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

Tissue pathways for cardiovascular pathology

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	May to 5 June. Responses and authors' comments are available to view on publication. **Dr Brian Rous** **Clinical Lead for Guideline Review**

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NICE has accredited the process used by the Royal College of Pathologists to produce its tissue pathways. Accreditation is valid for 5 years from 25 July 2017. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Foreword

The tissue pathways published by the Royal College of Pathologists (RCPath) are guidelines which enable pathologists to deal with routine surgical specimens in a consistent manner and to a high standard. This ensures that accurate 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 following stakeholders were contacted to consult on this document:

- UK Cardiac Pathology Network
- National Amyloidosis Centre, Royal Free Hospital, London.

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

The information used to develop this tissue pathway was obtained by undertaking a systematic search of the literature using PubMed and Embase databases. Key terms searched included cardiac, heart, histology, pathology, tissue sampling, as well as terms relating to individual pathologies (e.g. infective endocarditis and giant cell arteritis) and procedures (e.g. transplant, endarterectomy). Dates searched were between December 2018 and December 2023. Given the broad and fundamental nature of the information covered in this guideline, the previous version was taken as the basis for the scope of practice to be covered. Published evidence was evaluated using modified SIGN guidance (see Appendix A). Published guidance from key professional organisations and societies (e.g. RCPath, Association for European Cardiovascular Pathology) was also reviewed and evaluated for relevance to this document. Consensus of evidence in the guideline was achieved by expert review. Gaps in the evidence were identified by College members via feedback received during consultation.

A formal revision cycle for all tissue pathways takes place on a 5-yearly basis. However, each year, the College will ask the author/s of the tissue pathways, in conjunction with the relevant subspecialty adviser 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 2 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 College website.

The pathway has been reviewed by the Professional Guidelines team, Working Group on Cancer Services and Lay Advisory Group and was placed on the College website for consultation with the membership from 8 May to 5 June. All comments received from the Working Group and membership were addressed by the author to the satisfaction of the Chair of the Working Group and the Clinical Lead for Guideline Review.

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 Professional Guidelines team and are available on request. The authors have declared 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* (3rd edition). London, UK: CRC Press, 2022[1]
- Suvarna SK. *Cardiac Pathology: A Guide to Current Practice* (2nd edition). London, UK: Springer, 2019[2]
- Suvarna SK (ed.). *Atlas of Adult Autopsy: A Guide to Modern Practice*. London, UK: Springer, 2016.[3]

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.

1.2 General specimen information

Tissue specimens should be accompanied by a request (card/electronic) with identifying patient details (as a minimum 3 patient identifiers, e.g. patient's full name, address, date of birth and NHS number), name of the responsible consultant, date of the procedure and relevant clinical summary. The specimen container should be labelled with matching patient details.

2 Endomyocardial biopsy for the assessment of pathology of the native heart

2.1 Specimen submission

Endomyocardial biopsy (EMB) contributes significantly to sensitivity and specificity in unexplained cardiomyopathy.[4,5] The chief diagnostic limitation with EMBs is sampling error.[5–7] It is recommended that at least 4 to 5 biopsies are taken, each 1–2 mm in size;[4] but, for focal processes, more biopsy specimens are recommended. One should 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 additional pieces 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 to collect 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 °C. Alternatively, fresh material can be placed in an RNAlater for subsequent molecular analysis.[5,7]

For EM, a biopsy will be immediately placed in glutaraldehyde at room temperature to minimise contraction band artefacts.[8] This can be arranged by giving the clinicians small vials containing fresh EM fixative into which a single biopsy fragment can be placed. If an arrangement is set up to collect frozen tissue, the designated person could also place 1

biopsy fragment into EM fixative. If the specimen arrives in the laboratory in formalin, then 1 biopsy fragment is taken out and put into EM fixative immediately.

Specimens should be received with information as outlined in section 1.2. The accompanying clinical summary should include reference to all medications, including non-prescription and/or illicit drugs. Prescribed medications of particular importance include angiotensin-converting enzyme inhibitors and checkpoint inhibitors. In addition, the summary should also include co-existent medical conditions, especially multisystem diseases, such as collagen vascular diseases, thalassaemia, porphyria, amyloidosis and sarcoidosis. The results of echocardiographic and other cardiac imaging findings are always valuable. In addition, it is helpful if previous histology that 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 1 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 1 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.[8]

2.3 Sectioning and staining

Ideally, 5 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 2–3 (and up to 5) serial sections mounted on each slide at each level.[4,5,7]

At least 3–5 different levels are stained with haematoxylin and eosin (H&E).

Suggested stains include:

- 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
- modified Gömöri 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 should be investigated in all young male patients.[9] The tissue may need to be referred to specialist cardiac centres for these investigations. 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.[5,7]

2.4.2 Electron microscopy

Small samples of myocardium may be submitted for EM.[5–7] If a diagnosis is not reached by LM, then EM is performed on the glutaraldehyde sample or, if necessary, reprocessed paraffin tissue, with the caveat that 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.[5–7]

2.4.3 Infectious agents

Special stains (e.g. Grocott, PAS or ZN) are used in cases of granulomatous inflammation. If foamy macrophages are present, PAS is also performed to ascertain 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 an increasing number of causative mutations are recognised.[5,7] For most molecular biology purposes, snap-frozen tissue is optimal. EMBs often contain limited material. The residual deep-frozen tissue left after cryostat sections should be retained for diagnostic use. Some molecular biology methods (e.g. next generation sequencing [NGS]) may be applicable to paraffin-embedded myocardium; the technical limitations must be understood and collaboration with the local clinical genetics service is recommended when planning sample pathways.

