Significance and Value of Endomyocardial Biopsy Based on Our Own Experience

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Endomyocardial biopsy (EMB) has been established in parallel with the development of percutaneous catheter technology for the diagnosis of cardiac diseases. It was developed in the early 1960s in Japan by Drs. Konno, Sakakibara and Sekiguchi of Tokyo Women's Medical University. EMB is a valuable and useful, but invasive, modality for making a definite diagnosis in diseases such as myocarditis and secondary cardiomyopathies, which are often difficult to diagnose by imaging modality alone. In the field of heart transplantation, the histology of EMB helps monitor rejection to allografts. In cases of chronic heart failure, fibrosis and degeneration of cardiomyocytes are very important findings of heart remodeling. Recently, molecular biology technology has been applied to EMB specimens to get more detailed information. However, we must also recognize that EMB is an invasive examination that should not be performed without skillful cardiac catheterization experience to avoid complications. In this review as a message from pathologists, we present key cardiac histopathology using EMB, in a way that allows one to imagine whole cardiac pathological conditions. We also describe the current role of EMB and its significance in order to encourage young cardiologists to perform EMB to see another world of pathology.

Key Words: Cardiomyopathy; Endomyocardial biopsy; Pathological diagnosis

In recent years, many diagnostic imaging modalities have been developed, including echocardiography, computed tomography, magnetic resonance imaging (MRI), and scintigraphy (positron emission tomography: PET). Heart failure (HF) is usually diagnosed by conventional physical examination and additional imaging modalities on occasion.1 However, some of the remaining cases of HF require histological diagnosis by endomyocardial biopsy (EMB). Therefore, EMB is the gold standard for specifying the etiology of suspected cardiac diseases. Thanks to Drs. Konno and Sakakibara,2 and also Sekiguchi,3 EMB was developed and established in Japan. Pathologists who are not experts in cardiovascular diseases may find EMB specimens quite difficult to evaluate because many findings are nonspecific, and few diseases present specific findings. In addition to H&E pathology, immunohistochemistry and molecular biology are available to specify the pathogenesis. In this review, we hope to assist cardiologists in appreciating the benefits of EMB, with its high diagnostic value. We have experience of microscopic examination of over 5,000 EMB specimens, and we still find it difficult to evaluate but highly interesting to observe and diagnose.

General Aspects of EMB
Application of EMB and Guidelines
Although a consensus has been reached by committees such as the American Heart Association and the Association for European Cardiovascular Pathology and Society for Cardiovascular Pathology, the main application of EMB is diagnosing myocarditis, allograft rejection, or secondary cardiomyopathies such as sarcoidosis and amyloidosis.4 However, in Western countries, they may not perform EMB for accurate diagnosis of idiopathic cardiomyopathies such as dilated cardiomyopathy (DCM), which is probably related to cost-benefit ratio and procedure risk.5,6 Tricuspid valve injury, arrhythmia, hematoma, and hemopericardium caused by perforations may occur as complications of EMB. The risk of cardiac perforation is reported as 0.12–0.4%.* In Japan, cases of unexplained HF with onset in the recent 6 months are generally thought to have an indication for EMB. Our pathological diagnoses of 5,260 previous EMB specimens during the recent 12 years after heart transplantation (HTx) were as follows. The most frequent indication and diagnosis (31.5%) was for monitoring the status of allografts after HTx in our institution or abroad. The second most frequent diagnosis was DCM (23.8%). Hypertrophic cardiomyopathy (HCM) was 14.9% and myocarditis with histological evidence was 7.5%. Cardiac sarcoidosis with histological evidence was 4.6% and cardiac amyloidosis with histological evidence was 1.8%. Other rare cardiomyopathies, including arrhythmogenic right ventricular cardiomyopathy (ARVC) (0.7%) and mitochondrial cardiomyopathy (0.2%), were less than 1%. Nonspecific
changes such as hypertrophy only and nearly normal myocardium were 14.9%.

