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**POSITION PAPER**

# Heart Failure Association of the ESC, Heart Failure Society of America and Japanese Heart Failure Society Position statement on endomyocardial biopsy

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Endomyocardial biopsy (EMB) is an invasive procedure, globally most often used for the monitoring of heart transplant (HTx) rejection. In addition, EMB can have an important complementary role to the clinical assessment in establishing the diagnosis of diverse cardiac disorders, including myocarditis, cardiomyopathies, drug-related cardiotoxicity, amyloidosis, other infiltrative and storage disorders, and cardiac tumours. Improvements in EMB equipment and the development of new techniques for the analysis of EMB samples have significantly improved diagnostic precision of EMB. The present document is the result of the Trilateral Cooperation Project between the Heart Failure Association of the European Society of Cardiology, the Heart Failure Society of America, and the Japanese Heart Failure Society. It represents an expert consensus aiming to provide a comprehensive, up-to-date perspective on EMB, with a focus on the following main issues: (i) an overview of the practical approach to EMB, (ii) an update on indications for EMB, (iii) a revised plan for HTx rejection surveillance, (iv) the impact of multimodality imaging on EMB, and (v) the current clinical practice in the worldwide use of EMB.

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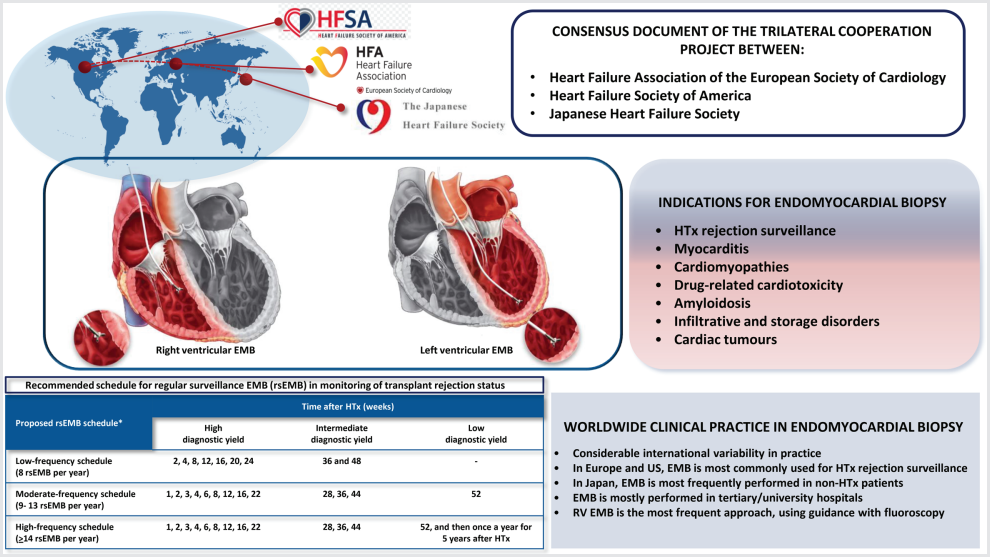
## Graphical Abstract




The contemporary perspective of endomyocardial biopsy.

**Keywords** Endomyocardial biopsy • Heart failure • Myocarditis • Cardiomyopathy • Amyloidosis • Sarcoidosis • Cardiotoxicity • Heart transplantation • Cardiac tumours

# Introduction

Endomyocardial biopsy (EMB) is an established invasive procedure most frequently used for the monitoring of heart transplant (HTx) rejection. EMB also has a complementary role to the clinical assessment in establishing the diagnosis of several cardiac disorders, including myocarditis, cardiomyopathies, drug-induced cardiotoxicity, amyloidosis, other infiltrative and storage disorders and cardiac tumours. Improvements in EMB equipment and a significant progress in the analysis of EMB samples have led to an improvement in diagnostic precision of EMB. This document is the result of the Trilateral Cooperation Project between the Heart Failure Association (HFA) of the European Society of Cardiology (ESC), the Heart Failure Society of America (HFSA), and the Japanese Heart Failure Society (JHFS). It was developed during the first Trilateral Cooperation Workshop held in Munich, in March 2019.

The role of EMB in the management of cardiovascular disorders has been previously discussed.[1,2] The present document, based on the Trilateral Cooperative Project between ESC-HFA/HFSA/JHFS, represents an expert consensus aiming to provide a comprehensive, up-to-date perspective on EMB, with a focus on the following main issues: (i) an overview of the practical approach to EMB, (ii) an update on the indications for EMB, (iii) a revised plan for HTx rejection surveillance, (iv) the impact of multimodality imaging on EMB, and (v) the current clinical practice in the worldwide use of EMB. All the relevant points are summarised in the *Graphical Abstract*.

# Historical milestones

Konno and Sakakibara first reported percutaneous EMB procedure (*Figure 1*), using a flexible bioptome with sharpened cusps that allowed EMB by pinching, as opposed to the surgical cutting technique used since 1950.[3,4] Subsequently, Sekiguchi described the use of EMB in diagnostic assessment of myocardial diseases such as glycogen storage disorders, sarcoidosis and myocarditis.[5] He proposed a systematic histopathological classification, including analysis of cardiomyocyte hypertrophy, degeneration, disarrangement and/or fragmentation of muscle bundles, as well as the extent of interstitial fibrosis, and endocardial thickening.[5,6]

Caves and Schultz modified the Konno-Sakakibara forceps to allow percutaneous biopsies through the right internal jugular vein under local anaesthesia with rapid tissue extraction.[7] The reusable Stanford Caves-Schultz bioptome and its subsequent modifications became the standard device for EMB for approximately two decades, predominantly used for monitoring of HTx rejection.[8,9] Since then, the use of EMB had extended to diverse cardiac diseases, including myocarditis, cardiomyopathies, drug-induced cardiotoxicity, amyloidosis, other infiltrative and storage disorders and cardiac tumours.

Simultaneously, the long-sheath technique was developed, which improved feasibility and safety of the procedure. In 1974, a flexible King's College bioptome was introduced by Richardson.[9] This bioptome, and its subsequent modifications, could be inserted

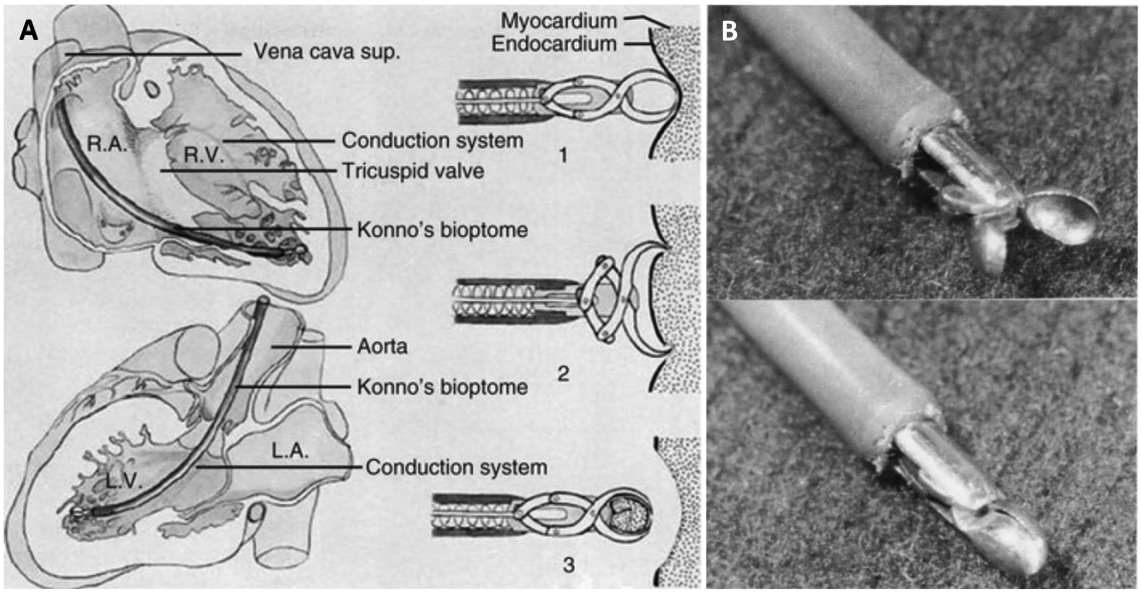



**Figure 1** (*A*) Original illustration by Konno and Sakakibara of the percutaneous technique of endomyocardial biopsy. (*B*) Opening and closing of the cutting claw at the tip of the catheter (3). LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Reproduced with permission from Konno and Sakakibara,[3] copyright Elsevier.