2.5 Report content

The report 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 regards 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’. [6] It is recommended that the nature and degree of infiltrate is clearly indicated,[5] as it is increasingly recognised that these criteria may need to be refined.

- 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 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.
- 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.[5]

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 – The recommendations in this section are based on non-analytic studies, such as case reports, case series or expert opinion (see references).]

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 International Society for Heart and Lung Transplantation (ISHLT) guidelines are used.[10–12]

3.1 Staffing and workload

2 or more pathologists in a unit should be competent in the reporting of cardiac transplant biopsies to provide cover for periods of leave. If an out-of-hours, on-call service is offered, it should be staffed by pathologists who contribute to the routine cardiac transplant pathology service. All pathologists reporting cardiac transplant biopsies should follow the revised ISHLT grading system.[10,11]

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 to obtain the required number of pieces, as this practice results in less representative sampling. Although the original ISHLT grading system required at least 4 pieces of myocardium, the trend has been to accept 3 evaluable samples as the absolute minimum for interpretation. Therefore, a minimum of 3, 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, 1 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.

Specimens should be received with information as outlined in section 1.2. The accompanying 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 CellSafe cassette or other method to avoid the loss of tiny specimens. All the biopsy fragments are embedded in 1 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 3 levels through the block, with at least 3 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 occasion, as suggested either by the clinical findings or by 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.[11]

Immunohistochemistry (immunoperoxidase or immunofluorescence) should be performed on a minimum of 2 biopsies during the first 2 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 includes 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 requires staining of more than 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 requires intravascular macrophages to be present in more than 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 must be assessed for its adequacy to exclude rejection; this should be documented in the report. To be adequate, a minimum of 3 pieces containing at least 50% myocardium free from previous biopsy site changes, scars or fat are 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 on 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 on.

3.6.5 Recurrence

Recurrence of the original cardiac disease may also be encountered in these biopsy specimens, for example, amyloidosis or myocarditis. 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 – The recommendations in this section are based on international expert society consensus developed on a body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies (see References).]

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.[7]

4.1 Specimen submission

Specimens should be received with information as outlined in section 1.2. 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 local protocol is required to ensure rapid transport to the laboratory, with refrigeration overnight if necessary. Samples with suspected infective endocarditis are best sampled in theatre prior to transfer of valve tissue for histology. This can alternatively be done in histopathology facilities if there is a laminar flow hood to supply sterile downdraft (Class II microbiological safety cabinet).

4.2 Specimen dissection

The key features of valve disease are typically macroscopic, not microscopic (although purely regurgitant valves may be anatomically normal if the pathology is due to disease in the surrounding tissues and if the regurgitation is mild). However, local pathology causing regurgitation should be itemised in terms of the valve(s) and their supporting tissues. Macroscopic photographs may be important for a permanent record and should be available at the time of writing of the microscopic report and final report sign-out.

The specimen is described carefully with particular note of calcification, fusion along the commissural lines, raphe formation and character, nodules, presence or absence of focal lesions, thrombus, vegetations and defects. The thickness and translucency are useful descriptors, as these are key features of myxoid change (e.g. floppy mitral valve [MV]). Chordae are examined for evidence of spontaneous rupture (pointed ends). When present, papillary muscle elements should be bisected longitudinally and examined for evidence of thrombi or ischaemia. Specimen dimensions (circumference and/or size of valve pieces) should be recorded. Where complete valves are submitted, comment should be made on cusp number if this is abnormal (e.g. in the case of bicuspid aortic valves [AV]). The possibility of dual pathologies should always 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 in Figure 1 (i.e. at least 1 perpendicular section to the line of closure, although more are possible). Note: calcified valves should be decalcified before section.

Figure 1: An example of appropriate blocking. Image courtesy of Dr Patrick Gallagher.

4.3 Sectioning and staining

Valves have 1 (minimum) representative sample from each leaflet, plus additional blocks of focal lesions. All sections are stained with H&E, although additional stains (EVG +/-AB) may be of use when considering aspects of connective tissue scarring and degeneration.

4.4 Further investigations and comments

- Microbiology sampling (a piece of the thrombus or vegetation); if available, fresh tissue.[13] In culture-negative endocarditis, submission of material may be appropriate for molecular assessment.
- Other stains include Alcian blue–diastase–PAS (ABDPAS) for connective tissue and fungi, Gram stain for bacteria (accompanied by a silver stain), EVG for connective tissues and Sirius or Congo red staining for amyloid.[7,14] 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).
- Immunostains for smooth muscle actin/muscle specific actin can be helpful to characterise fibromuscular plaques in carcinoid syndrome.

- Submission of tissue for ultrastructural studies is recommended for consideration of storage diseases.
- Evaluation of prosthetic heart valves (bioprosthetic and mechanical) is discussed elsewhere.[7]

[Level of evidence GPP – The recommendations in this section are based on the expert opinions of the authors.]

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.[15] Histological examination of all excised cardiac masses is mandatory.[1] The specimen is usually received in 10% formalin, which should be of adequate volume to ensure proper fixation. There should be a protocol for samples sent fresh out-of-hours, with refrigeration 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 received with information as outlined in section 1.2 along with a relevant clinical summary including erythrocyte sedimentation rate (ESR), if available. It is helpful if previous histology that is pertinent to the current specimen is reviewed.

5.2 Specimen dissection

The specimen is described in detail, including all 3 maximum dimensions, surface (e.g. glistening, gelatinous, papillary, fronded) and colour (e.g. yellow, tan, brown, grey). 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 to confirm excision and to exclude infiltrating malignancy. Liberal blocking of the lesion (possibly up to entirety if smaller than 30 mm) may be appropriate, but one might wish to reserve some tissue in case EM is to follow. For large tumours, judicious block selection is encouraged (e.g. 1 block/cm) and should include sections of macroscopically viable tumour (that will allow histologic characterisation) as well as areas of particular interest (e.g. cystic degeneration, haemorrhagic foci, necrosis).