**Appropriate Preparation of EMB Specimens**

Sufficient evaluation of EMB can be performed with H&E and Masson’s trichrome staining. If slices of specimens on glass slides are thicker than 7μm, interstitial fibrotic tissue is over-emphasized and may be confused with inflammatory cells or severe fibrosis. It is important to evaluate interstitial fibrosis by Masson’s trichrome staining for more accurate diagnosis. Additional snap-frozen sections are occasionally required with some immunostainings for diagnosis of muscular dystrophy and mitochondrial cardiomyopathy. When electron-microscopic evaluation is needed, specimens should be stored in 2.5% glutaraldehyde solution.

**Histology of EMB**

**Cardiomyocytes**

**Hypertrophy** Myocardial cell diameter is an important indicator of hypertrophy. Cardiomyocytes of the right ventricle that are ≤15μm in diameter are considered as not hypertrophic; a diameter of up to 20μm may indicate mild hypertrophy; up to 25μm may indicate moderate hypertrophy; between 25 and 30μm may be moderate to severe; and a diameter >30μm is compatible with severe hypertrophy, based on our experience and unpublished data (Figure 1). The mean minor axis length of 20–30 myocytes with central nuclei is used for myocyte diameter. Myocyte hypertrophy may occur for various reasons, but is generally associated with pressure overload such as systemic hypertension or valve stenosis. Cases of cardiomyopathy, especially HCM, almost always present with cell hypertrophy. However, in compensatory hypertrophy, cell size may depend on the severity of HF.

**Nuclear Enlargement and Deformation** Nuclear enlargement and bizarre nuclei tend to occur more frequently with hypertrophy of myocardial cells (Figure 1C,E). Hyperchromatic nuclei with irregular nuclear membrane are often seen in cases of chronic HF. Binuclear or greater nuclei are also noted in hypertrophied hearts (Figure 1E). Bizarre and deformed nuclei with increased chromatin are also common in severe cases of idiopathic cardiomyopathy. The deformed and enlarged nuclei of degenerative cells by routine H&E staining are often recognized as having deep in-folding of an irregular nuclear membrane and irregular distribution and aggregation of chromatin ultrastructurally. These electron-microscopic nuclear changes are nonspecific, but they may be related to decreased activity of nuclear function caused by cell damage, because chromatin consists of nuclear DNA. Apoptotic cells are very rarely observed in EMB specimens, but they can show nuclear changes such as condensed chromatin under the nuclear membrane, on electron microscopy, suggesting nuclear molecular ends of DNA fragments.

**Cellular Arrangement** Abnormal cell arrangement, called disarray (Figure 1B,D,F), is a well-known characteristic of HCM. The term “disarray” is often used to indicate HCM as a large deviation from the normal response to cardiac performance. However, cardiac diseases sometimes show cellular disarrangement because of fibrosis or hypertrophy. In addition, the junctional area of the septum and both ventricles, and the cardiac apex often present a nonspecific disarrangement as a non-pathological condition.

**Interstitial Tissues of the Myocardium**

**Interstitial Fibrosis (Figure 2)** There are 3 main patterns of fibrosis: interstitial, replacement, and perivascular fibrosis. Interstitial fibrosis refers to diffuse stromal fibrosis along each cardiomyocyte bundle or peri-cardiomyocytes. This pattern is nonspecific and often observed in specimens from HF cases. Replacement fibrosis appears to be a compensatory mechanism for myocardial necrosis and consequently influence tissue stiffness and ventricular cardiac function. Scar tissue, or replacement fibrosis, develops from loss of cardiomyocytes following inflammation such as myocarditis, sarcoidosis, or ischemia, and is also recognized in cases of HCM and muscular dystrophy. Perivascular fibrosis spreads radially around capillary and small arteries, and is frequently observed in myocardial tissue with pressure overload such as in cases of hypertensive heart disease and valvular disease, especially aortic stenosis. Our unpublished data from a comparative study of the fibrotic area between RV biopsies before HTx and the explanted hearts from the same 23 DCM patients showed no obvious differences in the fibrosis rate (Figure 2D). These data suggest that fibrosis on EMB may represent fibrosis in the whole heart.