through the long sheath using either jugular or subclavian veins, femoral veins, and right and left femoral arteries. The first study on radial approach using sheetless guiding catheters for left ventricular (LV) EMB was reported by Bagur and co-workers.[10] The safety of EMB was established both for the right and left ventricle.[11] With the improvement of the technique and tissue processing, EMB has gradually gained worldwide acceptance. Besides the significant progress in the technique, various imaging modalities were introduced for EMB guidance, and several new techniques were developed for tissue processing and viral genome detection (*Figure 2*).

# Practical approach to endomyocardial biopsy

## Selection of the access site

Endomyocardial biopsy is usually performed in a cardiac catheterisation laboratory, under fluoroscopic guidance, using jugular, femoral, or brachial veins, or femoral or radial arteries for vascular access.[12] Patient monitoring (heart rhythm, non-invasive blood pressure and blood oxygen saturation monitoring) is mandatory during the procedure. To minimise the risk of bleeding, an international normalised ratio should be $\leq 1.5-1.8$ and platelet count $\geq 50 \times 10^9$/L.[13]

The internal jugular vein is the most common access site for right ventricular (RV) EMB in HTx patients, whereas the right femoral vein is most frequently used in non-HTx patients. Other access sites include brachial venous access for RV EMB,[12] and right femoral and radial arteries for LV EMB. Radial access is associated with fewer vascular complications, earlier ambulation and lower costs; however, radial thrombosis may occur if the inner vessel diameter is small ($\leq$2.5 mm) and peak systolic velocity is low.

Endomyocardial biopsy is most commonly performed as a single procedure in HTx patients, while in non-HTx patients it can be combined with right heart catheterisation, coronary angiography, and/or electrophysiological study for the purpose of electroanatomic voltage mapping-guided procedure.[14]

## The number of endomyocardial biopsy procedures per operator for the maintenance of procedural skill

The number of EMBs per operator required to maintain the procedural skill may vary between institutions and is not accurately defined. Training and yearly volumes for operators should be consistent with the recommendations of the appropriate medical societies. The opinion of the Trilateral Cooperative Project experts is that a range between 20 and 50 procedures per operator per year may be reasonable. The report of the American College of Cardiology Competency Management Committee recommends 50 EMBs per operator per year.[15] In addition to the procedural skill, it is essential that an experienced cardiac pathologist is available for the timely analysis and communication of EMB findings.

Details of EMB technique are described in the online supplementary *Appendix 1* and a video tutorial on EMB procedure as it is performed in expert centres in Europe, the US and Japan is available online (*online supplementary video links*).

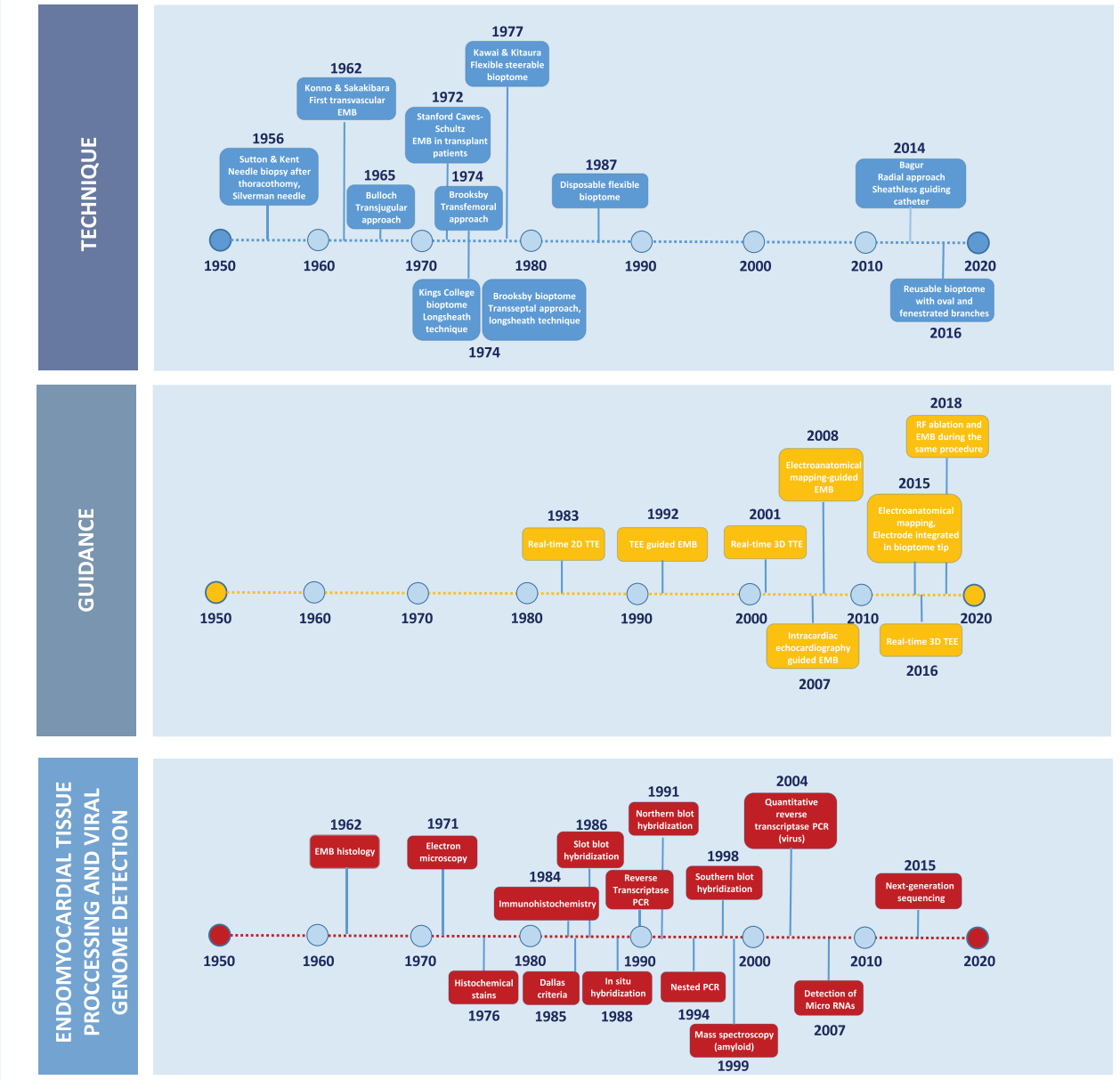



**Figure 2** Historical cornerstones in the development of endomyocardial biopsy (EMB): procedural technique (*upper panel*), imaging guidance (*middle panel*), myocardial tissue processing (*lower panel*). 3D, three-dimensional; PCR, polymerase chain reaction; RF, radiofrequency; TEE, transoesophageal echocardiography; TTE, transthoracic echocardiography.