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 may be carried out as necessary.

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

5.4 Further investigations and comments

Immunostaining may be helpful to confirm a benign entity and exclude malignancy, as well as to ascertain the primary origin of a metastatic neoplasm in conjunction with clinical and radiologic data. Relevant immunohistochemical panels (e.g. CD34, CD31, calretinin, smooth muscle alpha-actin (SMA), desmin, Ki67, cytokeratin, melan-A, S100, CD45) should be selected according to histopathologic appearances and clinical information.[16]

5.5 Report content

Cardiac masses encompass a broad differential diagnosis that includes non-neoplastic and neoplastic entities (primary [benign vs malignant] and metastatic). The histopathological report should include the macroscopic description and the name of the preferred entity and comment on the completeness of excision based on evaluation of the stalk/base/attached normal cardiac tissue. Metastases are the most common tumours in the heart, especially melanomas, lymphomas and carcinomas of lung, breast and oesophageal primary origin.[17] The most common primary tumour diagnoses will be the cardiac myxoma and papillary fibroelastoma.[17,18] Other diagnoses will be 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 cardiac pathologist and/or soft tissue tumour pathologist is advised. The case then enters the cancer tissue pathway and best practice in soft tissue tumour management should be followed.

[Level of evidence D – The recommendations in this section are based on non-analytic studies, such as case reports, case series or expert opinion (see References).]

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 (including carotid endarterectomy specimens).

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.

Specimens should be received with information as outlined in section 1.2. It is helpful if previous histology that is pertinent to the current specimen is identified and reviewed.

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 measurements taken of the overall dimensions and the thickness of the aortic wall.[19] Particular note should be made of the presence of inflammatory features (i.e. thickening of the wall or a 'tree bark' appearance), 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.[19] Useful pathological examination concentrates on possible predisposing factors.

6.3 Sectioning and staining

In general, 6 pieces of representative aorta (submitted in 2 cassettes) are sufficient for initial screening. If there is a clinical history of vasculitis or macro-/microscopic features suggestive of aortitis, then additional tissue should be examined (up to 12 blocks or at least 1 block per cm). Further detail related to sampling is provided in Stone *et al.*[19] Sections should be embedded 'on end' and all sections primarily stained with H&E, ABDPAS and EVG or Alcian blue–EVG (ABEVG). Alizarin red staining is optional; it 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

distinguishes predominantly acid mucopolysaccharides from neutral mucopolysaccharides. However, deposition of mucopolysaccharides without tissue destruction is usually insignificant. Thus, AB +/- 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 international consensus statements of the pathology of the aorta are applicable to other large vessels; all reports should follow these guidelines.[19,20]

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)[21,22] 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 should be described. 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 on 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 mucoid extracellular matrix accumulation (MEMA) or myxoid degeneration (previously called cystic medionecrosis) are seen as 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, Ehlers–Danlos, Loeys–Dietz and related connective disorders (e.g. osteogenesis imperfecta). A consensus grading system has been proposed and should be based on the worst area(s) sampled from multiple slides and aorta sections.[20]

6.4.4 Aortitis/vasculitis

Any description of vasculitis should conform to the international consensus document.[19] 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, scarring, adventitial versus intimal involvement, thrombosis and the presence/distribution of eosinophils, neutrophils, macrophages, plasma cells and T and B lymphocytes. Appropriate immunostains should be available.

6.5 Further investigations and comments

Knowledge of the pathology may guide 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 – The recommendations in this section are based on non-analytic studies such as case reports, case series or expert opinion (see References).]

7 Temporal artery specimens

This pathway applies to temporal artery biopsies (TAB) taken to establish the diagnosis of giant cell arteritis (GCA). It is acknowledged that there is a current lack of agreement regarding the diagnostic features and classification of inflammation observed in TAB sections.[23] There remains discussion and debate about which reporting parameters are important; recent studies have sought to determine consensus in this challenging area.[24,25] The below represents the recommended best practice until such time as validated reporting criteria are established.

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.

Specimens should be received with information as outlined in section 1.2. The clinical summary should include the degree of suspicion for GCA, relevant past medical history, ultrasound findings (if performed) and laboratory markers of inflammation (C-reactive protein (CRP) and 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.

7.2 Specimen dissection

There is evidence that the size of the biopsy and extent of sampling are critical to diagnostic sensitivity.[26–28] 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 millimetres. A series of transverse sections are made, producing segments 3–5 mm long, with up to 3 segments placed in each cassette. The segments should be 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 although this must balance with local laboratory cost/work realities.[26,28] As a minimum, the artery biopsy is examined through at least 3 histological ‘levels’, which should be requested ‘up-front’. If negative, additional levels (3 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 this practice did not reach consensus in the recent Delphi study.[24]

7.4 Further investigations and comments

Identification of a destructive infiltrate of macrophages and giant cells on H&E is diagnostic of GCA. Notably, the diagnosis may be made in the presence of a destructive infiltrate of macrophages without fully formed giant cells. Since the disease is focal and often variable, serial sections or multiple levels may be required to identify diagnostic features. Timely communication with clinicians is essential, especially if the biopsy has been submitted for urgent diagnosis prior to commencing steroids. However, a decision to discontinue

steroids may also be taken dependent on the biopsy findings, to reduce the potential for serious side effects. This, again, highlights the need for expediency.

