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Endomyocardial Biopsy: A Clinical Research Tool and a Useful Diagnostic Method

Julián González, Francisco Salgado,
Francisco Azzato, Giuseppe Ambrosio and
Jose Milei

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1. Introduction

The routine indication of endomyocardial biopsy (EMB) in myocarditis has long been a matter of debate [1]. Although always claimed as the ultimate diagnostic tool for myocarditis, its low sensitivity, low availability, high cost, and the inherent risks of an invasive procedure have led many physicians to avoid performing it. Yet, at present EMB continues to be the “gold standard” for the diagnosis of myocarditis [2].

Since its introduction in the early 1960s by Sakakibara and Konno many improvements have been made in the technique and some progress has been made in the analysis of the samples. The introduction of the Dallas Criteria [3] in 1986 was the first effort to make histological diagnosis more consistent, but still they have a very low sensitivity and lack prognostic value in many clinical studies [4-7].

After the Dallas criteria, the use of immunohistochemistry to better identify mononuclear cells infiltrating myocardial tissue added significant sensitivity to histological diagnosis [8, 9]. Also, introduction of polymerase chain reaction (PCR) applied to isolation of viral genomes from EMB samples became a promising tool. Both proved to carry prognostic value in some studies, but results have been not consistent in all publications.

Moreover, development of noninvasive methods to assess myocardial injury in myocarditis, particularly magnetic resonance image (MRI), provides a very interesting alternative to EMB, although some authors suggest that they may be complementary [10].

In this chapter we will review the most relevant evidence of the clinical usefulness of EMB and all these developing techniques.

2. Technical issues on endomyocardial biopsies

The first approach to obtain tissue samples from the heart was proposed in the 1950s by Vim and Silverman by using a needle introduced through a limited thoracotomy. The high incidence of pneumothorax and cardiac tamponade made this technique not accepted [11]. It was in 1962 that for the first time Sakakibara and Konno reported their technique of EMB introducing the bioptome in order to sample the endocardium [12]. After development of the bioptome, many improvements have been made in terms of flexibility and maneuverability, making the procedure safer and easier.

The possibility of peripheral vein access made the right ventricle the most attractive site for sampling, especially the interventricular septum because it is thicker than the right ventricular free wall and it is located in the natural path of blood flow [11]. Anyway, if needed, the left ventricle may be reached through the femoral artery and across the aortic valve [13].

According to current recommendations of the International Society of Heart and Lung Transplantation [14] and the American Heart Association, American College of Cardiology and European Society of Cardiology [2] a minimum of 4 -5 samples of 1 – 2 mm³ in size should be collected at room temperature to prevent contraction band artifacts. Additional samples may be taken if special procedures are required as immunohistochemistry (IHC), transmission electron microscopy, and/or polymerase chain reaction.

Complications of EMB have been prospectively studied by Decker et al. [15] in 546 consecutive procedures. The overall complications rate was 6%, 2.7% related to sheath insertion and 3.3% related to the biopsy procedure itself. Perforation was observed in only 3 patients (0.5%) with 2 deaths attributable to perforations (0.3%). The detailed report is summarized in table 1.

Related to Sheath Insertion = 15 (2.7%)
Arterial puncture during local anesthesia = 12 (2%)
Vasovagal reaction = 2 (0.4%)
Prolonged venous oozing after sheath removal = 1 (0.2%)
Biopsy Procedure = 18 (3.3%)
Arrhythmias = 6 (1.1%)
Conduction abnormalities = 5 (1%)
Pain without perforation = 4 (0.7%)
Perforation = 3(0.5%), 2 patients died (0.3%)

Table 1. Complications of EMB (Deckers et al. [15])

3. Current recommendations for the use of endomyocardial biopsies

In an attempt to better determine the clinical use of EMB, a committee of experts from the American Heart Association, the American College of Cardiologists and the European Society of Cardiology developed a consensus statement about when EMB was to be used in 14 clinical scenarios [2]. It is remarkable that in only 2 of those scenarios the recommendation reaches recommendation level I. Table 2 summarizes the 14 clinical situations, the level of recommendation, and evidence for the use and clinical value of EBM.

Nº	Clinical Scenario	EMB usefulness	Level of recom.	Level of evid.
1	New-onset heart failure of <2 weeks' duration associated with a normal-size or dilated left ventricle and hemodynamic compromise	Distinguish between lymphocytic myocarditis (good prognosis) and GCM or NEM that require immunosuppressant treatment.	I	B
2	New-onset heart failure of 2 weeks' to 3 months' duration associated with dilated left ventricle and new-onset ventricular arrhythmias, second- or third-degree heart block, or failure to respond to usual care within 1 to 2 weeks	Distinguish between lymphocytic myocarditis (good prognosis) and GCM that requires immunosuppressant treatment.	I	B
3	Heart failure of >3 months' duration associated with dilated left ventricle and new-onset ventricular arrhythmias, second- or third-degree heart block, or failure to respond to usual care within 1 to 2 weeks	Cardiac sarcoidosis is a special differential diagnosis in this setting. Sarcoidosis responds very well to corticosteroid treatment. GCM is also a possibility in this scenario.	Ila	C
4	Heart failure associated with a DCM of any duration associated with suspected allergic reaction and/or eosinophilia	Detect HSM and stop offending medication and start high dose corticosteroids.	Ila	C
5	Heart failure associated with suspected anthracycline cardiomyopathy	Although anthracycline toxicity can be detected by means of noninvasive test, EMB has better sensitivity to detect earlier stages and stop offending drug earlier. Requires TEM.	Ila	C
6	Heart failure associated with unexplained restrictive cardiomyopathy	Although a great progress has been made in the use of noninvasive tests such as CMR in the assessment of restrictive	Ila	C