## Selection of endomyocardial biopsy site, sampling error and biopsy of non-cardiac tissues

The most common site of EMB is RV EMB (*Figure 3*), but occasionally LV (*Figure 4*) or biventricular EMB may be needed. The decision on EMB site should be based on the clinical indication, findings of preprocedural imaging, and on the operator expertise.[16] A study of 755 patients with suspected myocarditis and non-ischaemic cardiomyopathy (including infiltrative and storage disorders) indicated that biventricular EMB can increase diagnostic accuracy compared with selective LV or RV EMB.[11] Sampling error is the major limitation of the diagnostic utility of EMB. It is suggested that at least five samples should be taken from different sites in the right and left ventricle in order to reduce the risk of sampling error in the setting of diseases with focal pattern or intracardiac tumours.[1,17]

In patients with infiltrative and storage disorders affecting multiple organs, biopsies taken from the most affected organ are most likely to provide the diagnosis, but occasionally their utility may be hampered by low sensitivity. In patients with

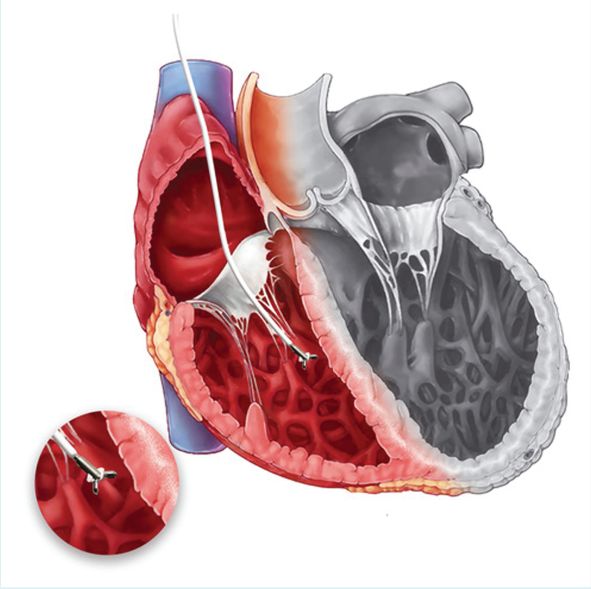


**Figure 3** An artistic presentation of right ventricular endomyocardial biopsy. Endomyocardial biopsy samples are typically taken from the interventricular septum.

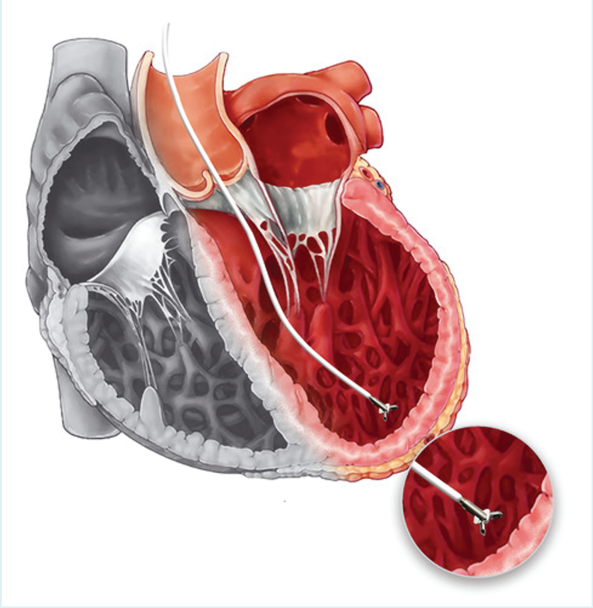


**Figure 4** An artistic presentation of left ventricular endomyocardial biopsy. Endomyocardial biopsy samples are typically taken from the ventricular apex.

amyloidosis, abdominal fat pad biopsies have a sensitivity of 75% for immunoglobulin light-chain (AL) amyloidosis, but the sensitivity is significantly lower in both hereditary and wild-type transthyretin amyloidosis (ATTR) (~45% and ~15%, respectively) and thus, a negative result does not rule out cardiac involvement.[18]

## Imaging guidance

In most centres, EMB is performed using fluoroscopic guidance; however, novel guidance modalities have emerged aiming to improve the feasibility and enable targeted EMB. The role of imaging in EMB guidance is twofold. Firstly, preprocedural imaging with echocardiography, cardiac magnetic resonance (CMR) imaging, computed tomography and/or positron emission tomography (PET) can be used to direct EMB to the specific sites of myocardial disease. Secondly, procedural imaging (e.g. real-time three-dimensional echocardiography) can be performed simultaneously with fluoroscopy to improve the accuracy of the EMB procedure.[19] Intracardiac echocardiography has also been successfully employed to guide EMB of cardiac tumours.[20]

Preprocedural diagnostics with CMR has been demonstrated to improve diagnostic performance of EMB in several cardiac disorders. CMR-directed EMB can improve procedural accuracy in diseases with focal pattern (e.g. sarcoidosis),[21] and in the setting of soft tissue masses, which may be difficult to visualize by fluoroscopy.[22] Likewise, a small study suggested that directing EMB to the regions of late gadolinium enhancement on CMR can increase diagnostic utility in myocarditis.[23] However, a larger study failed to confirm this finding, perhaps because late gadolinium enhancement is a non-specific sign, which may correspond to both acute necrosis/inflammation, as well as fibrosis in myocarditis.[11] Since T2 mapping has a greater sensitivity for detecting inflammation, this technique may be further explored for directing EMB to the most affected regions of the heart in myocarditis and other inflammatory disorders.[24] However, small cohort studies of patients with cardiomyopathies indicate that the concordance between CMR and EMB findings is only partial and that these procedures have a complementary role in diagnostic assessment.[25,26]

Electroanatomic voltage mapping has been used for the guidance of EMB in diseases with focal pattern associated with ventricular arrhythmias (myocarditis, sarcoidosis and arrhythmogenic right ventricular cardiomyopathy, ARVC).[27,28] Areas of low-voltage or abnormal electrogram on electroanatomic voltage mapping have a high sensitivity and specificity to identify the pathological substrate.[27] EMB procedure may be further facilitated by using bioptomes with an integrated electrode at the tip, as well as with the use of three-dimensional electroanatomic voltage mapping systems and intracardiac echocardiography.[29,30]

## Complications

Endomyocardial biopsy is associated with a low rate of major complications (~1%),[11,16] which can be classified as major and minor (*Table 1*). Patient characteristics, EMB site, procedural volume and operator expertise are the most important determinants of EMB risk (details in online supplementary *Table S1*). The risk of major complications is lower in HTx recipients compared with non-HTx patients (0.19% vs. 0.70%).[31] Haemodynamically unstable patients with acute or advanced heart failure (HF) and those with dilated ventricles may be at a higher risk of cardiac perforation, tamponade and malignant arrhythmias.[32] Cardiac perforation and tamponade are more frequently observed with RV than with LV EMB[16] but

LV EMB is more frequently complicated by stroke or systemic embolism. High-volume centres have a lower complication rate compared with low-volume centres, and high procedural volume has been identified as an independent predictor of a lower risk of major complications.[33]

There is a risk of tricuspid valve damage during EMB, both at the valvular and sub-valvular level.[34] The risk of complication can be minimised by using a correctly located long sheath across the tricuspid valve with the tip in the right ventricle, to avoid repeated exposure of the valve leaflets to the bioptome. Infection/sepsis is a very rare risk of EMB if the procedure follows recommendations for the aseptic technique.

The risk of periprocedural mortality is low (0–0.07%),[16,35] and most frequently caused by stroke, malignant arrhythmias, high-degree atrioventricular block, and cardiac tamponade.[36] The risk of stroke and systemic embolism can be decreased by identification of a thrombus (an absolute contraindication for EMB) and administration of low-dose heparin during the procedure in patients with high thromboembolic risk.[1]

Management of cardiac perforation during EMB includes immediate pericardiocentesis and autotransfusion from the pericardium to a large central vein (femoral or jugular) until the bleeding has stopped.[37] If cardiac perforation has occurred, these patients require close monitoring and consultation with a cardiac surgical service. Urgent surgical repair of the perforation site may be required in patients with ongoing bleeding or instability related to the perforation.