There are 4 validated histological patterns of GCA; while knowledge about the correlation of these with clinical features is incomplete, it is recommended that reporting of the prevalent pattern is undertaken where possible.[29] As a minimum, the following microscopic features should be included: the location and extent of inflammatory cells and granulomatous infiltration in the artery wall; the presence/absence of intimal hyperplasia, fibrosis and luminal occlusion (< or >50%); and the status of the tunica media (intact/disrupted).[24] The reporting of neoangiogenesis is controversial, with conflicting literature as to its association with the ischaemic complications of GCA. However, if present, the inclusion of neoangiogenesis in TAB reports is recommended.

It should be noted that there is overlap in the histological features for GCA, age-related changes and atherosclerosis, meaning it can be difficult to distinguish between these entities. Intimal thickening is non-diagnostic and can be seen as an age-related phenomenon. Likewise, intimal elastosis is often seen in hypertension and progressive age and is not diagnostic of 'healed arteritis', which is itself a term that should be avoided due to lack of a clear definition.

Histochemical stains and immunohistochemistry are not usually needed but an EVG stain may help to identify a strong single internal elastic lamina with a thick parallel-oriented media (identifying the specimen as an artery), to determine intimal reduplication of the internal elastic lamina (indicating arteriosclerosis and age-related changes) and/or to identify breaks in the internal elastic lamina. ABDPAS is of limited use, although it may help to define recent intimal thickening. Immunohistochemistry for CD68 and CD3 is confirmatory only and is not routinely required.

[Level of evidence D – The recommendations in this section are based on non-analytic studies such as case reports, case series or expert opinion (see References).]

8 Pulmonary thromboendarterectomy 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. Rarely, such specimens can also contain pulmonary artery (PA) intimal sarcomas or tumour emboli derived from other primary sites. These recommendations are based on peer-reviewed evidence.[30–33]

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 local protocol is required to ensure rapid transport to the laboratory. Specimens should be received with information as outlined in section 1.2.

8.2 Specimen dissection

The specimen usually consists of irregular vascular casts and fragments of variably 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 to include cross sections of larger proximal vascular casts and smaller distal branches. If any focal, unusual appearances are noted, these areas are sampled more extensively.

8.3 Embedding

Multiple fragments may be embedded in 1 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

1 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 PA sarcomas or tumour emboli from other primary sites, including lung, breast, kidney, liver, pancreas and the gastrointestinal (GI) tract, may clinically present as pulmonary thromboembolism and may, therefore, be encountered in pulmonary thromboendarterectomy (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. A 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 media is often included in the specimen and the report should contain an approximate estimation of such amount. If there is a significant amount of medial or adventitial tissue, this should be pointed out in the report and the clinician should be 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 – The recommendations in this section are based on the expert opinions of the authors.]

9 Heart dissection – explants post cardiac transplantation

This pathway applies to explanted hearts received after cardiac transplantation and is in line with more widely accepted general guidelines.[1,2,34,35]

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 towels 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. Specimens should be received with information as outlined in section 1.2. A copy of the transplant referral letter is useful and any removal of tissues for graft harvest (e.g. valves) or research should be indicated.

9.2 Specimen dissection

The specimen usually consists of atrial cuffs, ventricles and outflow tracts. The explanted heart may have had pieces removed for research purposes and 1 or more valves may have been harvested for use as homografts. The following set of assessments will suffice for most hearts: (1) weigh the fixed specimen received; (2) describe the completeness of the chambers, attached great vessels and the epicardial surface; (3) detail any wires or catheters from implantable defibrillator, pacemaker or ventricular assist devices, etc.; (4) check the atrial appendages for thrombus; (5) examine the mitral and tricuspid valves (TVs) from the atria and record any abnormality; and (6) 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 2 planes) for the left ventricle (LV) and wall thickness and cavity dimensions for the RV. After the rest of the examination is complete, isolated ventricular weights may 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 5 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, right ventricular outflow tract (RVOT), both atria and any focal lesions. 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 an 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), ABDPAS (for storage disorders), and Phosphotungstic acid-haematoxylin (PTAH) stain (to highlight contraction bands) may be required, depending on the histopathological appearances on H&E staining and the clinical history. Immunohistochemistry for CD3, CD20 and CD68, etc. may be performed as required (to demonstrate inflammatory cell subsets in myocarditis).

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 – The recommendations in this section are based on the expert opinions of the authors.]

10 Autopsy cardiac dissection

10.1 Introduction

Alongside complementary College guidelines[36,37] and the books referenced above,[1–3] European-wide guidance[34] exists covering this type of specimen in detail. These form the basis for this section along with, unless otherwise stated, references.[21,38–41]

The UK Cardiac Pathology Network (UKCPN) has now been established with support from patient groups. The network aims to 'promote best practice in the pathological investigation of cardiovascular disease and sudden cardiac death of probable cardiac origin in the United Kingdom and other countries'. The network website contains the contact details for specialists who can offer advice and accept digital or tissue referrals.

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. Most 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 medico-legal instructions is essential before the autopsy and is 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 requirements for samples for special investigations, including EM, microbiology and DNA extraction, should be considered before starting the dissection to optimise sampling.

10.1.2 Photography

Photography may be required (particularly in complex cases including congenital heart disease and post-surgical deaths) and digital recording facilities should be available in any mortuary. Stepwise photographs of the dissection through the chambers and of a mid-ventricular transverse section (with ruler) are very helpful as a record of the case and for onward referral, but 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, 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 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; however, as with other referral practices, depending on the trusts concerned, there may be cost implications to be considered. Further discussion of the financial consequences is beyond the scope of this guideline.