Nº	Clinical Scenario	EMB usefulness	Level of recom.	Level of evid.
		cardiomyopathy, EMB still remains the only diagnostic tool for many of them.		
7	Suspected cardiac tumors	When diagnosis is not possible through other methods. Not recommended in typical myxoma because of embolization risk.	IIa	C
8	Unexplained cardiomyopathy in children	Differential diagnosis	IIa	C
9	New-onset heart failure of 2 weeks' to 3 months' duration associated with a dilated left ventricle, without new-onset ventricular arrhythmias or second- or third-degree heart block, that responds to usual care within 1 to 2 weeks	Seldom GCM can be diagnosed in this setting. EMB should not be performed routinely.	IIb	B
10	Heart failure of >3 months' duration associated with a dilated left ventricle, without new ventricular arrhythmias or second- or third-degree heart block, that responds to usual care within 1 to 2 weeks	In recent trials patients showing enhanced expression of HLA molecules in EMB had some benefit from immunosuppressant therapy. Hemochromatosis may be a differential diagnosis in this setting.	IIb	C
11	Heart failure associated with unexplained HCM	Some entities, specially infiltrating diseases that can thicken heart walls, can be diagnosed with EMB (Pompe's and Fabry's diseases, amyloidosis).	IIb	C
12	Suspected ARVD/C	Rarely needed because CMR generally establishes the diagnosis.	IIb	C
13	Unexplained ventricular arrhythmias	Generally shows myocarditis or nonspecific findings.	IIb	C
14	Unexplained atrial fibrillation	Not recommended	III	C

CRM, Cardiac Magnetic Resonance; DCM, Dilated Cardiomyopathy; GCM, Giant Cell Myocarditis; HSM, Hypersensitivity Myocarditis; NEM, Necrotizing Eosinophilic Myocarditis; TEM, Transmission Electron Microscopy.

Table 2. Clinical Recommendations for the Use of EMB [2].

4. The anatomopathological picture of different types of myocarditis

We will briefly describe the pathological features of the main pathologies cited in this chapter that constitute the differential diagnosis of lymphocytic myocarditis:

- Lymphocytic myocarditis
- Giant cell myocarditis
- Sarcoidosis
- Hypersensitivity myocarditis
- Eosinophilic myocarditis

4.1. Lymphocytic myocarditis

The pathological picture of lymphocytic myocarditis is the infiltration of myocardium by activated T lymphocytes, with or without signs of myocyte injury, as illustrated by the EMB sample of a patient with cytomegalovirus (CMV) myocarditis shown in figures 1-3. Figure 3 also shows the characteristic nuclear inclusions of CMV infection. Histological findings are generally diffuse but may be focal in nature (figure 4) making multiple samples and immunohistochemistry necessary for greater diagnostic accuracy.

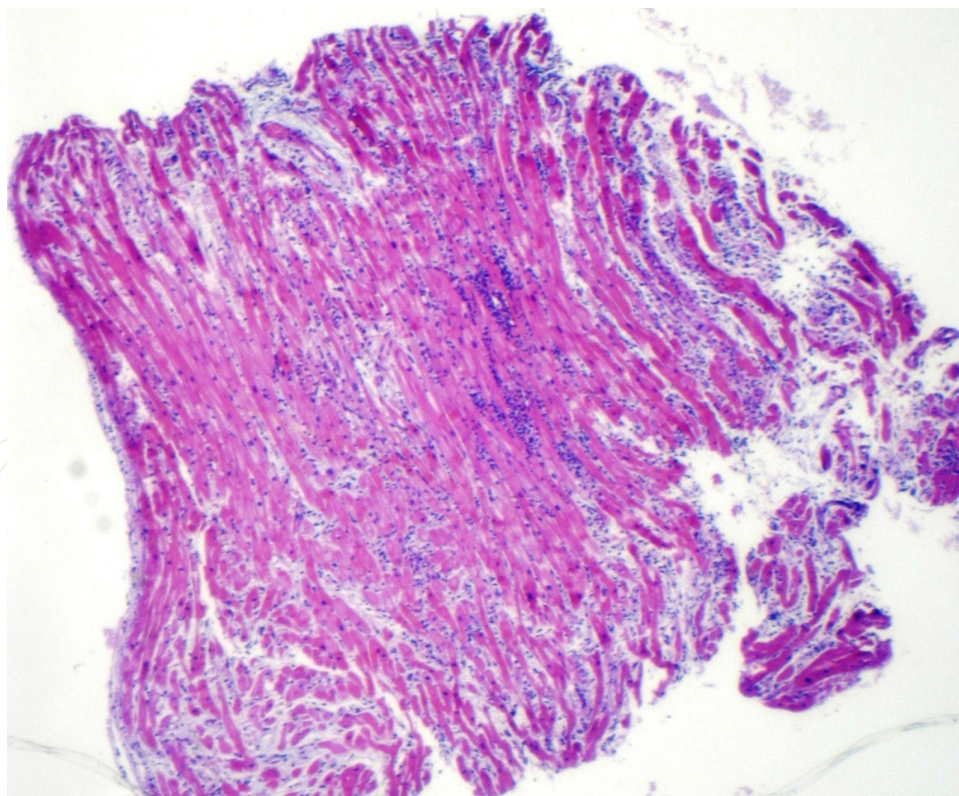


Figure 1. Myocarditis. Endomyocardial biopsy demonstrating a diffuse infiltration of lymphocytes. H-E. 40 X.