## Evaluation of endomyocardial biopsy samples

The choice of the technique for the analysis of EMB specimens depends on the clinical presentation and suspected underlying cardiac disorder. First, the pathologist performing the analysis should be well trained in specimen processing and proficient in analysis techniques. Standardized diagnostic criteria for histopathological analyses (e.g. Dallas criteria for myocarditis) should be used to minimise EMB reporting variability. Second, the use of vital stains is indicated to demonstrate myocyte hypertrophy, patterns of myocyte disarray or vacuolization. Infiltrative disorders such as amyloidosis can be characterized by Congo red stain, immunohistochemistry, immunogold electron microscopy and mass spectroscopy. Immunostaining can be used to quantify resident and infiltrating macrophages, myofibroblasts, and lymphocytes. Quantitative polymerase chain reaction (PCR), reverse transcription (RT)-PCR and direct sequencing should be used to identify infectious agents.[38] Simultaneously, blood samples should be assessed with PCR to identify systemic infection, and to exclude potential contamination of heart tissue by persistently/latently infected blood cells.[17] Electron microscopy is useful to detect and quantify changes in cardiomyopathies and storage disease.

The most frequent indication for a repeat EMB procedure is the follow-up of graft rejection status after HTx. Rarely, a repeat EMB may be considered if sampling error is suspected in a patient with unexplained deterioration of HF and/or malignant rhythm disorders, when EMB findings may provide information pertinent to further management.[17]

Details on EMB sample processing and analyses are presented in *Table 2* and considered in the online supplementary *Appendix S2*. In addition, typical histopathological findings of the normal myocardium, lymphocytic myocarditis, HTx rejection and cardiac amyloidosis are presented in *Figure 5*.

# Indications for endomyocardial biopsy

Endomyocardial biopsy can provide important histological, immunohistochemical, and molecular information about the heart. Since EMB is an invasive procedure with limited availability, risk and benefits of the procedure should be taken into account. In establishing an indication for EMB, it is important to identify clinical situations in which EMB can complement the diagnostic process in order to confirm clinically suspected diagnosis and provide information relevant for the management. Diagnostic value of EMB also depends on the myocardial disease (i.e. lower sensitivity in diseases with focal involvement), and on the centre's proficiency in sample processing and analysis. The most frequent indications for EMB are summarised in *Table 3*.

## Clinically suspected myocarditis

Endomyocardial biopsy is indicated in patients with fulminant/acute myocarditis presenting with cardiogenic shock or acute HF and LV dysfunction, with or without malignant ventricular arrhythmias and/or conduction abnormalities. It may also be considered in haemodynamically stable patients with clinical symptoms and diagnostic criteria (electrocardiographic abnormalities, elevated troponin levels, imaging findings) suggestive of myocarditis, in the absence of significant coronary artery disease.[17]

A retrospective registry-based analysis of 220 patients (mean age 42 years) from the US, Europe and Japan with acute myocarditis and LV dysfunction has shown that patients with fulminant myocarditis have significantly worse short-term (60-day mortality/HTx rate: 27.8% vs. 1.8%) and long-term prognosis (7-year mortality/HTx rate: 43.0% vs. 9.0%) compared with non-fulminant course and that EMB-proven diagnosis of giant cell myocarditis carries the worst prognosis.[39] A recent analysis of 443 individuals with suspected myocarditis has shown that among high-risk patients with LV dysfunction, sustained ventricular arrhythmias and/or haemodynamic instability ($n$ = 118, EMB performed in 56 patients) EMB-established diagnosis (89.3%) offered information relevant for the management and prognosis (e.g. institution of immunosuppressive therapy in giant cell myocarditis, sarcoidosis or eosinophilic myocarditis).[40] In addition, EMB can provide differential diagnosis in patients with severe clinical course, when non-invasive assessment is inconclusive or unfeasible.[40] Accordingly, in unexplained acute HF with haemodynamic compromise, a cohort study of 851 patients demonstrated that EMB provided a diagnosis in 39%, and that the most common finding was acute myocarditis.[41] In

**Table 1 Major and minor complications of endomyocardial biopsy**

| Major complications | Minor complications |
|---|---|
| Death (0–0.07%) | Chest pain (transient) (0–1.8%) |
| Cardiac perforation/haemopericardium/tamponade (0–6.9%) | Deep vein thrombosis (0.23–3.8%) |
| Pneumothorax/air embolism (0–0.8%) | Puncture site haematoma/nerve palsy (0–0.64%) |
| Thromboembolism (0–0.32%) | Hypotension/vaso-vagal syncope (0–4.3%) |
| Valvular trauma (0.02–1.1%) | Arterial trauma/vascular damage/fistulae (0.32–2.8%) |
| Severe arrhythmias/atrioventricular block (0–11%) | |

Detailed description of complications according to the centre volume, access site, type of endomyocardial biopsy procedure and patient characteristics as well as references are provided in online supplementary *Table S1*.

this study, EMB-based diagnosis resulted in a change of therapy in almost a third of patients, and most clinical decisions concerned the institution or withholding of immunosuppressive medications.[41]

The common histological types of myocarditis include lymphocytic, eosinophilic, giant cell and granulomatous myocarditis (cardiac sarcoidosis). The most prevalent is lymphocytic myocarditis caused by viral infection, autoimmunity or drug-toxicity, which is frequently associated with HF of various severity. Eosinophilic myocarditis is characterised by eosinophilic infiltrate in the heart and is often accompanied by peripheral blood eosinophilia. Giant cell myocarditis is rare (~1% of acute myocarditis cases) but it may take the fulminant course and carries a poor prognosis.[39] EMB has a high sensitivity (80%) and positive predictive value (71%) for giant cell myocarditis, especially if performed within 2–4 weeks of symptom onset.[42] Non-caseating granulomatous myocarditis is the usual histopathological finding in patients with cardiac sarcoidosis.[43] EMB may be indicated in suspected cardiac sarcoidosis (electrocardiographic abnormalities, unexplained syncope, or palpitations), if imaging studies (echocardiography, CMR, $^{18}$fluorodeoxyglucose PET) and lymph node or lung biopsy render inconclusive results, as well as in cases of isolated cardiac involvement.[44] The major drawback is a low sensitivity of EMB due to the focal nature of myocardial involvement, revealing non-caseating granulomatous infiltrates in ~25% of patients.[44] Small case series have suggested that sensitivity can be improved with an electrogram-guided approach targeting areas with low amplitude and/or abnormal electrogram appearance,[45] or with preprocedural CMR-guided EMB.[21]

Endomyocardial biopsy is rarely indicated in individuals with suspected COVID-19 myocarditis. EMB and autopsy findings support the presence of SARS-CoV-2 in the myocardium,[46,47] and histopathological studies suggest that increased interstitial macrophage infiltration and lymphocytic myocarditis are the most common findings.[46,48]

The diagnostic value of EMB in clinically suspected myocarditis increases if the procedure is performed 2–4 weeks after symptom onset[1,17] and the sample is analysed with the use of immunohistochemistry. A recent meta-analysis (61 publications with a total of 10 491 patients) indicated that the use of immunohistochemistry can increase the detection rate of inflammation in EMB specimens to ~51%.[49]

## Dilated cardiomyopathy

In patients with dilated cardiomyopathy (DCM), EMB may be indicated in the setting of decompensated HF with moderate-to-severe LV dysfunction, refractory to standard HF treatment, with a recent onset of the clinical syndrome, exclusion of other specific aetiologies, absence of severe LV remodelling and negative familial history and/or genetic testing for cardiomyopathy. In this setting, EMB can be used to confirm inflammatory cardiomyopathy with a higher sensitivity compared with CMR.[49] EMB may also have a role in the assessment of Borrelia burgdorferi involvement in unexplained DCM in endemic regions for Lyme disease.[50] A study of 110 individuals with recent-onset DCM has demonstrated that Borrelia burgdorferi genome was present in 20% of EMB samples.[51]