10.2 Macroscopic assessment

The heart is initially examined in situ within the thoracic cavity, having exposed the pericardium and lung tissues by removal of the anterior chest wall. The removal of the heart is described in detail elsewhere.[42]

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

At this point, the following should be examined:

- connections of the major arteries
- external architecture of the heart and the pericardium (including measurement and assessment of any pericardial effusions or additional pathology present) prior to removal of the cardiac tissue
- presence or absence of arterial bypass grafts e.g. saphenous vein grafts (SVGs) and left internal mammary artery (LIMA) grafts
- electrical pacemaker connections, ideally identified early and preserved intact with the cardiac tissue (i.e. not disrupted) during evisceration if there is suspicion of pacemaker-related pathology (see section 10.4).[37]

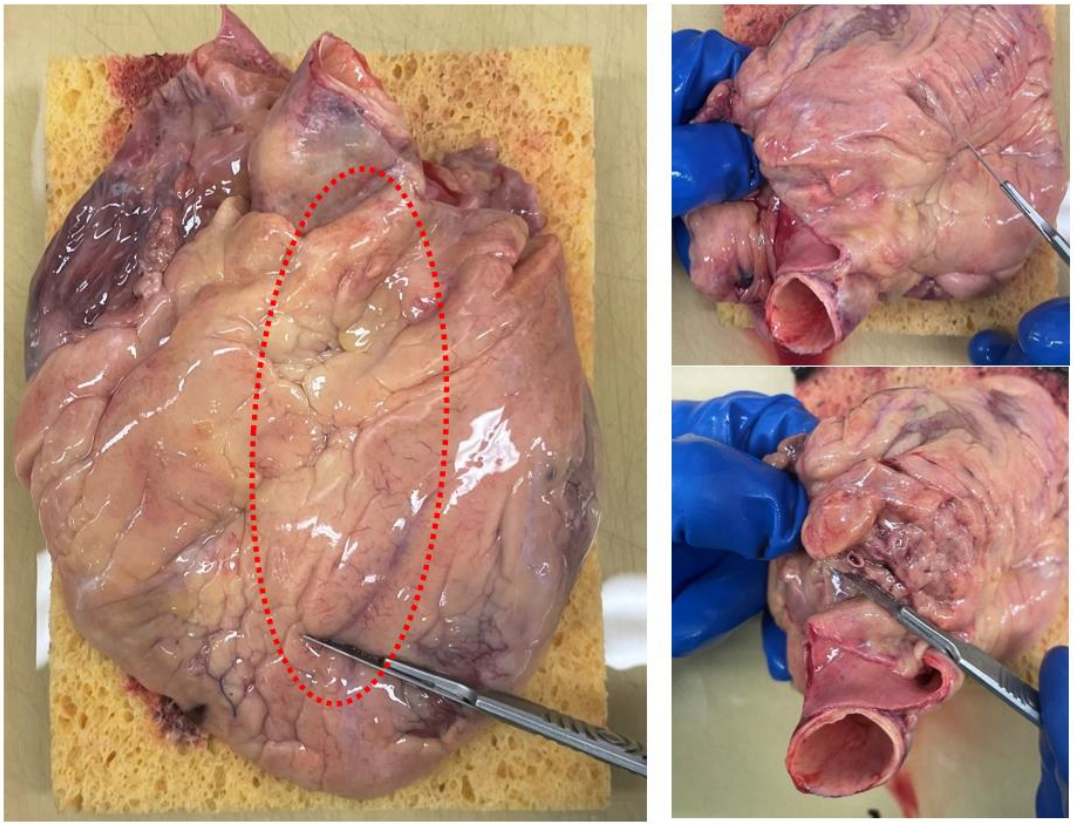
10.2.1 Great vessels

- Open the PA about 10 mm above the valve, with fingertip palpation of the inner proximal pulmonary arteries (to avoid missing proximal pulmonary emboli).
- Then, 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 4 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 triangular and rectangular auricular appendages, respectively. 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 Figure 2).
- It is now generally agreed that cutting coronary arteries longitudinally can destroy thrombi/emboli and make assessment of stenosis impossible.[38,39] A sharp scalpel blade is essential. Blunt blades are ineffective and dangerous. This procedure will be more difficult with heavily calcified coronaries.

Figure 2: Dissection and visual inspection of the left anterior descending (highlighted by the red circle); note that the heart may need to be rotated to aid dissection (images courtesy of Dr Whyte and Professor Suvarna).



There are various solutions for assessing heavily calcified arteries.

- Scissors may be required to transect heavily calcified arteries, although this may lead to plaque distortion, making true assessment of percentage stenosis more challenging.
- 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.

- 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.
- Where practical, some pathologists advocate assessing the coronary vessels after perfusion-fixation.

Coronary artery inspection

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 orifice.
- Identify the circumflex and local branches (diagonal/obtuse marginals OM1/OM2).
- Identify the right coronary artery in the sulcus between the atrial appendage and RV.
- Similar examination should run around the right side of the heart towards the anterior marginal and posterior interventricular descending artery (PIVD).
- Stable stenosis over 70% correlates reasonably well with ischaemic sudden cardiac death (SCD). Milder stenoses do not reliably predict ischaemic SCD taken in isolation but may produce local thrombosis and SCD.

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.