**Inflammatory Cell Infiltration** In the evaluation of
Significance and Value of EMB

Endocardial Thickening

The endocardium often thickens with proliferation of collagen fibers in cardiomyopathy. In cases of endocardial fibroelastosis, proliferation of elastic fibers is also observed. For the confirmation of elastic fiber proliferation, elastica van Gieson staining is needed. The presence of \( \geq 10 \) elastic fiber layers in endocardial fibrous thickening is considered abnormal proliferation.

Arteriopathy of Arterioles in the Myocardium

EMB specimens occasionally contain small arteries of the peripheral coronary arteries. Some arterioles with thickened walls caused by high blood pressure or arteriosclerosis are sometimes seen. In HCM, small arteries in the myocardium often show dysplastic thickened walls.

Inflammatory cells in EMB, the infiltration of lymphocytes, macrophages and eosinophils is important (Figure 3). A few lymphocytes are sometimes found in stroma, and thus it is important to determine whether this finding has pathological significance indicating myocarditis. In some cases, nuclei of fibroblasts in interstitial tissue look like inflammatory cells. The appearance of eosinophils may be consistent with eosinophilic myocarditis (Figure 3E), which is discussed later. However, these eosinophils may infiltrate because of hypersensitivity to drugs such as catecholamines for HF. Because degranulated eosinophils are quite similar in appearance to neutrophils, they can be mistaken for neutrophils because of a few eosinophil granules remaining in the cytoplasm after degranulation. In viral myocarditis, no neutrophils are present except in the early stage of severe inflammation. If a microabscess is found in the myocardium with sepsis or similar, the patient might have bacterial myocarditis.

Fat Infiltration

Fat infiltration is also commonly found in EMB. Aged women and obese patients in particular tend to have large fat deposits in the epicardium, and the presence of adipose tissue in the myocardium often has no pathological significance. Replacement by adipose tissue often seen in the terminal stage of myocardial degeneration and fibrosis. In ARVC, replacement by fibrofatty tissue of myocardium is an important histological characteristic.

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EMB specimens occasionally contain small arteries of the peripheral coronary arteries. Some arterioles with thickened walls caused by high blood pressure or arteriosclerosis are sometimes seen. In HCM, small arteries in the myocardium often show dysplastic thickened walls. When the myocardial arteriolosclerosis is seen in a patient with chest...
pain and no stenosis of epicardial coronary arteries, possible microvascular angina is suggested.17

Representative Diseases Requiring Diagnosis With EMB (Secondary Cardiomyopathy)
Guidelines emphasize the importance of EMB diagnosis for the diseases described below.

Myocarditis
Myocarditis is an inflammatory disease caused by viral, autoimmune, or idiopathic infection. The key diagnostic finding of myocarditis is damaged myocardium with mostly lymphocytic infiltration (Figure 3A–C). When specimens show a large amount of inflammatory cells destroying cardiomyocytes, it is quite easy to diagnose. However, EMB specimens show variable findings over the time course of the disease because fibrosis progresses and inflammatory cells may disappear.4 If inflammatory cell infiltration remains as focal lesions, myocarditis will not be seen by possible sampling error.19 In Japan, myocarditis has been classified into 3 types as a histological classification in the 2009 Japanese Circulation Society guidelines (Table 1).20 These classifications are important for treatment strategy.

Lymphocytic Type
Most cases of myocarditis induced by a number of different viral infections may develop lymphocytic, mostly T lymphocyte, infiltration (Figure 3A, B). Myocarditis accompanied by giant cells as well as lymphocytes, and needs to be differentiated from sarcoidosis as discussed in the relevant section. Prominent myocardial injury may be severe, damaging the cardiomyocytes (Figure 3D, E). Giant cell myocarditis is often accompanied by focal eosinophilic infiltration (Figure 3F).21

Eosinophilic Type
Prominent eosinophilic infiltration and degranulation of eosinophils are seen in the stroma. Myocyte necrosis sometimes occurs. Eosinophil numbers in peripheral blood are not always elevated. Cases may be caused by viruses, or by allergic hypersensitive reactions to drugs or food (Figure 3G). In eosinophilic myocarditis, eosinophils may preferentially infiltrate the endocardial layer. It may overlap with Löeffler endocarditis.22,23