## Cardiotoxicity of cancer therapy

Immune checkpoint inhibitors (ICI) represent a novel, highly effective class of anti-neoplastic drugs but their use can result in cardiac toxicity in up to 5% of cases, including myocarditis, non-inflammatory LV dysfunction, myocardial infarction and arrhythmias.[52] ICI-mediated myocarditis and pericarditis occur early (>75% cases in first four cycles), more frequently in patients on combined ICIs and can be severe or fatal in up to 50%.[53,54] EMB is indicated in suspected ICI-mediated cardiotoxicity, if CMR or $^{18}$F-fluorodeoxyglucose PET-computed tomography yield uncertain findings and/or the patients cannot undergo non-invasive assessment due to haemodynamically instability.[55] In patients with confirmed ICI-mediated myocarditis, ICI treatment should be discontinued and high-dose immunosuppression should be instituted, in addition to standard HF care.[52] If active inflammation has been ruled out by EMB, then ICI treatment re-challenge may be considered once LV function has stabilized or recovered with standard HF drugs.[52]

Endomyocardial biopsy has been used to document and assess the degree of anthracycline-related cardiotoxicity.[56] However, EMB is not routinely recommended in patients with anthracycline-related cardiotoxicity and HF when there is a clear causal relationship. EMB may be considered in rare cases when there is clinical uncertainty as to the cause of HF (e.g. suspected myocarditis). The role of EMB in cyclophosphamide-induced

**Table 2 Sample processing, analysis and characteristic findings according to clinical presentation**

| Disease | EMB processing/staining | Possible findings |
|---|---|---|
| Myocarditis, DCM | **Histopathology** Haematoxylin and eosin, Mason or Mallory trichrome, Elastic van Gieson, PAS, Heidenhein's AZAN, and Methylene blue stain (Trypanosoma cruzii) | **Dallas criteria for myocarditis:** inflammatory infiltrates associated with myocyte degeneration and necrosis of non-ischaemic origin (active or borderline).<br>**Lymphocytic myocarditis:** patchy or diffuse inflammatory infiltrate mostly of lymphocytes and macrophages [viral infections, immune-mediated myocarditis (systemic lupus erythematosus, polymyositis/dermatomyositis, rheumatoid arthritis, organ-specific autoimmune disorders, etc.)].<br>**Giant cell myocarditis:** myocyte necrosis and diffuse or multifocal inflammatory infiltrates, with T lymphocytes, macrophage-derived multinucleated giant cells and eosinophilic granulocytes.<br>**Granulomatous myocarditis:** non-necrotizing granulomas with macrophages and multinucleated giant cells, surrounded by fibrosis and a lymphocytic infiltrate (sarcoidosis).<br>**Eosinophilic myocarditis:** interstitial inflammatory infiltrate dominated by eosinophils, often without myocyte damage, frequently accompanied by peripheral eosinophilia (hypersensitivity, parasitic infection, Churg–Strauss syndrome, endomyocardial fibrosis). |
| | **Quantitative real-time PCR** for enteroviruses, adenoviruses, herpesviruses (cytomegalovirus, herpes simplex, Epstein–Barr, human herpesvirus 6), parvovirus B19, influenza A and B, and SARS-CoV-2 virus + Borrelia | **Infection confirmed or not by (RT-) PCR** |
| | **Immunohistochemistry**<br>CD3 (T cells), CD68 (macrophages), MHC II, alpha SM-myofibroblasts | **Myocarditis confirmed by immunohistochemistry:** ≥14 leucocytes/mm$^2$ including up to 4 monocytes/mm$^2$ with the presence of CD3+ T-lymphocytes ≥7 cells/mm$^2$ |
| DCM, ARVC | Histology and PCR as above, additional immunohistochemical stains for lamin A/C, dystrophin, and plakoglobin (ARVC) | **DCM:** non-specific histopathology including hypertrophy and vacuolar changes of myocytes, interstitial fibrosis, foci of micro-scarring.<br>**ARVC:** progressive myocyte atrophy/loss with fibrous or fibro-fatty myocardial replacement. |
| Storage diseases | PAS, Congo Red, sulfate alcian blue, or S/T thioflavin, Sudan black or Oil Red O (lipid deposits), Prussian Blue (iron), TEM (Anderson–Fabry, Danon) | PAS+ sarcoplasmic vacuoles and lysosomal glycogen accumulation (Pompe disease); PAS+ and LAMP2 absence, autophagic granules in TEM (Danon disease), PAS+ and lamellar bodies (Anderson–Fabry), Congo Red+ and interstitial deposits (amyloidosis); brownish perinuclear granules in myocytes highlighted in blue by Prussian Blue stain (iron storage disease). |
| Tumours | Standard histopathology + immunohistochemistry for specific tumours | Differential diagnosis between benign and malignant tumours, and in malignant tumour subtyping. |
| Heart transplantation | Haematoxylin and eosin, Giemsa, Movat, Masson trichrome, Weigert-Van Gieson, Ziehl Nielsen, PAS, Gram, Gomori, CD31, CD34, CD45, CD68, C4d | **Cellular rejection:** Grade 0R (no rejection); Grade 1R (mild) interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage; Grade 2R (moderate), ≥2 foci of infiltrate with associated myocyte damage; Grade 3R (severe) diffuse infiltrate with multifocal myocyte damage, oedema, haemorrhage, or vasculitis.<br>**Humoral rejection:** capillary injury, endothelial cell swelling and aggregation of intravascular macrophages (positive staining for C4d or C3d fragments of complement by endothelial cells). |

ARVC, arrhythmogenic right ventricular cardiomyopathy; CD, cluster of differentiation; DCM, dilated cardiomyopathy, EMB, endomyocardial biopsy; LAMP2, lysosome-associated membrane protein 2; MHC II, major histocompatibility complex type II; PAS, periodic acid Schiff; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; TEM, transmission electron microscopy.