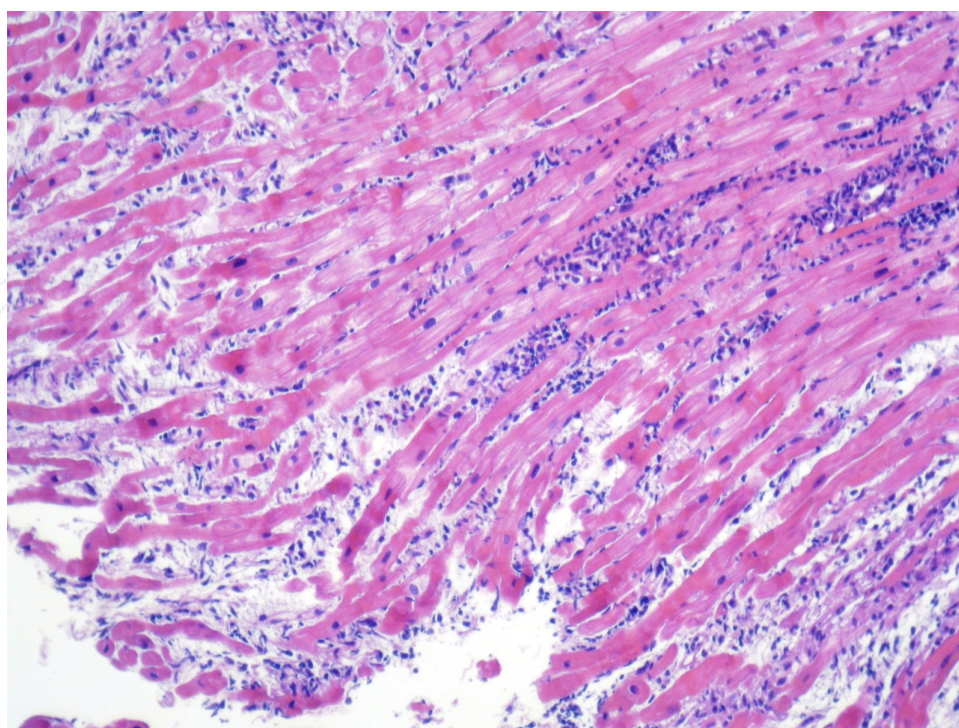


Figure 2. Myocarditis. Biopsy sample of the case illustrated in Figure 1. A dense infiltrate of lymphocytes and myocyte necrosis is evident. H-E- 100X.

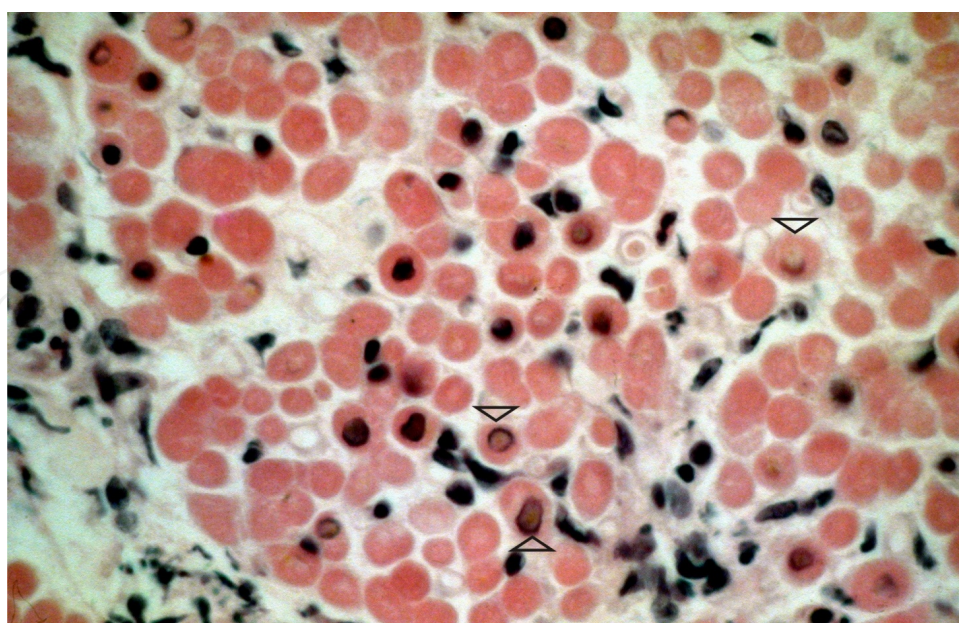


Figure 3. Myocarditis. Biopsy sample of the case illustrated in Figures 1 and 2. Lymphocytic myocarditis by cytomegalovirus infection. Note the characteristic "owl's eye" nuclear inclusions (arrows). H-E. 400X

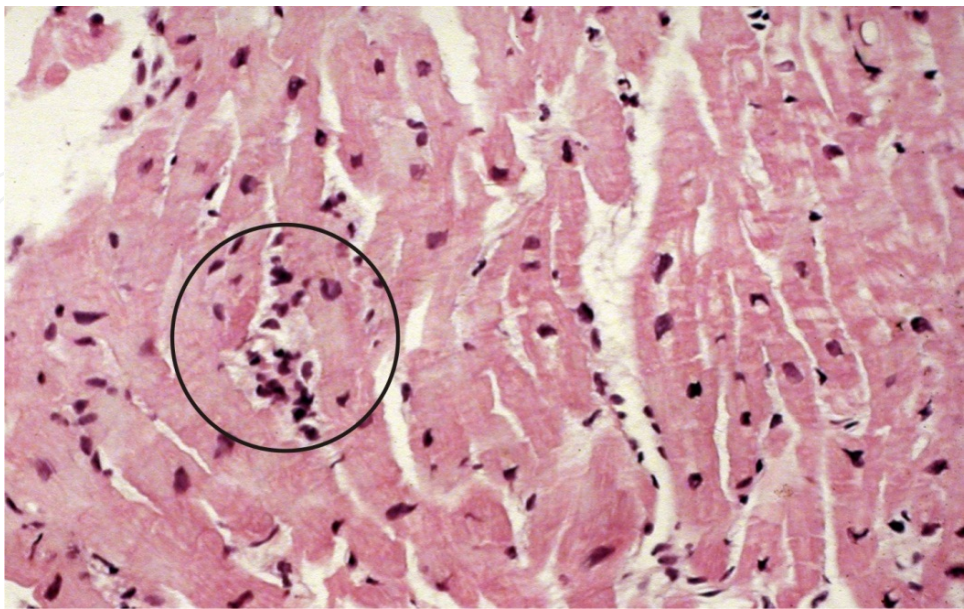


Figure 4. Focal myocarditis. Inflammation is quite focal. Note necrotic myocytes infiltrated by lymphocytes (circle) H-E 200X.

