
12 There is an explicit link between the recommendations and the supporting evidence	2–11
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	2–11
16 The different options for management of the condition or health issue are clearly presented	2–11
17 Key recommendations are easily identifiable	2–11
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	2–11
20 The potential resource implications of applying the recommendations have been considered	2–11
21 The guideline presents monitoring and/or auditing criteria	12
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interest of guideline development group members have been recorded and addressed	Foreword