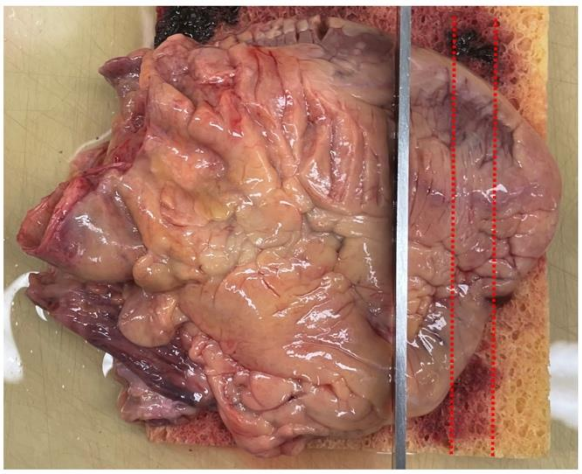
Coronary stents are increasingly common but are still a contentious area. Coronary metal stents cannot be dissected with scalpel or scissors without crushing the metal device and distorting/damaging the tissues. If the stent requires investigation of the lumen, then this is removed en bloc for resin 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 a 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 re-stenotic tissue.

There is a method described for dissolution of the metal stent, although this is not commonly practiced.[2] Likewise, radiology rarely may be employed. For further discussion please refer to the previously mentioned additional College guidance.[37] It should be noted that self-dissolving stents are being trialled in a few centres.

10.2.3 Myocardium

At least 3 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 Figure 3).

Figure 3: Transverse slicing of the ventricular tissue. Image courtesy of Dr Whyte and Professor Suvarna.

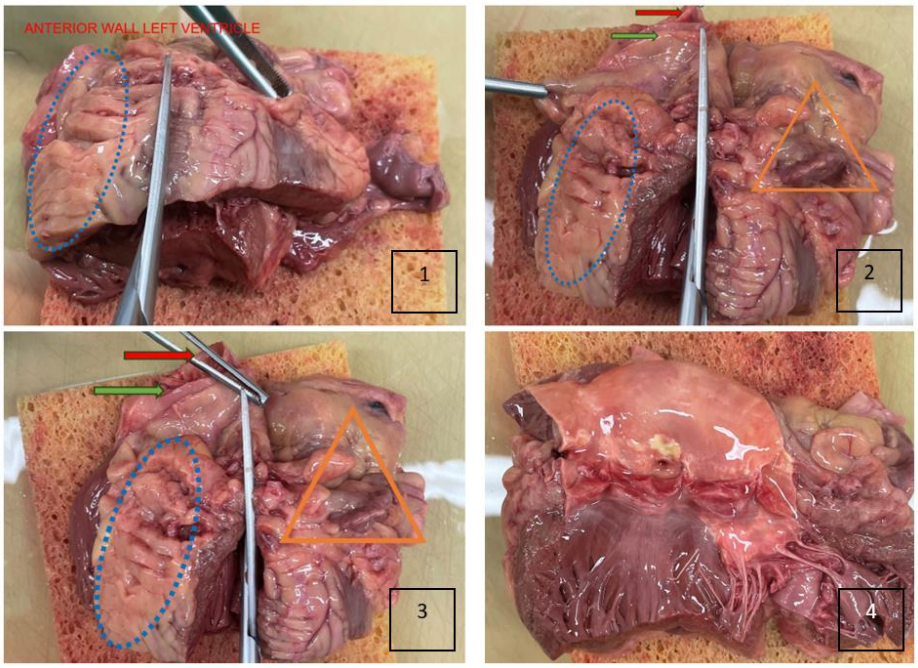


The cardiac chambers are opened along the posterior aspect of the right atrium and ventricle, approximately 10 mm to the right of the septum and PIVD. This posterior approach allows direct inspection of the complete TV and the right-sided atrial/auricular and ventricular tissues. The cut is continued onto the front of the RV and upwards through the RVOT and PV. The circumferences of the TV and PV and thickness of the RVOT are recorded, along with other comments on macroscopic appearance (e.g. fatty infiltration, vegetation).

The chambers of the left heart are similarly inspected by opening the atrioventricular walls posteriorly, 10 mm to the left of the atrial septum and PIVD. This allows direct inspection of all the left-sided chambers and auricle without further cuts. To assess the left ventricular outflow tract (LVOT) and AV, a cut is made on the anterior aspect of the LV running along the edge of the anterior free wall and in parallel to the LAD up to the leaflet of the MV. Between the auricle and the left main stem, this cut is then angled to the left, so it opens the LVOT and AV (Figure 4). This approach has the added benefit of keeping the MV

intact (a lateral approach to the LV may disrupt the valve making demonstration of pathology more challenging). The MV/AV circumferences and thickness of the LVOT are recorded, along with other findings as per the right-hand side.

Figure 4: 1. The cut is made on the anterior wall of the LV parallel to the LAD. 2. When at approximately the level of the appendage the cut is angled into the LOFT. 3. The direction of the cut into the LVOT is illustrated by the instruments. 4. The opened aortic valve – note the calcification in this example. Blue oval = dissected LAD (overlying septum), orange triangle = auricle, red arrow = aorta, green arrow = pulmonary vein. Image courtesy of Dr Whyte and Professor Suvarna.



To assess hypertrophy objectively, it is often helpful to measure the thickness (mm) of the ventricular free walls 10 mm below the AV.[38] 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 and septum (Fulton weights). This is a destructive procedure and should be used only where genuinely informative (i.e. chronic or fibrotic lung disease) and where the heart requires no further examination.

10.3 Histological sampling

If a decision is made to sample for histology, then blocks are taken from areas of myocardial tissue of relevance, in the context of the appropriate consent and medico-legal requirements. Frank macroscopic cardiac disease (e.g. myocardial infarction, tamponade) does not automatically need histology, but microscopic examination may be of benefit with providing additional information in addition to the macroscopic impression (for example, assessing the pattern and dating of infarctive lesions).

In a non-cardiac death case, it may be appropriate to simply take a representative sample of the LV (+/-) RV. If a cardiac cause of death is considered to be the primary lesion, then macroscopic pathology and relevant areas should be sampled as described below.