In order to better standardize histological diagnosis, Dallas criteria have been developed (table 3), for first and subsequent biopsies. **Active myocarditis** is defined as the presence of lymphocytes infiltrating myocardium plus evidence of myocyte injury (excluding contraction bands, a common artifact in EMB samples). **Borderline myocarditis** is defined as milder infiltrates without evidence of myocyte injury.

For subsequent biopsies, **ongoing** myocarditis, **resolving** (healing) **myocarditis** (figure 5) and **resolved** (healed) **myocarditis** categories have been created if infiltrates are the same as first biopsy, less than the first biopsy or have disappeared respectively.

First biopsy
Active myocarditis, with or without fibrosis
Borderline myocarditis
No myocarditis
Subsequent biopsy
Ongoing (persistent) myocarditis, with or without fibrosis
Healing (resolving) myocarditis, with or without fibrosis
Healed (resolved) myocarditis, with or without fibrosis

Table 3. Dallas criteria for the diagnosis of myocarditis

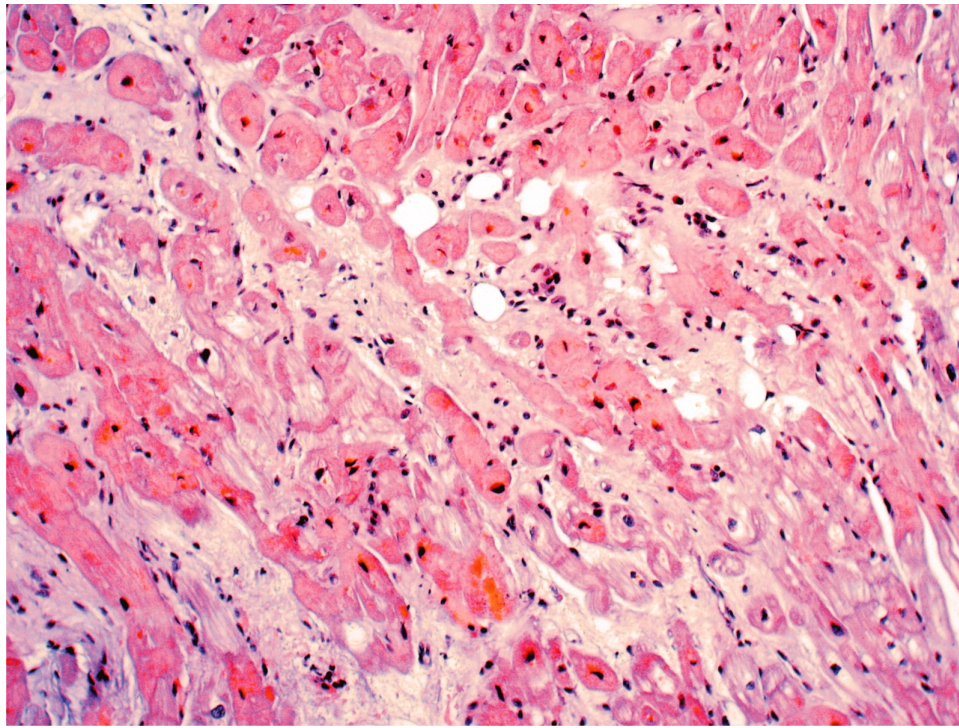


Figure 5. Healing myocarditis. Diffuse lymphocytic infiltrate is mingled with interstitial fibrosis. Note the scattered atrophic myocytes. H-E 200X.

4.2. Giant Cell Myocarditis (GCM)

This specific form of myocarditis of unknown cause is particularly aggressive with a high mortality. Extensive myocyte necrosis with an intensive infiltrate of lymphocytes, plasma cells and eosinophils are seen. The most striking characteristic, which names the disease, is the presence of giant multinucleated cells in the borders of necrotic areas (figure 6). Multinucleated cells are originated from macrophages. The most abundant cells in the remaining infiltrates are CD8+ T-lymphocytes. The main differential diagnosis of GCM is sarcoidosis, which is differentiated for:

- Eosinophils are abundant in GCM and absent in sarcoidosis
- Fibrotic scarring is more prominent in sarcoidosis
- No granulomas are seen un GCM
- Sarcoidosis may affectepicardium, never affected by GCM