Granulomatous Type
Typical cardiac sarcoidosis sometimes shows non-caseous epithelioid granulomas with fibrosis. Many cases of granulomatous myocarditis exhibit eosinophil infiltration (Figure 4A).24

The pathological diagnosis of myocarditis by EMB is

### Table 1. Classification of Myocarditis by Different Categories in JCS Guidelines 2009

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Cell type</th>
<th>Clinical type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Lymphocytic type</td>
<td>Acute</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Giant cell type</td>
<td>Fulminant</td>
</tr>
<tr>
<td>Fungi</td>
<td>Eosinophilic type</td>
<td>Chronic (prolonged)</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>Granulomatous type</td>
<td>(latent)</td>
</tr>
<tr>
<td>Protozoa, parasites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other causes of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs, chemical substances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergy, autoimmune</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen disease, Kawasaki disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiation, heart stroke</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown cause, idiopathic</td>
<td></td>
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</tbody>
</table>

1. Ishibashi-Ueda H et al.
2. Representative Diseases Requiring Diagnosis With EMB (Secondary Cardiomyopathy).
3. Guidelines emphasize the importance of EMB diagnosis for the diseases described below.
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5. If inflammatory cell infiltration remains as focal lesions, myocarditis will not be seen by possible sampling error.
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7. These classifications are important for treatment strategy.

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   - Myocarditis accompanied by giant cells as well as lymphocytes, and needs to be differentiated from sarcoidosis as discussed in the relevant section. Prominent myocardial injury may be severe, damaging the cardiomyocytes (Figure 3D, E).
10. Giant cell myocarditis is often accompanied by focal eosinophilic infiltration (Figure 3F).
11. Eosinophilic Type
   - Prominent eosinophilic infiltration and degranulation of eosinophils are seen in the stroma. Myocyte necrosis sometimes occurs. Eosinophil numbers in peripheral blood are not always elevated. Cases may be caused by viruses, or by allergic hypersensitive reactions to drugs or food (Figure 3G).
12. In eosinophilic myocarditis, eosinophils may preferentially infiltrate the endocardial layer. It may overlap with Löeffler endocarditis.
13. Granulomatous Type
   - Typical cardiac sarcoidosis sometimes shows non-caseous epithelioid granulomas with fibrosis. Many cases of granulomatous myocarditis exhibit eosinophil infiltration (Figure 4A).
14. The pathological diagnosis of myocarditis by EMB is...

Figure 4. Secondary cardiomyopathies. (A) Cardiac sarcoidosis. (Left) Photomicrograph shows a few giant cells in fibrous scar background in EMB (Masson’s trichrome). (Middle) Multinucleated giant cells with mononuclear cells in a granuloma (H&E). (Right) Macrograph shows septal thinning with fibrosis of autopsy case of DCM-like cardiac sarcoidosis. (B) Cardiac amyloidosis. (Left) The loupé micrograph shows diffuse subendocardial deposit of amyloid (blue area). (Middle) EMB with Masson’s trichrome stain shows massive amyloid deposit (blue-gray part). (Right upper panel) Higher magnification of positive immunohistochemistry for TTR as a pericellular deposit. (Lower panel) Ultrastructure of amyloid fibril. (C) Fabry disease. (Left) Vacularated and enlarged cardiomyocytes are prominent (H&E). (Middle) Granular Gb3 accumulation in cytoplasm by immunohistochemistry. (Right) Concentric lamellar bodies in cytoplasm are found by electron microscopy (x1,500). EMB, endomyocardial biopsy; TTR, transthyretin; Gb3, globotriaosylceramide.
Table 2. EMB Diagnosis of Myocarditis: The Dallas Criteria