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). Please also see section 10.3.3 on sudden arrhythmic death syndrome (SADS).

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. A record should be made of the degree of vascular occlusion/stenosis (as a percentage) along with site, quality and size of areas of infarction and/or patchy fibrosis. Transverse chamber diameter of the ventricles and outflow tracts can be of assistance when assessing the degree of cardiac failure. The Fulton Index (dissected weight of the RV compared with the combined weight of the LV and septum)[3] is of use when assessing right ventricular hypertrophy in the setting of pulmonary hypertension (especially when considered against ante-mortem data). Valvular heart disease is recorded and may require histology/further investigation in some settings (e.g. atypical fibrosis or calcification, suspected infective endocarditis).[13]

Well-defined myocardial ischaemic damage does not automatically require histological sampling as part of the autopsy, 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 [+/- decalcification] and background/damaged myocardial tissue, equivalent to 1 to 4 blocks).

Cardiac involvement by systemic disease often requires tissue sampling. In general, 1 to 2 blocks suffice, although wider sampling may be governed by the nature of disorder and ante-mortem pathophysiology. Thus, metastatic disease to the heart could require just 1 block, but consideration/exclusion of granulomatous myocarditis (e.g. cases of sarcoid) might need at least 4 to 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 decalcification may be required before histology.

10.3.3 Sudden death and no pathology seen (sudden arrhythmic death syndrome)

SCDs without clear macroscopic pathology may require referral of the intact heart for specialist cardiac pathology review. If sampling is undertaken, then, as a general rule, blocks should include:

- atria (often high; right next to the SAN)
- interventricular septum
- RVOT
- mapped LV blocks from anterior, lateral, apical and posterior regions.

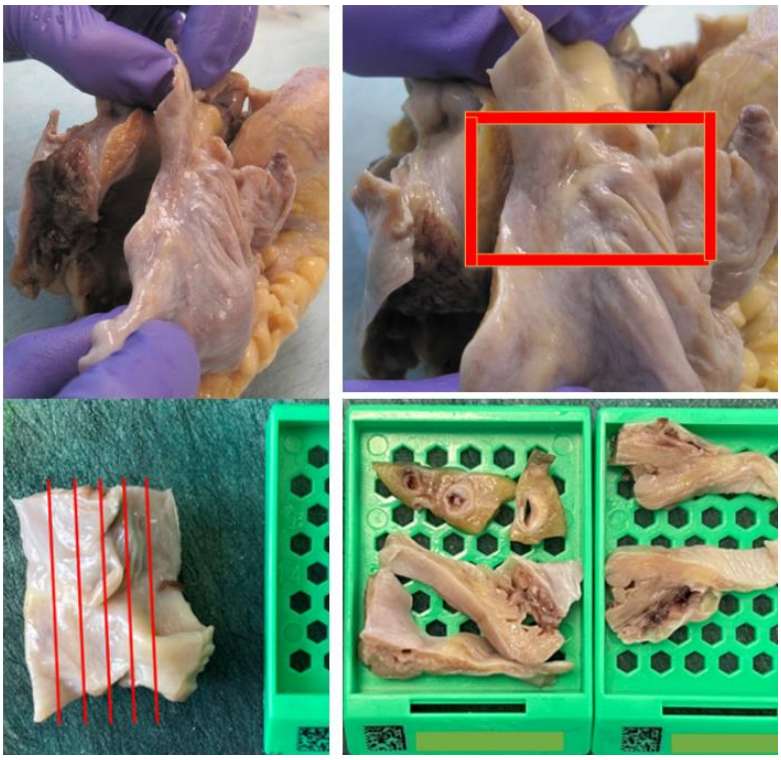
Complex cardiac cases may also require further blocks including full transverse section (jumbo blocks) of the RV, septum and LV. Ideally, the heart should be retained for a period of time, in case further blocks are needed, but this will have to fit with the needs of the relatives. Consideration of sampling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

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 below).

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 (Figure 5).