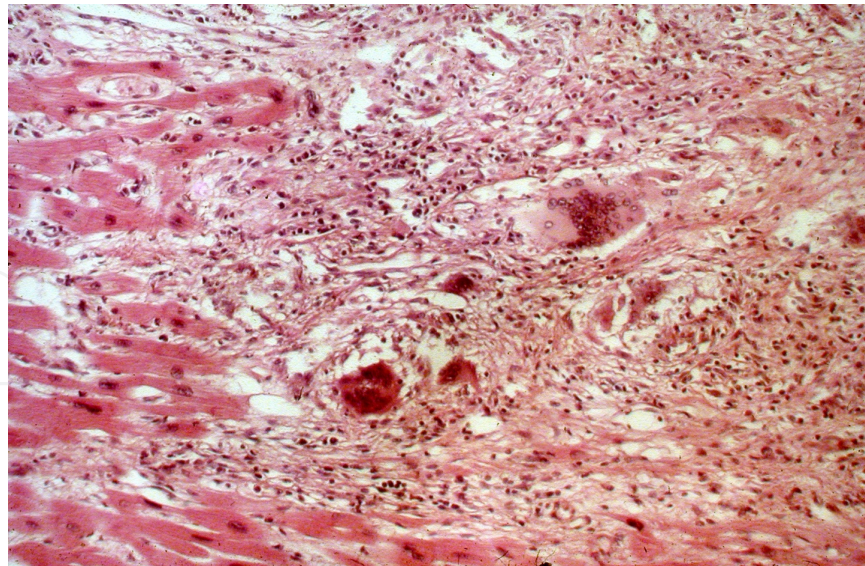


Figure 6. Giant cell myocarditis. A dense infiltrate of lymphocytes with prominent giant cells is observed. Note the absence of well-established granulomas. H-E 200X.

4.3. Sarcoidosis

Sarcoidosis is a systemic disease that may affect the myocardium. The presence of granulomas on EMBs may reach 20% of cases. The compromise is patchy and EMBs may be negative. Non-caseating granulomas consisting of histiocytes, giant cells, lymphocytes and plasma cells are the most prominent feature of the disease. Focal infiltrates of lymphocytes are seen, but they lack eosinophils seen in GCM. Patchy fibrosis is also a frequent finding (figure 7).

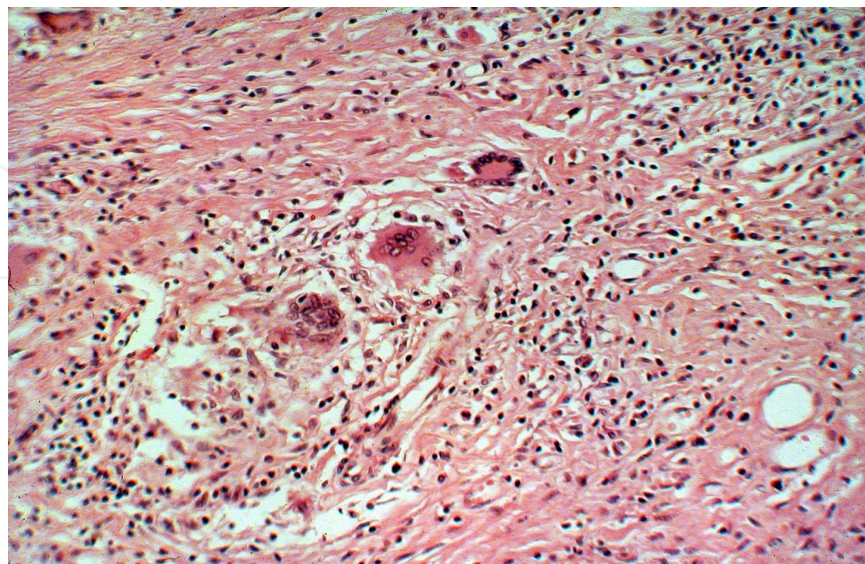


Figure 7. Sarcoidosis. Endomyocardial biopsy demonstrates a well-established, non-necrotizing granuloma. Giant cells are evident. H-E 200X.

4.4. Hypersensitivity myocarditis

Although not very common, hypersensitivity to drugs may involve the myocardium. The suspicion of this entity should arise when a patient presents with acute heart failure in the context of a hypersensitivity reaction to a drug. Tissue samples show a chronic perivascular infiltrates with lymphocytes, macrophages and plasma cells, with a prominence of eosinophils. Myocyte injury may be seen but is not a prominent feature. Fibrosis is absent.

4.5. Eosinophilic myocarditis

Myocarditis may be present up to in 25% of patients with hypereosinophilic syndrome. Extensive infiltration with eosinophils is present in this type of myocarditis (figure 8) but two distinctive features help distinguishing it from hypersensitivity myocarditis: the presence of myocyte necrosis and the presence of intracavitary thrombi containing eosinophils, which can also be seen in the lumen of intramyocardial coronary vessels.