<table>
<thead>
<tr>
<th>Classification</th>
<th>First biopsy</th>
<th>Subsequent biopsy</th>
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<tbody>
<tr>
<td><strong>Definition of idiopathic myocarditis:</strong> “an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes not typical of the ischemic damage associated with coronary artery disease”</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory infiltrate</strong></td>
<td>Focal, confluent, diffuse</td>
<td>Endocardial, interstitial</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td>Mild, moderate, severe</td>
<td>Mild, moderate, severe</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>Lymphocytic, eosinophilic, granulomatous, giant cell, neutrophilic, mixed</td>
<td>Perivascular, replacement</td>
</tr>
<tr>
<td><strong>Extent</strong></td>
<td>Mild, moderate, severe</td>
<td></td>
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Cardiac Sarcoidosis

Patients with sarcoidosis show well-known findings of eye lesions and swelling of the bilateral hilar lymph nodes. However, cardiac lesions are also important for prognosis associated with lethal arrhythmia and HF. In some cases, lesions of sarcoidosis are only found in the heart. Diagnosis of sarcoidosis is easy to confirm when the EMB shows epithelioid granulomas containing multinucleated giant cells (Figure 4A). However, there is a high frequency of false negatives and only 20–30% of cases in which sarcoidosis is suspected clinically are able to be confirmed with EMB. In addition, inflammation repeats a cycle of relapse and remission. Granulomas may be replaced with fibrosis without giant cells or epithelioid cells in the inactive state of sarcoidosis. Thus, in cases of clinically suspected sarcoidosis, when burn-out fibrosis with only a few lymphocytes are seen in the specimens, deep serial sections are required to find granulomas. In our data, evidence of dendritic cells and M2 macrophages in a non-granulomatous area of the EMB may be helpful for suspected diagnosis of cardiac sarcoidosis. Recently, improvements have been made in diagnostic imaging that captures inflammatory lesions such as gallium scintigraphy and PET. The diagnostic guidelines of the Japanese Circulation Society for cardiac sarcoidosis using new diagnostic modalities will be published soon. In a report from our institution, 74 consecutive patients who were initially diagnosed as cardiac sarcoidosis by EMB or other modalities were evaluated for the presence of basilar thinning of the interventricular septum (IVS) by echography, because this feature is often recognized among patients with poor prognostic cardiac sarcoidosis (Figure 4A, Right).

Cardiac Amyloidosis (Figure 4B)

Amyloidosis is a systemic disease with amorphous deposits of amyloid substance. Amyloid deposits in the heart are found in the stroma and small arterial walls. It is well known for positive staining with Congo-red, and for exhibiting apple green birefringence on polarizing microscopy. In cardiac amyloidosis, light-chain (AL) amyloidosis tends to exhibit HF, mainly involving diastolic dysfunction and diverse arrhythmia, and the prognosis is quite poor. Because of the diffusely spread amyloid material in stroma and vessel walls, sampling error is relatively rare. Our previous unpublished data showed 62% confirmation of histological amyloidosis by EMB among cases of suspected amyloidosis clinically. With Mason’s trichrome staining, the blue or green areas of stroma turn gray because of amyloid deposition (Figure 4B, Middle). Immunohistochemistry is very helpful for typing the amyloid protein. Clinically, as the heart becomes firm because of amyloid deposition, diastolic dysfunction may advance, and some cases may be diagnosed as restrictive cardiomyopathy.
(RCM) or HCM. In our experience, when amyloid deposits are found in clinical HCM cases, the echocardiographic record should be rechecked. In addition, there is a high incidence of transthyretin-derived amyloidosis (TTR amyloidosis) in elderly patients (≥70 years) with cardiac amyloidosis. AL and TTR amyloidosis can be differentiated with immunostaining (Figure 4B, Right).