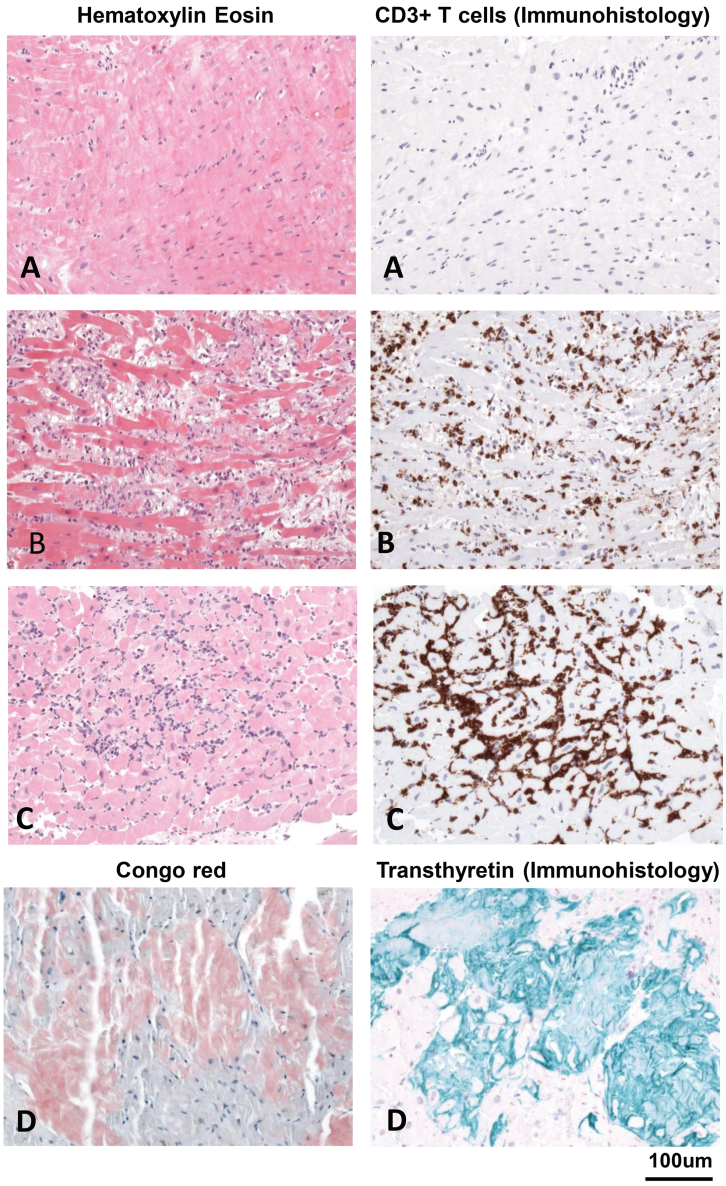



**Figure 5** Typical histopathological findings of the normal myocardium (*A*), lymphocytic myocarditis (*B*), cellular heart transplant rejection (*C*) and cardiac amyloidosis (*D*). (*A*) Normal myocardium: no myocyte necrosis, inflammation or fibrosis. (*B*) Acute lymphocytic myocarditis: many necrotic myocytes (light pink) and numerous CD3+ T cells and other immune cells (e.g. CD68+ macrophages). (*C*) Acute cellular heart transplant rejection: significant amounts of inflammatory cells including CD3+ T cells. (*D*) Cardiac amyloidosis: Congo red staining and subtyping by immunohistochemistry defines cardiac amyloidosis (presented in the figure: transthyretin amyloidosis).

cardiotoxicity, and other cancer therapy-induced HF is less well-established[57] and EMB is not indicated.

## Unexplained ventricular arrhythmias, conduction disorders and syncope

Endomyocardial biopsy may be indicated in patients with unexplained ventricular arrhythmias/syncope (ventricular fibrillation or tachycardia, frequent multifocal ventricular premature complexes/non-sustained ventricular tachycardia), refractory to treatment, without obvious cardiac disease or with minimal structural changes in order to identify potentially treatable aetiologies, such as myocarditis, ARVC, or sarcoidosis.[44,58,59] Ventricular arrhythmias may be the only symptom of myocarditis and sarcoidosis,[44,60] as well as the first presentation of ARVC in patients with subtle structural abnormalities, that may challenge diagnostic evaluation. Given the focal nature of cardiac sarcoidosis and ARVC, undirected EMB can be false negative and electroanatomic voltage mapping guidance may be considered to increase diagnostic yield.[45,61]

**Table 3 Indications for endomyocardial biopsy**

| Clinical presentation | Endomyocardial biopsy finding |
|---|---|
| • Suspected fulminant myocarditis or acute myocarditis with acute HF, LV dysfunction and/or rhythm disorders.<br>• Suspected myocarditis in haemodynamically stable patients. | Myocarditis type:<br>• Lymphocytic myocarditis<br>• Eosinophilic myocarditis<br>• Giant cell myocarditis<br>• Granulomatous myocarditis |
| Dilated cardiomyopathy with recent onset HF, moderate-to-severe LV dysfunction, refractory to standard treatment (following exclusion of specific aetiologies). | Myocyte abnormalities, focal or diffuse fibrosis and inflammatory infiltrates (inflammatory cardiomyopathy). |
| Suspected ICI-mediated cardiotoxicity: acute HF with/without haemodynamic instability early after drug initiation (~ first 4 cycles) | ICI-mediated myocarditis |
| High-degree atrioventricular block, syncope and/or unexplained ventricular arrhythmias (ventricular fibrillation, ventricular tachycardia, frequent multifocal premature ventricular complexes), refractory to treatment, without obvious cardiac disease or with minimal structural abnormalities. | • Myocarditis<br>• Arrhythmogenic right ventricular cardiomyopathy<br>• Cardiac sarcoidosis |
| Autoimmune disorders with progressive HF unresponsive to treatment with/without sustained ventricular arrhythmias and/or conduction abnormalities. | • Autoimmune myocarditis<br>• Viral myocarditis<br>• Vasculitis/vasculopathy |
| MINOCA/takotsubo syndrome with progressive LV dysfunction and HF with/without ventricular arrhythmias or conduction abnormalities. | Differential diagnosis of myocarditis |
| Unexplained restrictive or hypertrophic cardiomyopathy. | • Amyloidosis<br>• Infiltrative/storage disorders (Anderson–Fabry disease, glycogen storage diseases, sarcoidosis, haemochromatosis) |
| Cardiac tumours. | Histopathological diagnosis |
| • Routine surveillance EMB<br>• Symptom-triggered EMB | HTx rejection status |

EMB, endomyocardial biopsy; HF, heart failure; HTx, heart transplant; ICI, immune checkpoint inhibitor; LV, left ventricular; MINOCA, myocardial infarction without obstructive coronary artery disease.

Endomyocardial biopsy may be useful in patients with new-onset bradycardia and conduction abnormalities, when clinical presentation is suggestive of a treatable aetiology (e.g. myocarditis, amyloidosis, sarcoidosis).[62,63] Electroanatomic voltage mapping guidance may be useful, as suggested by a cohort of patients with unexplained atrioventricular block, where a comprehensive evaluation, including electroanatomic voltage mapping guided-EMB, demonstrated cardiac sarcoidosis in 34%.[64]

## Autoimmune disorders

Endomyocardial biopsy is rarely indicated in autoimmune disorders (systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, polymyositis/dermatomyositis, etc.), but it may be considered in patients with progressive HF unresponsive to usual treatment, as well as in patients with sustained ventricular arrhythmias and/or conduction abnormalities, when there is a high clinical suspicion of myocarditis or vasculitis. In a small study of patients with systemic sclerosis and HF, greater extent of EMB-detected inflammation and fibrosis correlated with serious adverse events.[65] Likewise, EMB in patients with systemic lupus erythematosus can provide confirmation of lupus myocarditis, hydroxychloroquine-induced cardiotoxicity and/or coronary vasculitis/vasculopathy.[66,67]

## Myocardial infarction without obstructive coronary artery disease and takotsubo syndrome

Endomyocardial biopsy is rarely indicated in myocardial infarction without obstructive coronary artery disease (MINOCA) and in takotsubo syndrome. In may be considered for the purpose of differential diagnosis of myocarditis in the setting of progressive LV dysfunction and HF despite standard therapy, with or without life-threatening ventricular arrhythmias/conduction abnormalities.[68]

## Restrictive and hypertrophic cardiomyopathy

Endomyocardial biopsy may be considered in patients with restrictive and hypertrophic cardiomyopathy if the aetiology of cardiomyopathy remains inconclusive following non-invasive assessment, and there is clinical suspicion of infiltrative or storage disorder (amyloidosis, sarcoidosis, Anderson–Fabry disease, and glycogen storage diseases) with available treatment options.[69–71] In patients with cardiac amyloidosis, differentiating between AL amyloidosis and wild-type or hereditary ATTR amyloidosis has

important therapeutic implications.[72] EMB is highly sensitive and specific for cardiac amyloidosis,[73] and may be considered if non-invasive assessment provides inconclusive or discordant results (e.g. abnormal serum free light-chain assay and a positive $^{99m}$Tc 3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy), or in patients with plasma cell dyscrasia and ambiguous imaging results.[72,74] Congo red staining and immunohistochemistry are the standard techniques used to characterize the type of amyloid fibrils in EMB specimens, but newer technologies, such as immunoelectron microscopy and laser dissection mass spectrometry appear superior to immunohistochemistry in identifying amyloid protein type.[75,76] In individuals with LV hypertrophy and suspected Anderson–Fabry disease, who do not meet all diagnostic criteria, EMB can be performed to confirm the diagnosis.[71] Rarely, EMB may be indicated in the presence of iron overload and unequivocal imaging results to confirm cardiac haemochromatosis.[77]

### Tumours of the heart

In patients with cardiac tumours, multimodality imaging plays the pivotal role in the identification and characterisation of cardiac masses. EMB may be indicated in patients with primary or metastatic cardiac tumours when non-invasive assessment and/or biopsy of non-cardiac tissues have been inconclusive, and histological diagnosis is relevant for the prognosis and treatment.[1] EMB is not indicated for intracardiac masses with high embolic potential, such as left-sided tumours or typical cardiac myxomas. EMB guidance with transthoracic, transoesophageal and intracardiac echocardiography can improve the efficacy and safety of the procedure.[20,78]

## Monitoring of heart transplant rejection status

Despite advances in cardiac imaging and availability of novel biomarkers, EMB remains the 'gold-standard' for the detection of HTx rejection. EMB after HTx can be scheduled according to a protocol for routine surveillance EMB (rsEMB) in asymptomatic patients, and it is also performed in patients with worsening clinical status, as a symptom triggered EMB (stEMB).