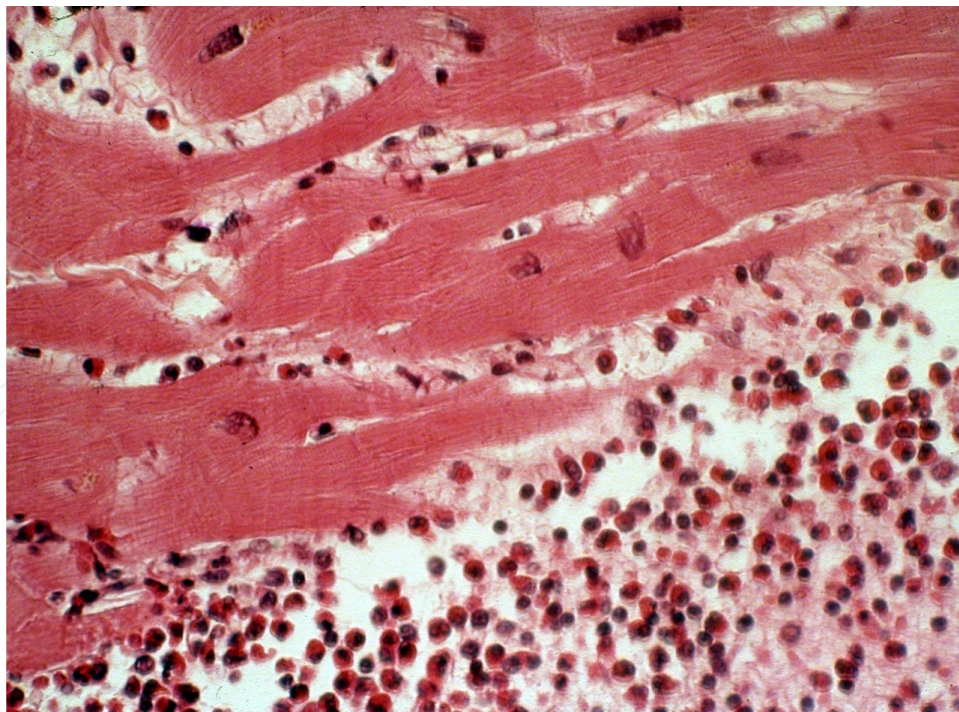


Figure 8. Hypereosinophilia. The interstitial infiltrate is suggestive of hypersensitivity myocarditis. H-E 200X

5. The role of endomyocardial biopsy in the management of myocarditis

Endomyocardial biopsy is still considered the “gold standard” for diagnosis of viral myocarditis. The use of Dallas criteria, although questioned, remains almost universal. The development of IHC and PCR for processing EMB samples widened its usefulness.

5.1. The rise, decline and validity of the Dallas criteria

The Dallas criteria for histopathological diagnosis of myocarditis were introduced in 1986 [3] in the intent of standardizing the way in which EMB would be analyzed and became, since then, a “gold standard” for the definitive diagnosis of myocarditis.

As previously stated, **active myocarditis** was defined as the presence of inflammatory infiltrates associated with myocardial injury not characteristic of ischemic heart disease, and **borderline myocarditis** was defined as a less intensive infiltrate without evidence of myocyte damage.

Furthermore, most clinical investigation on myocarditis have used the Dallas criteria as the main inclusion criteria [16]. The main weakness of Dallas criteria is low sensitivity (about 25%) to detect infiltrates in myocardial samples, mainly due to: 1) the patchy nature of myocardial infiltrates makes sampling error a great concern, 2) the lack of consistent interpretation of EMB samples, even among most experienced pathologists.

The issue of sampling error has been addressed by many authors. Chow and Hauck published on postmortem EMB showing that one sample had a sensitivity of 25% to detect myocarditis, and that 5 samples were needed to raise this figure to 66% [17, 18]. Similar experience has been published with the use of EMB to detect allograft rejection [19, 20].

On the other hand, the lack of interobserver agreement in the interpretation of histological samples shows that the Dallas criteria did not achieve completely their goal. It is remarkable that of the 111 patients enrolled in the Myocarditis Treatment Trial (positive EMB according to Dallas criteria required as inclusion condition) only 64% had the diagnosis confirmed by the expert pathologist panel [21]. In another study where 7 expert pathologists examined the EMB of 16 patients with dilated cardiomyopathy (DCM), interpretation of samples varied remarkably. Diagnosis of myocarditis was made in 11 patients at least by 1 pathologist. But only in 3 patients, three pathologists agreed in the diagnosis, and in 5, two pathologists agreed, showing that even for expert pathologists, interpretation of EMB is quite variable [22].

Some investigators showed that many patients with a clinical presentation suggestive of myocarditis were negative for Dallas criteria but had a PCR positive for viral genomes in the EMB. Martin et al. studied 34 children with clinical presentation suggestive of myocarditis. Twenty-six of the 34 samples were positive for viral genomes but only 13 of the 26 were positive for Dallas criteria [23]. Pauschinger et al. found that 24 of 94 patients with idiopathic dilated cardiomyopathy (DCM), all of them negative for Dallas criteria, were positive for viral genomes [24]. In another study, Pauschinger et al. demonstrated positive PCR for enteroviruses in 45 patients with idiopathic DCM; only 6 were positive for Dallas criteria [25]. Why et

al. showed in 120 patients with DCM that 41 were positive for enterovirus genomes in their EMB, but only 5 were positive for Dallas criteria [26].

Dallas criteria also lack prognostic value. Grogan et al. compared the clinical outcome in 27 patients with myocarditis and 58 patients with idiopathic DMC; presence of myocarditis did not affect prognosis [4]. Angelini et al. followed 42 patients with biopsy proven myocarditis, 26 with active myocarditis and 16 with borderline myocarditis also according to Dallas criteria. Heart failure was more frequent in the borderline myocarditis (BM) group than in the acute myocarditis (AM) group. They concluded that myocyte necrosis does not carry prognostic value [5]. Caforio et al. studied 174 patients, with active myocarditis (n=85) or borderline myocarditis (n=89). They concluded that IHC enhanced EMB sensitivity for the diagnosis of myocarditis and that Dallas criteria lacked prognostic value [6]. Kindermann et al. followed 181 patients with clinically suspected myocarditis in whom EMB was performed. Dallas criteria were positive only in 69 patients (38%), but sensitivity was increased by the use of IHC, which showed inflammation in 91 patients. Dallas criteria also proved of no prognostic value in that study [7].

Moreover, Dallas criteria did not show predictive value to select patients for immunosuppressant therapy. Clinical trials using immunosuppressant treatment for myocarditis did not show, in general, a better outcome in patients who received treatment compared to those who received placebo, even though, some patients improved markedly their left ventricular function after treatment. Dallas criteria did not predict which patients were to improve [21, 27].

The need of new criteria to make the definite diagnosis has been claimed for many authors, but as shown in the papers cited, the Dallas criteria supported by immunohistochemistry remain, at present the “gold standard” for the diagnosis of myocarditis.

5.2. The role of immunohistochemistry

The main problem with the histopathological diagnosis of myocarditis in routine samples is the differentiation between interstitial lymphocytes and other types of cells, mainly fibroblasts and histiocytes.