Metabolic and Storage Diseases

Metabolic and storage diseases of the heart may occur through various enzyme deficiencies caused by genetic abnormalities. One of the most representative diseases is Fabry (Anderson-Fabry) disease, which has a relatively high incidence rate. Metabolic and storage diseases, including amyloidosis, may be diagnosed as HCM initially. Fabry disease is caused by a lack/decrease of activity of α-galactosidase A, which is a hydrolase in lysosome through an X-linked recessive inheritance. In this disease, glycosphingolipid is deposited in cardiomyocytes without being metabolized. Vacuolar changes are seen in the cytoplasm. In severe cases, cardiomyocytes have a lace-like pattern of vacuolation (Figure 4C, Left). Globotriaosylceramide (Gb3) immunostaining is helpful for diagnosis (Figure 4C, Middle). These changes in Fabry disease by EMB are very helpful not only for diagnosis, but also for monitoring the efficacy of enzyme-replacement therapy. Furthermore, transmission electronic microscopy (TEM) can confirm the deposition of ceramide trihexoside exhibiting a concentric lamellar body, supporting the diagnosis (Figure 4C, Right).

Primary Cardiomyopathies

After the possibilities of secondary cardiomyopathy have been eliminated, idiopathic or primary cardiomyopathies should be considered. Idiopathic/primary cardiomyopathies are classified in a slightly different manner between the USA, Europe, and Japan. However, in general, idiopathic cardiomyopathy (primary cardiomyopathy) is classified into DCM, HCM, ARVC, and RCM. Originally, it was defined by morphology because of unknown heterogeneous causes and various etiologies. Therefore, many disease types for which the cause has been analyzed with gene analysis are still imprecisely treated because of being considered a type of idiopathic cardiomyopathy. In Western countries, it is often roughly classified into ischemic cardiomyopathy and non-ischemic cardiomyopathy, and it is important to identify first that the case is non-ischemic without significant stenosis of coronary arteries. Histologically, it is basically a nonspecific lesion with cardiomyocyte hypertrophy and fibrosis, and it is often impossible to make a complete diagnosis with only EMB findings. It is important to perform microscopic examination based on sufficient clinical information, and making a diagnosis based only on histological findings should be avoided.

DCM

DCM is a primary heart muscle disease characterized by significant dilatation of both ventricles and thinning of the walls with poor contractility. EMB often shows interstitial fibrosis around cardiomyocytes (Figure 2). The cardiomyocytes show a mixture of atrophy through degenerative compensatory hypertrophy, exhibiting marked variation in size. Attenuation of myofibers is also seen. These changes are nonspecific. The endocardium often thickens with fibrous proliferation and mural thrombi. Inflammatory cell infiltration is not usually recognized, but T lymphocytes (CD3) and macrophages may infiltrate on some occasions. In recent years, the concept of inflammatory DCM has been proposed, and it is possible that DCM may result from an inflammatory mechanism. Almost 30% of cases among DCM have a familiar occurrence. A left ventricular assist device (LVAD) is sometimes applied in severe HF cases for LV recovery or as a bridge to HTx. Hemodynamic predictors for LVAD weaning have been reported. However, histopathological markers that reflect sufficient improvement of the patient’s own cardiac function to allow weaning from LVAD are still unclear. We have performed histopathological analysis seeking predictive markers of recovery from endstage HF in patients with LVAD. As unpublished data, we compared the routine histology of a LVAD successful weaning group consisting of 11 DCM cases (10 males, mean age 27±9 years) and of a no-LVAD weaning of 13 DCM cases (12 males, mean age 33±13 years) from 1994 to 2007. More severe fibrosis among the cases of no-LVAD weaning was seen (P=0.0009). In addition to H&E and Masson’s trichrome staining, more intense Tenascin C localization in the interstitium by immunohistochemistry, which shows cardiac remodeling, was recognized as a predictive marker for the removal of LVAD.

HCM

Many cases of HCM are hereditary and have been reported to exhibit mutation of the genes encoding sarcomeric protein. Depending on the type of genetic abnormality, the phenotype is likely to differ slightly. However, no reports have summarized this. In younger cases or those with a family history of the disease, histological changes tend to be severe. However, in cases of onset at an advanced age, the histological finding are often not notable. HCM is usually diagnosed by non-invasive methods without EMB. However, histology will be helpful in the differential diagnosis of secondary cardiomyopathies such as Fabry disease and amyloidosis. Hypertrophic cardiomyocytes and cellular/fascicular disarray are important findings on EMB (Figure 1B,D). Replacement fibrosis is characteristic, in addition to interstitial fibrosis or plexiform fibrosis. In cases of severe fibrosis, medial and intimal thickening of the small arteries may be significant, so-called small intramural coronary arteriole dysplasia (SICAD). In addition, in cases of severe fibrosis, the cardiac chambers are enlarged and gross forms may become similar to DCM (i.e., dilated phase HCM (d-HCM)). HCM does not always present myocardial disarrangement throughout the heart. Even within the same specimen, some areas may exhibit complicated disarray, while disarray is hardly recognized in other areas.