At present, there is a lack of consensus on the optimal timing and frequency of rsEMB. In the era of potent immunosuppressive regimens, a decline in diagnostic utility was observed with surveillance protocols that utilise frequent rsEMB procedures. A diagnostic yield of 1.39% for detecting clinically silent acute rejection was described with a protocol of 14 rsEMB procedures per patient in the first year after HTx.[79] Another study reported a diagnostic yield of ~3% in the first 6 months after HTx and of 0% in the next 6 months, with a protocol involving an average of $8.7 \pm 3.7$ rsEMB procedures in months 0–6, and $2.0 \pm 2.1$ rsEMB procedures in months 6–12.[80] Recently, a low-frequency protocol for rsEMB was tested in 282 HTx patients and demonstrated morbidity and mortality comparable with the high-frequency protocol data in the International Society for Heart and Lung Transplantation Registry.[81] In this study, rsEMB was performed monthly for the first 6 months (with the first rsEMB being scheduled 1 month after HTx), and subsequently at months 9 and 12. Despite this relatively low frequency of rsEMB procedures, only six unscheduled stEMB procedures were required, resulting in a change of treatment in only two patients.

### Revised schedule for heart transplant rejection surveillance

Currently, most HTx protocols suggest performing rsEMB every week during the first month, every second week for the next several months, and then once monthly for the first 12 months. Thereafter, rsEMB are often continued at variable frequency for years, despite a low risk of late rejection and with a low cost-effectiveness.[82] Recently, non-invasive surveillance of HTx rejection with the combined use of novel techniques, such as gene expression profiling and donor-derived cell-free DNA has shown high negative predictive validity for acute graft rejection, which may decrease the need for rsEMB.[83] In the future, multicentre prospective clinical trials should be planned to test the optimal approach to rsEMB after HTx. Based on the available data on diagnostic yield of EMB according to the time after HTx, the following schedule for rsEMB is suggested (*Figure 6*).

## Contraindications

In most instances, contraindications for EMB are consistent with contraindications for cardiac catheterisation (*Table 4*). Additional caution is required in patients with recent pacemaker implantation (increased risk of lead dislodgement for RV EMB), marked ventricular wall thinning and hypercontractility (high risk of ventricular perforation).[84]

## Multimodality imaging and endomyocardial biopsy

Multimodality imaging including standard two-dimensional, three-dimensional, speckle-tracking and intracardiac echocardiography, CMR, computed tomography and nuclear imaging techniques (e.g. $^{18}$F-fluorodeoxyglucose PET), represent key non-invasive diagnostic tools in the evaluation of patients with suspected myocarditis, cardiomyopathies, cardiotoxicity, infiltrative or storage disorders and cardiac tumours. These imaging techniques allow identification of cardiac structural and functional alterations, tissue characterisation, exclusion of significant coronary artery disease or pericardial involvement, and the assessment of myocardial perfusion and metabolism (*Table 5*).[85–91] In most instances, modern imaging techniques in combination with laboratory analyses, biomarkers, genetic testing and/or biopsy of non-cardiac tissues can provide the diagnosis without a requirement for EMB, thus narrowing the scope of clinical situations in which EMB may be necessary.

Nevertheless, EMB cannot be fully substituted by cardiac imaging. CMR and nuclear imaging are often limited by access issues and

| Proposed rsEMB schedule* | Time after HTx (weeks) | | |
|---|---|---|---|
| | High diagnostic yield | Intermediate diagnostic yield | Low diagnostic yield |
| Low-frequency schedule (8 rsEMB per year) | 2, 4, 8, 12, 16, 20, 24 | 36 and 48 | - |
| Moderate-frequency schedule (9-13 rsEMB per year) | 1, 2, 3, 4, 6, 8, 12, 16, 22 | 28, 36, 44 | 52 |
| High-frequency schedule (≥14 rsEMB per year) | 1, 2, 3, 4, 6, 8, 12, 16, 22 | 28, 36, 44 | 52, and then once a year for 5 years after HTx |

**Figure 6** Recommended schedule for the routine surveillance endomyocardial biopsies (rsEMB) in the monitoring of heart transplant (HTx) rejection status. High pre-test diagnostic probability is highlighted in green, intermediate in yellow and low in blue. *If rsEMB reveals more than grade 1 rejection or if there is ongoing clinical concern for the patient, a follow-up EMB should be considered.

**Table 4 Contraindications for endomyocardial biopsy**

Absolute contraindications

- Intracardiac thrombus
- Ventricular aneurysm
- Severe tricuspid, pulmonary or aortic stenosis
- Aortic and tricuspid mechanical prosthesis

Relative contraindications

- Active bleeding
- Infection and fever
- Infective endocarditis
- Pregnancy
- Recent cerebrovascular accident/TIA (<1 month)
- Uncontrolled hypertension
- Thin ventricular wall (for the biopsy of the myocardium)
- Coagulopathy
- Contrast media hypersensitivity[a]
- Uncooperative patient

TIA, transient ischaemic attack.
[a]Contrast media are rarely used for endomyocardial biopsy and is an infrequent contraindication for the procedure.

well-recognised contraindications to CMR and cannot be applied in haemodynamically unstable/claustrophobic patients. Also, EMB may be the only viable diagnostic option in patients with malignant ventricular arrhythmias, frequent ventricular ectopic beats and fast atrial fibrillation with irregular R-R intervals, as well as in those with rapid/relentless disease progression, in whom establishing histological diagnosis can significantly impact further treatment (e.g. fulminant myocarditis).

## The role of endomyocardial biopsy in prognosis and risk assessment

Available data indicate that EMB may have a role in evaluation of prognosis and risk stratification of patients with several cardiac disorders. EMB-confirmed lymphocytic myocarditis is associated with a more favourable outcome in comparison with giant cell myocarditis, which confers a poor prognosis.[39] Viral persistence in the myocardium in patients with LV dysfunction is associated with a deterioration in LV function, while spontaneous viral elimination usually leads to a significant recovery.[92]

Endomyocardial biopsy-detected morphological changes in the myocardium may also inform on the prognosis in DCM. Focal derangement and diffuse myofilament lysis in EMB samples are predictors of readmissions for worsening HF in patients with DCM, while diffuse myofilament lysis is as an independent predictor of mortality.[93] Furthermore, findings of ultrastructural changes, fibrosis, apoptosis, hypertrophy, vascular density, inflammation, and viral persistence may indicate adverse prognosis in DCM.[93,94] An analysis of EMB samples from 182 patients demonstrated an association between increased immune cell activity in the myocardium and poor long-term prognosis.[95]

Endomyocardial biopsy remains the gold standard for the surveillance of graft rejection in HTx recipients, with implications for the treatment and long-term prognosis.[96–99]