Schnitt et al. published a pioneer work in 50 consecutive EMBs assessed by two independent observers [28]. The use of an immunoperoxidase technique to stain specifically leucocyte common antigen (CLA, now CD45A) had a better interobserver concordance ($r=0.83$) than hematoxylin – eosin (H&E) samples ($r=0.63$) in identifying lymphocytes. Intraobserver concordance between IHC and H&E-identified lymphocytes was poor ($r=0.28$ and $r=0.14$ respectively). The main drawback of CLA antibodies is that it also stains mast cells and histiocytes. They did not study the impact of the technique in the diagnosis of myocarditis [28].

One of us (JM) emphasized in a pioneer paper in 1990, the need of immunohistochemical staining of lymphocytes for the reliable diagnosis of myocarditis in EMB. The diagnosis of myocarditis was established in 27 patients according to routine staining of EMB samples. We analyzed those samples using antibodies to CLA, κ and λ immunoglobulin light chains and T cell receptor (TCR). Only 14 out of the 27 biopsies showed to have true myocarditis [8]. The technique proved to be useful for diagnosis of myocarditis as a cause of sudden death (figure 9) [30].

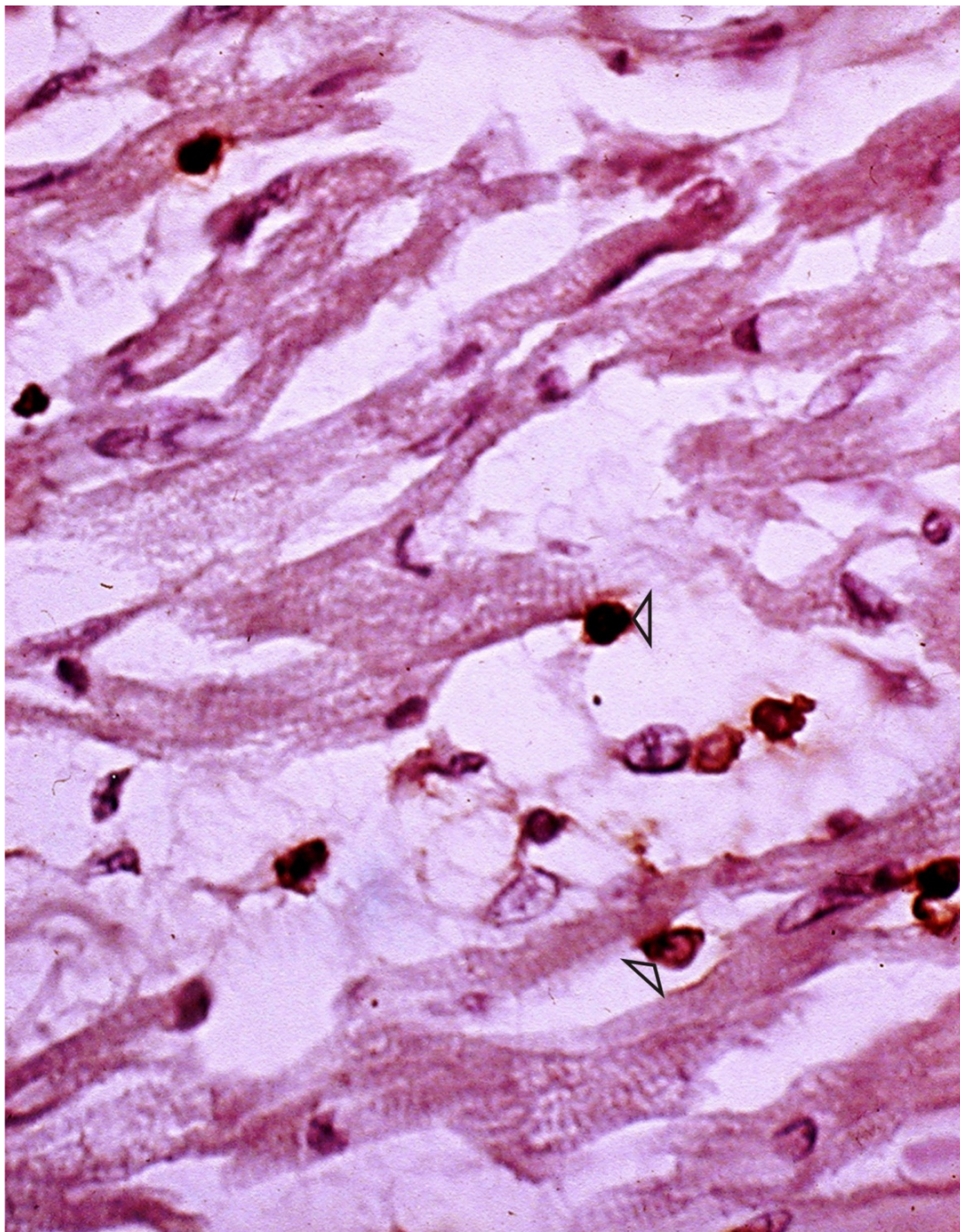


Figure 9. Diffuse myocarditis in a 6 year-old boy found underwater in a swimming pool. There are extensive myocardial injury and marked interstitial edema and apposition of T- lymphocytes to the sarcolemma of necrotic myocytes. Immunoperoxidase for T- lymphocytes. Note the classic picnotic nuclei and cytoplasmic positivity (arrows) X200 [30].

After these papers, new markers and new antibodies have been developed and IHC diagnosis has become more sophisticated. Kühl et al. studied the biopsies of 170 patients with DCM with no history of previous viral disease. EMB were performed and processed for H&E to determine the presence of myocarditis according to Dallas criteria, and for immunohistochemistry using antibodies to CD45RA, CD2, CD3, CD4, CD8, CD45R0 and HLA class I. Only 5% of samples

were positive for Dallas criteria, but 48% showed positive staining for one or more of the antibodies, showing a very higher sensitivity of immunohistochemistry to show inflammatory changes in DCM [29].