RCM

RCM is characterized by diastolic dysfunction of both ventricles observed in secondary cardiomyopathies such as amyloidosis, Löffler endocarditis and endocardial fibroelastosis. Histologically, the cardiomyocyte hypertrophy and abnormal arrangement, and interstitial fibrosis of RCM are similar to those of other cardiomyopathies.

ARVC

ARVC is a quite rare disease named after the arrhythmogenesis that is morphologically characterized by RV enlargement and fibrofatty change of the myocardium. If
lesions advance, the same lesions may develop on the LV wall. Initial lesions are often found around the tricuspid annulus at the base of the RV and just below the pulmonary valve. As EMB specimens are not collected from this area, the rate of sampling error is high. ARVC is said to cause ventricular arrhythmia through genetic defects of desmosomal proteins. In addition to gene abnormalities, evidence of inflammation has also been reported. Thus, as is the case for other types of cardiomyopathy, the etiology appears to be diverse. In typical cases with a family history, immunostaining of desmosome proteins with gene abnormality may be used to make a diagnosis. As the RV often shows adipose tissue interposition, caution must be taken that interposition of nonspecific adipose tissue in the myocardium, which is common in aged female patients, is not diagnosed excessively. The important findings of ARVC are fibro-fatty replacement and myocardial atrophy.

Heart Transplant-Related Pathology

The pathological examination of a recipient explanted heart is a good opportunity to review the pretransplant evaluation and clinical diagnosis. Most of all cases of diagnosis by EMB before HTx are not different from the final pathological diagnosis of the explanted heart. In our hospital, 2 recipients with a pre-HTx clinical diagnosis of DCM revealed cardiac sarcoidosis in the scar stage with ventricular dilatation on pathological examination of their explanted hearts. The explanted heart from another DCM case showed noticeable abnormal cell arrangement and disarray only in the ventricular septum, accompanied by thickened small arteries (i.e., SICAD). These findings confirmed that the pathology in this case was the dilated phase of HCM rather than DCM. The quite rare cardiomyopathy called triglyceride deposit cardiomyovascularopathy (TGCV), which was recently proposed in Japan, was discovered as a result of detailed examination of a recipient heart. Most recipients are implanted with a LVAD for chronic HF while waiting for HTx. Morphological changes of the cardiac valves often occur during long-term LVAD support.

Diagnosis of Allograft Rejection

As with the transplantation of other organs, immunosuppressive agents must be carefully adjusted after HTx. Myocardial biopsy is important when evaluating rejection following HTx. The first biopsy is usually performed within 7 days after the transplant, and biopsies are then performed weekly for 3 weeks. If there is no problem, the interval is increased gradually month by month and year by year. In our hospital, EMB for the evaluation of rejection is performed using 4 specimens sampled from the RV septum via the right internal jugular or the right femoral vein. For sample preparation, 3 stages of serial sections are prepared so that the whole tissue segment can be observed.

Determination of Acute Cellular Rejection (ACR) (Figure 5A)

The infiltration pattern and inflammatory cell type in the myocardium must be identified. The grading proposed by The International Society for Heart & Lung Transplantation (ISHLT) in 1990 is applied to the evaluation of ACR. Currently, the standards revised in 2004 (ISHLT 2004) are generally used (Table 3). ISHLT 1990 divided Grades 0–4 into 7 grades, but many cases that were identified as Grade 2 do not require treatment, and not many progress to 3A or more. It has been reported that some cases are mixed, exhibiting the finding of the “Quilty” effect (Figure 5C), which does not require treatment. In ISHLT 2004, Grade 2 was combined with Grade 1R (“R” refers to revised), which does not require treatment, and cases of Grade 3A or greater were considered to be Grade 2R, a group that may require treatment.