## Therapeutic implications of endomyocardial biopsy

Endomyocardial biopsy can provide information valuable for the treatment of several cardiac disorders. Data from the few randomised trials in patients with myocarditis support institution of immunosuppressive therapy in the setting of EMB-proven, virus-negative myocarditis with circulating cardiac autoantibodies[100] and in giant cell myocarditis.[101] Based on small observational cohorts, clinical experience and expert opinion, immunosuppressive therapy can be instituted in virus-negative eosinophilic myocarditis, ICI-mediated myocarditis, cardiac sarcoidosis and myocarditis associated with autoimmune diseases.[17,52,102–104] In patients with myocarditis of unknown aetiology, a clinical trial failed to demonstrate a beneficial effect of immunosuppression on LV function and survival.[105]

**Table 5 Sensitivity and specificity of magnetic resonance and nuclear imaging techniques in myocarditis, amyloidosis and sarcoidosis**

| Disease | Method | Finding | Sensitivity | Specificity |
|---|---|---|---|---|
| Myocarditis[85–87] | | | | |
| Early phase (<14 days from symptom onset) | CMR | **T1 weighted imaging**: early gadolinium enhancement is suggestive of hyperaemia and capillary leak. LGE is suggestive of cell necrosis and fibrosis. **T2 weighted imaging**: presence of myocardial oedema (typically subepicardial) | 67% | 91% |
| Late phase (>14 days after symptom onset) | CMR | **T2 weighted imaging:** imaging modality with the greatest diagnostic accuracy | 71% | 72% |
| Amyloidosis[88,89] | | | | |
| | CMR | **Increased T1 weighted imaging**, ECV Diffuse global subendocardial LGE | 85% | 92% |
| | Nuclear imaging ($^{99m}$Tc pyrophosphate, or $^{99m}$Tc-hydroxymethylene-diphosphonate full body scan) | Typical finding: positive uptake in ATTR cardiac amyloidosis. | >90% | >90% |
| Sarcoidosis[90,91] | | | | |
| | $^{18}$F-fluorodeoxyglucose positron emission tomography | Active inflammation and scar | 89% | 78% |
| | CMR | **T2 weighted imaging**: inflammation, focal wall thickening, myocardial fibrosis. Typical finding: subepicardial and mid wall LGE on basal septum and/or inferolateral wall | 93% | 85% |

ATTR, transthyretin amyloidosis; CMR, cardiac magnetic resonance; ECV, extracellular volume; LGE, late gadolinium enhancement.

In patients with DCM, therapeutic implications of EMB-proven virus-negative myocardial inflammation (i.e. inflammatory cardiomyopathy) have been addressed in two randomised trials. In the TIMIC study ($n$ = 85), 6 months of prednisone and azathioprine treatment resulted in a significant improvement in LV function compared with placebo without major adverse effects.[106] Another trial ($n$ = 84) reported that 3 months of immunosuppressive therapy vs. placebo provided a significant improvement in LV ejection fraction that was maintained at 2-year follow-up, although there was no difference in survival.[107] A propensity score-matched retrospective analysis of patients receiving immunosuppressive therapy ($n$ = 90) vs. standard care ($n$ = 90) also demonstrated beneficial effects of immunosuppression on HTx-free survival and improvement in LV function after a median follow-up of 12 months.[108] In an observational study or 110 patients with Lyme disease associated cardiomyopathy, an improvement in cardiac function was described with antibiotic treatment in addition to standard HF medications.[51]

In patients with active viral infection, several treatment options have been investigated, including intravenous immunoglobulins, interferon-alfa and beta, ganciclovir, acyclovir and valacyclovir.[109–112] A phase II randomised trial of 143 patients with EMB-proven enterovirus, adenovirus, and/or parvovirus B19 presence in the myocardium has demonstrated that 24 weeks of interferon beta-1b vs. placebo resulted in effective viral clearance or reduction in viral load.[113] Likewise, rituximab has shown promising results in a small series of patients with cardiomyopathy and CD20+ B lymphocytes in EMB samples.[114] Presently, recommendations for the routine clinical use cannot be given for these medications, pending further clinical evaluation.

Endomyocardial biopsy findings also have therapeutic implications for individuals with storage disorders for which specific enzyme replacement therapies are available (Anderson–Fabry disease, glycogen storage disorders), as well as in the management of amyloidosis and in HTx rejection.

## Worldwide use of endomyocardial biopsy: current clinical practice

There is a considerable international variability in the clinical practice of EMB. In most countries the procedure is more frequently used for the surveillance of HTx rejection than for other

indications.[97,115] However, in Japan, EMB is more frequently performed in non-HTx patients because of the low rate of HTx procedures.[116,117] According to a nationwide study in Japan reporting on 9508 adult patients (EMB performed in 2010–2013), the most common indication was DCM (35%), followed by sarcoidosis (7.3%), amyloidosis (4.2%), and myocarditis (3.4%), whereas HTx patients accounted for only 3.6% of EMB indications.[33] By contrast, in a large US survey (2002–2014), the most frequent indication for EMB was HTx rejection surveillance (71%), followed by the assessment of cardiomyopathies, amyloidosis, myocarditis and sarcoidosis.[84] Similarly, in a large single-centre study from Brazil reporting on 5347 EMB procedures (1978–2011), HTx rejection surveillance was the most common indication in 67% of patients, while the assessment of cardiomyopathies and cardiac tumours accounted for 33% and 1% of EMB procedures, respectively.[35]

The overwhelming majority of EMB procedures are performed in tertiary or university hospitals (99% of HTx and 94% of non-HTx EMBs).[31] RV EMB is the most frequently used approach, while LV EMB is less frequent, especially in the USA. A large single-centre European non-HTx study ($n = 4221$, over 28 years) indicates that LV EMB can be safely performed (84% of patients) and provide incremental diagnostic information to RV EMB.[16] Guidance with fluoroscopy was used in 98% of the procedures in the Brazilian study, whereas two-dimensional echocardiography and guidance with both fluoroscopy and two-dimensional echocardiography were used significantly less often (1.6% and 1.0%, respectively), mostly for cardiac tumours.[35] In this study, the right internal jugular vein was used as an access site in 97% of the procedures, followed by the left internal jugular vein (0.6%), femoral (0.5%), or subclavian approach (0.3%).[35] Similar practice is applied in HTx centres in Germany, where internal jugular vein is the prevailing vascular access site in 95% of EMB procedures, while femoral access is used in 4.6%.[118] By contrast, most of the specialized centres in other countries report performing EMB in the non-HTx population using femoral veins and/or arteries.[119,120]

# Future perspectives

Endomyocardial biopsy has gained global acceptance in the surveillance of HTx rejection and in diagnostic assessment of select patients with myocarditis, cardiomyopathies, cardiotoxicity of cancer drugs, infiltrative and storage disorders and cardiac tumours. In addition, EMB was instrumental in describing the pathophysiology of ICI-mediated cardiotoxicity[52] and myocardial involvement in SARS-CoV-2 infection.[48]

Future improvement in technologies is expected to provide more flexible and steerable guidance catheters, as well as the possibility of integrating EMB with high-resolution imaging modalities.[121] Innovations in cardio-pathology, including new-generation PCR tools, confocal laser scanning microscopy and super-resolution microscopy with high-contrast and high-resolution fluorescent imaging, will likely improve the diagnostic yield of EMB.[122]

Presently, there is an unmet need to develop a network of regional and national centres with a standardized expertise in EMB practice. This issue can be addressed though the implementation of Heart Failure Quality of Care Centres, providing multidisciplinary care of HF patients, including the availability of EMB in tertiary level centres.[123] The high level of expertise provided by these centres will increase diagnostic value of EMB, open new clinical perspectives and decrease the risk of complications. These centres should build multidisciplinary teams with complementary competences in EMB procedure, evaluation of samples, interpretation of the results and clinical expertise in patient management. The teams should include HF specialists, electrophysiologists, experts in imaging, cardio-pathology, molecular biology, and clinical genetics.

Endomyocardial biopsy has only partially fulfilled its earlier expectations. Its future role will be determined by advances made in non-invasive assessment of cardiac disorders, progress in translational sciences and the development of new, targeted therapeutic options.

# Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Conflict of interest**: none declared.

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