Feeley et al. showed that antibodies anti CD45R0 were very accurate for the diagnosis of myocardial inflammation in a series of 163 routine autopsies in a general hospital. The only 5 samples that showed more than 14 CD45R0 positive cells per high power field belonged to transplanted patients, of whom three with cardiac rejection and one with a lymphoproliferative disorder [30]. Although not designed to study myocarditis, Krous et al. showed that staining with anti CD3 (T lymphocytes) and CD68 (macrophages) was useful to differentiate myocarditis from sudden infant death syndrome and suffocation in EMB of children [31]. And as previously reported, in our hands immunohistochemical staining allowed the diagnosis of unapparent myocarditis as a cause of sudden death in children [32].

In a paper by Caforio et al. immunohistochemistry has been used to reinforce Dallas criteria. More than half of borderline myocarditis diagnosis would have been missed with H&E alone [6]. In this connection, also Kindermann et al showed in their study that only 69 (38%) out of 181 EMB samples were positive for Dallas criteria while 91 (50%) were positive using CD3, CD68 and HLA class II antibodies [7].

5.3. The role of polymerase chain reaction

In the early 1990s many authors published series of cases showing the isolation of different viral genomes with PCR [33-37], but these papers were mainly descriptive of the presence of certain types of viruses in EMB samples and did not assess prognostic or therapeutic value of these findings. However, almost a decade after PCR also proved to be of prognostic value [36]. Frustaci et al. treated 41 patients with biopsy proven myocarditis who presented with ongoing heart failure with complete standard immunosuppressant treatment. Viral genomes were present in biopsy specimens of 17 non responders (85%), including enterovirus (n=5), Epstein-Barr virus (n=5) adenovirus (n=4), both adenovirus and enterovirus (n=1), influenza A virus (n=1), parvovirus-B19 (n=1), and in 3 responders, who were all positive for hepatitis C virus. Cardiac autoantibodies were present in 19 responders (90%) and in none of the nonresponders. The presence of viral genomes was independently associated with failure of immunosuppression to improve ventricular function [38]. Conversely, Camargo et al. demonstrated that children with chronic myocarditis have a favorable response to immunosuppressant therapy independently of the presence or not of viral genomes in EMB [39].

Kytö et al. showed in a retrospective analysis of autopsies of 40 fatal myocarditis that viral nucleic acids were found in the hearts of 17 patients (43%), including CMV (15 patients), parvovirus B19 (4 patients), enterovirus (1 patient), and human herpes virus 6 (1 patient). In 4 patients, CMV DNA was found in addition to parvovirus B19 or enterovirus genomes. No adenoviruses, rhinoviruses, or influenza viruses were detected in that study of fatal myocarditis. In 67% of the patients in whom PCR was positive for CMV, *in situ* hybridization revealed viral DNA in cardiomyocytes. Only 1 of these patients was immunocompromised. From these findings it can be concluded that the finding of CMV genome in EMB biopsies of patients with myocarditis carries a particularly bad prognosis [40].

Wilmot et al. also demonstrated the prognostic value of PCR in fulminant myocarditis in 16 children treated with mechanical circulatory support. PCR results were available from 15 patients and were positive in 11. Viral presence was associated with death or need for transplantation ($P = 0.011$). Upon histological analysis, absence of viral infection and lack of myocardial inflammation were associated with recovery (P values 0.011 and 0.044, respectively) [41].

Mavrogeni et al. followed a cohort of 85 patients with myocarditis. In 71 patients CRM was positive and in 50 EMB was performed. Chlamydia, herpes virus and parvovirus B19 were present in 80 % of EMB samples. In 7 patients with clinical deterioration 1 year after, EMB showed persistence of infectious agent genomes [42].

Viral myocarditis is a known cause of sudden death. In this connection, PCR has been performed in post-mortem samples of patients with sudden death. The test proved to be of diagnostic usefulness in some cases [43, 44].

6. Endomyocardial biopsy as a research tool

The role of EMB as a research tool cannot be undervalued. Almost all papers cited in this chapter have been conducted on EMB samples. Many developments relative to heart disease are due to basic science investigations using EMB. In this regard, many advances in the understanding of genetic expression in the failing heart have been made thanks to the possibility of obtaining heart muscle samples [45-48].

In the specific field of myocarditis, EMB will surely allow to identify better predictors of mortality, need of transplantation and response to certain drugs or therapeutic strategies by the discover of new molecular markers of inflammation, tissue damage or survival. With PCR the prognostic value of viral genome presence will be better defined promptly and, in the future, the expression of certain myocyte genes will surely introduce a new tool to predict outcomes.

7. Conclusions

As shown by the data revised here, EMB is an important diagnostic tool in myocarditis. It still remains the gold standard for the definite diagnosis. Dallas criteria, although severely questioned by many authors, still remain a reference method to establish diagnosis and are generally required as inclusion criteria in clinical investigation. On the other hand, it helps distinguishing lymphocytic myocarditis from other entities, like giant cell myocarditis, necrotizing eosinophilic myocarditis or sarcoidosis, which may guide treatment and prognosis.

The introduction of IHC and PCR provided new tools for evaluating EMB samples. Although not yet standardized adequately, they have shown to give valuable prognostic and therapeutic information. They have become routine testing in myocarditis.

Author details

Julián González¹, Francisco Salgado¹, Francisco Azzato¹, Giuseppe Ambrosio² and Jose Milei¹

¹ Instituto de Investigaciones Cardiológicas Prof. A. Taquini – UBA – CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina

² University of Perugia School of Medicine, Perugia, Italy

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