Antibody-Mediated Rejection (AMR) (Figure 5B)

This refers to a rejection reaction of B lymphocytes because of a humoral factor such as HLA. It often occurs within 4 weeks of the transplant, but it can also occur after that time. Serum panel reactive antibody is clinically useful when screening for AMR, and serum donor-specific antigen is a predictor. AMR is suspected clinically when there is cardiac dysfunction in the absence of ACR. Histologically, cases of AMR exhibit enlargement of capillary endothelial cells, the appearance of CD68-positive macrophage in capillaries, and the deposition of complement (C3d and C4d) on capillary walls as immune-phenotypic markers, together with interstitial edema. These findings were confirmed by ISHLT 2004 as AMR 1. AMR may not have notable inflammatory cell infiltration and cases might not
be able to be clearly identified with normal H&E staining alone (Figure 5B, Left). The evaluation of complement (C3d and C4d) deposition by immunostaining such as fluorescent and immunohistochemistry (Figure 5B, Right) is important. However, as the period of C3d positivity is short, many cases are likely to only exhibit C4d positivity. In order to standardize the pathological evaluation of AMR and observe subsequent organ prognosis and clinical course, a new pathological AMR (pAMR) grading system has been proposed. 74–80 We have experienced 3 cases (3.2%) showing (definite) AMR clinically and immunohistochemically among 94 HTx recipients over 15 years. Plasmapheresis worked to remove humoral factors causing AMR in these cases. Cardiac allograft vasculopathy (CAV) is also an important pathology related to long-term prognosis. Epicardial coronary arteries are usually affected by so-called chronic rejection characterized by concentric intimal thickening of multifactorial immune or non-immune etiology. Coronary artery obstruction may develop into silent myocardial infarct. We follow CAV progression of HTx recipients by annual intravascular ultrasound examination and EMB. 81,82 Some cases show microvasculopathy with medial hypertrophy of small intramural coronary arteries (Figure 5D). 83

Conclusions

Although EMB may be considered a minor modality because it is invasive and often provides nonspecific findings, the histology obtained by EMB offers a lot of information that can determine both medical therapy and the patient’s prognosis. We hope that cardiologists effectively utilize evidence from EMB.

Acknowledgments

We greatly appreciate Professor Morie Sekiguchi, who passed away prematurely in 2016, for his contribution to highlighting the role of EMB not only in Japan but worldwide. He founded the organization of Cardiac Biopsy Conference (CABIC) in 1979 and raised a lot of cardiologists and pathologists through cardiac pathology. We thank Dr. Chikao Yutani who was a former director of the pathology department at the National Cerebral and Cardiovascular Center. Also, we deeply thank Mrs. Yukako Miyatake for assistance with the manuscript.

Disclosures

None of the authors have any conflicts of interest or financial relationships to declare.

References


| Table 3. Comparison of the 1990 and 2004 Grading System of ISHLT for Acute Cellular Rejection |
|---------------------------------------------|-------------------|
| Grade 0                                     | No rejection       |
| Grade 1, mild                               | Focal perivascular and/or interstitial infiltrates, no myocyte damage |
| 1A-Focal                                    | Focal perivascular and/or interstitial infiltrates, no myocyte damage |
| 1B-Diffuse                                  | Diffuse infiltrate, no myocyte damage |
| Grade 2                                     | 1 focus of infiltrate with associated myocyte damage |
| Grade 3, moderate                           | Multifocal infiltrate with myocyte damage |
| 3A-Focal                                    | Multifocal infiltrate with myocyte damage |
| 3B-Diffuse                                  | Diffuse infiltrate with myocyte damage |
| Grade 4 severe                              | Diffuse, polymorphous infiltrate with extensive myocyte damage ±edema ±hemorrhage ±vasculitis |
| 1990                                        | 2004              |
| 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 ±edema ±hemorrhage ±vasculitis |