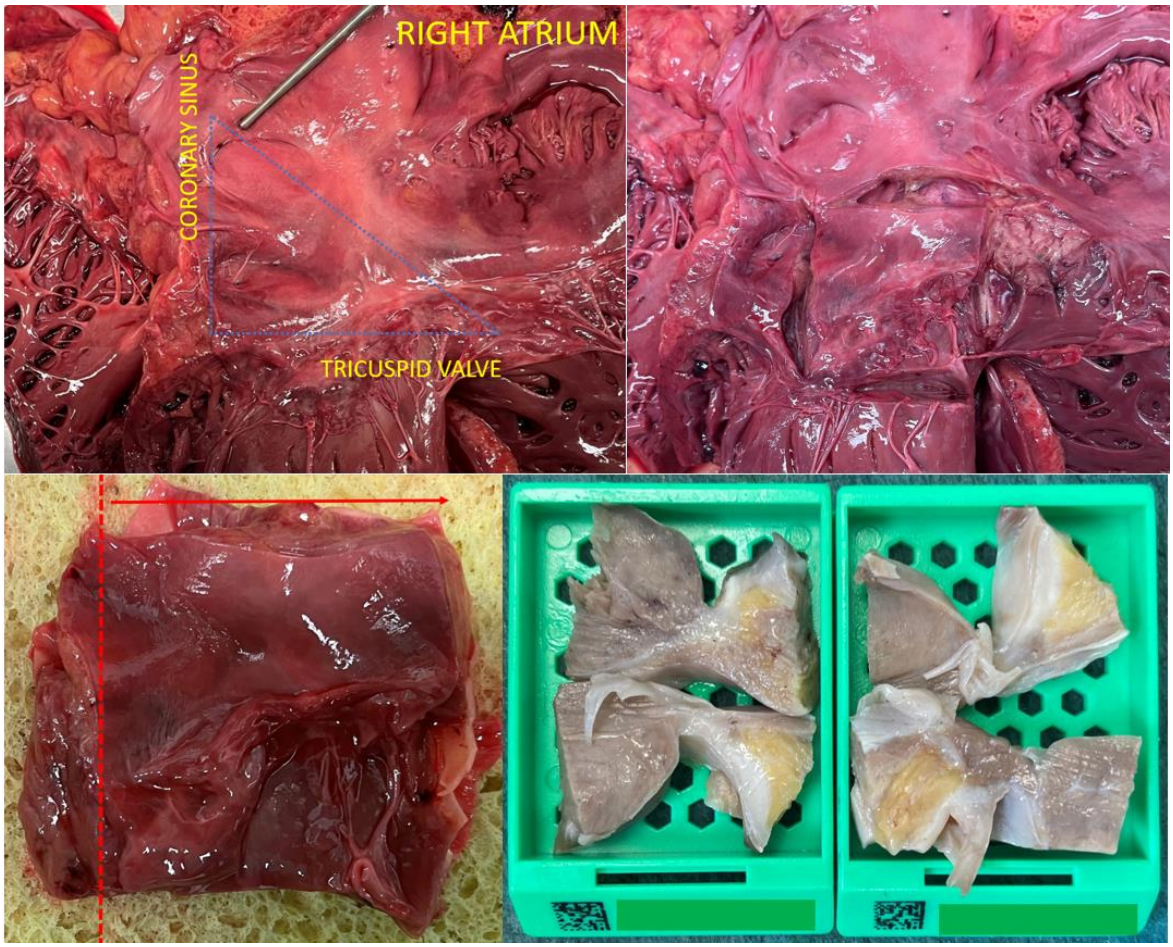
Figure 5: Top left: The region of the SAN between the opened SVC and the right auricle with the region of interest to be removed highlighted in red (top right). Bottom left: The tissue is sliced longitudinally as indicated by the red lines. Bottom right: Blocking of the slices to allow identification of the SAN. NB: This illustration is using fixed tissue, but the technique is the same on fresh. Image courtesy of Dr Whyte and Professor Suvarna.



The atrioventricular node (AVN) is at the apex of the triangle of Koch (Figure 6). 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).

Figure 6: Top left: The approximate region of the triangle of Koch containing the membranous septum and AVN. Top right: Scored area representing the tissue block to take. Bottom left: Once fixed, transverse slice through the block. Bottom right: Example of blocked tissue. Image courtesy of Dr Whyte and Professor Suvarna.



Remove this region 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. For further discussion of the devices listed below, please refer to the College guidelines: *Guidance for pathologists conducting post-mortem examinations on individuals with implanted medical devices*.[37]

10.4.1 Vascular access lines

These lines are inspected externally at the start of the autopsy and then cut flush with the skin. The internal portion should be left in situ so that their 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 1 piece with the thoracic organ block. It should be noted that some intracardiac pacemakers now exist with no generator unit or long lead components. Microbiology sampling, histology and/or photography may be appropriate.

Important considerations include:

- 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 2 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.

For atrioventricular valve samples, this can be achieved by opening an atrium along the posterior aspect alongside the septum and then turning 90° to run the incision along/laterally around the atrial base immediately above the atrioventricular groove to approximately 50% of the atrial chamber. 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.

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 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.

- 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 or primary complications (e.g. haemorrhage and infection) at the point of dissection.

- The primary focus is on the grafted vessels, their attachments/engraftments and consideration of the background vasculature.
- Transversely cut the vessels from the aortic root along the graft, or from the LIMA, at 3–5 mm intervals 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. As with native coronary arteries, 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 consideration of issues regarding tissue retention is required.

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 timescale 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. The recommendations in this section are based on the expert opinions of the authors.]

11 Coronary endarterectomy specimens

Most primary coronary interventions are based on angioplasty and stenting without tissue removal. However, coronary endarterectomies are sometimes performed to approach difficult targets during coronary artery bypass grafting[43] and atherectomy is increasingly used to modify heavily calcified lesions prior to stenting;[44] hence, these specimens will be received in some centres. Best practice is evolving. The evidence base for optimal handling is somewhat sparse but is summarised below.

11.1 Macroscopic description

Specimens should be received with information as outlined in section 1.2. These specimens tend to be small and irregular and may or may not resemble an actual artery. Make a general description, taking note of the overall shape, dimensions (in millimetres) 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 1 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.[22] 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 on, but its presence is common and does not indicate likely complication.[40]

[Level of evidence GPP – The recommendations in this section are based on the expert opinions of the authors.]

12 Criteria for audit

The following are recommended by the RCPath as key performance indicators:[45,46]

- histopathology cases that are reported, confirmed and authorised within 7 and 10 calendar days of the procedure.
- standard: 80% of cases must be reported within 7 calendar days and 90% within 10 calendar days.

In memoriam: Professor S Kim Suvarna (1960–2024)

The College and the authors of this guideline wish to express their deepest gratitude and pay tribute to the late Professor Suvarna, a distinguished member and contributor to this guideline. His memory lives on through the impact of his work. We are honoured to have had the privilege of collaborating with him.

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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 1 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 II guideline monitoring sheet

The cancer datasets of the Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this dataset 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	Introduction
2 The health question(s) covered by the guideline is (are) specifically described	Introduction
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword
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	Introduction
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 and Introduction
12 There is an explicit link between the recommendations and the supporting evidence	All sections
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	All sections
16 The different options for management of the condition or health issue are clearly presented	All sections

17 Key recommendations are easily identifiable	All sections
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	Appendix A
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	Section 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