Endomiokardna biopsija: juče, danas i sutra

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Apstrakt

Glavni ciljevi u ovom radu su bili da se prikaže aktuelno znanje o primarnim bolestima srčanog mišića, kardiomiopatijama (KMP), da se da njihova klasifikacija, dijagnostičke mogućnosti endomiokardne biopsije (EMB), da se utvrdi vrednost EMB u izboru najbolje terapije. Uz mnoge kontroverze, EMB se danas smatra široko prihvaćenom metodom, sa malo komplikacija, i izvodi se rutinski u mnogim kardiološkim centrima. Iako smo mi otpočeli analizu EMB još u osamdesetim godinama, danas se EMB u Beogradu relativno retko radi. Jako je teško dati odgovor – zašto?

KMP se dele na dve osnovne grupe, prema funkcionalnim i strukturalnim, patološkim karakteristikama, na idiopatske i specifične, odnosno na primarne i sekundarne. Idiopatska grupa se zatim deli na 5 podgrupa: hipertrofičnu, dilatacionu, restriktivnu, aritmogenu KMP desne komore, i neklasifikovane KMP. Specifične KMP se dele na 8 podgrupa, baziranih na etiopatogenetskim karakteristikama. Na infektivne, sa virusnim miokarditisom (VMK) kao najčešćim entitetom, na metabolične, uključujući i endokrine poremećaje i bolesti nakupljanja. Defekti u deficitu oligoelemenata i vitamina, zahvaćenost srca u bolestima vezivnog tkiva, kao i granulomi i neoplazme takađe spadaju u ove grupe oboljenja. Poremećaji (pre)osetljivosti i toksične reakcije, sa dugim nizom substanci koje mogu da utiču na srce, su vrlo česte u današnjoj patologiji. Na kraju, u poslednju grupu bi spadala oštećenja srca nastala u sklopu različitih sistemskih sindroma, ipak relativno retkih.

EMB je u svetu u znatnom porastu primene, posebno u dijagnostici akutnog odbacivanja kod srčane trnasplantacije, dijagnostici KMP, posebno VMK, sa malom stopom komplikacija, te se smatra, efikasnom, korisnom i sigurnom procedurom.

Ključne reči: endomiokardna biopsija, primarna bolest srčanog mišića, kardiomiopatija, dijagnoze, lečenje, immunomodulation, ljudi

Endomyocardial biopsy: yesterday, today and tomorrow

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Abstract

The major goals of this presentation are to give the updated knowledge of primary heart muscle disease, cardiomyopathies (CMP), their classification, the diagnostic possibilities using endomyocardial biopsy (EMB) and to estimate the value of EMB in the choice of the right therapeutic approach. Despite many controversies, EMB is today a widely accepted method, with low percentage of complications for analysing CMPs, and is considered to be a routine procedure in many cardiological centers. Inspite the fact that we started with the use of EMB in 80's, it is not performed often today in Belgrade, like many yeras ago [(2)]. It's difficult to say why ?

The CMPs may be subdivided, according to the functional and structural features, into two groups: idiopathic and specific, or primary and secundary. Idipathic group is consisted of 5 subgroups: hypertrophic, dilated, restrictive, arrithmogenic right ventricular CMP and unclassified.

Specific CMPs may be subdivided into 8 groups mainly based on etiopathogenetic characteristics: infective, with viral myocarditis as the most common entity, metabolic, including endocrine disorders and infiltration and storage diseases. Deficiency disorders and heart involvement in connective tissue disorders are also included. Granulomas, neoplasms and neuromuscular disorders are also wery often presented with cardiac disfunction and structural abnormalities. Sensitivity and toxic reactions with long list of substances wich may affect the heart are probably the most present today. Finally, the last group represent miscellaneous systemic syndromes with heart affection.

EMB has been increasingly used in the diagnosis of CMPs, with special influnce on diagnosis of heart transplant rejection, myocarditis, treatment modalities of different types of myocarditis, with low complication rate, considered effective, usefull and safe procedure.

Key words: endomyocardial biopsy, primary heart muscle disease, cardiomyopathy, diagnosis, treatment, immunomodulation, human

Introduction

The major goals of this presentation are to give the updated knowledge of primary heart muscle disease, of diagnostic possibilities using endomyocardial biopsy (EMB) and to estimate the value of EMB in the choice of the right therapeutic approach. Despite many controversies, EMB is today a widely accepted method, with low percentage of complications for analysing cardiomyopathies (CMPs), and is considered to be a routine procedure in many cardiological centers [1]. Inspite the fact that we started with the use of EMB in 80's, it is not performed often today in Belgrade, like many yeras ago [2]. It's difficult to say why?

INDICATIONS OF EMB

Although the technique of EMB was introduced in the early 1960`s for diagnosing myocarditis and primary myocardial diseases (Sakakibara and Konno [3], it became popular in the 1970 when "Stanford technique" was introduced for evaluation of cardiac rejection. Unlike King`s College bioptome technique [4], where left ventricular biopsy is usually performed [5], Caves-Schultz or Stanford bioptome uses percutanous approach through the right internal jugular vein. Several biopsy specimens can be obtained, and possible frozen section for immunohistochemical analysis could be done.

Controversial issues concerning EMB and its clinical indications could be found in the literature [6-8]. It is universally agreed that the value of EMB in the diagnosis and management of cardiac allograft rejection is well established, in the assessment of anthracycline cardiotoxicity, and myocarditis and secondary myocardial diseases can be readily diagnosed by EMB. Today, there is little dispute about main indications and contraindications for EMB, listed in Tables 1 and 2.

Evaluation of cardiac allograft rejection
Monitoring anthracycline cardiotoxicity
Diagnosis of inflammatory myocarditis
Distinction between restrictive and constrictive heart disease
Diagnosis of specific cadiomyopathies (storage diseases, etc.)
Diagnosis of neoplasm (primary and metastatic)
Idiopathic chest pain
Idiopathic arrhythmia
Idiopathic cardiomyopathies

Table 1. Main indications for EMB

The EMB may provide confirmatory morphologic data in many idiopathic or specific CMPs. While right ventricular biopsy is easier to perform and likely to make the diagnosis, there may be specific indications for left EMB.

The contraindications to EMB are few. Bleeding disorders are the most common contraindication. The presence of intraventricular mural thrombus, if involving the left ventricle, may predispose to systemic embolisation, and intracardiac shunts may carry a risk of paradoxical systemic embolisation [9]. Prior myocardial infarction and arrhythmogenic right ventricular CMP may also carry a risk. EMB is a technique that can be mastered by a cardiologist with basic cardiac catheterization training. The equipment is standard, and multiple

sequential biopsies can be obtained from a single patient, the technique is safer and has fewer serious complications than conventional percutaneous liver or kidney biopsies.

EMB EVALUATION OF CARDIAC ALLOGRAFT REJECTION

Two major problems arise following successful cardiac transplantation: acute rejection and infections, due to immunosuppression treatment. Untreated, of unnecessary overtreated, acute rejection in heart transplant patients will frequently result in patient dearth or his marked disability. Therefore, much attention has been paid to monitor the degree of cardiac allograft rejection and thereby to determine the most appropriate immuno-suppression regimen, especially if myocarditis was present before the transplant [10].

Many different techniques have been used to evaluate the evidence of rejection, such as: electrocardiographic monitoring, echocardiography radioactively labelled different cells or isotopes, magnetic resonance imaging, etc, So far, none of these techniques have shown to be sensitive enough to replace endomyocardial biopsy (EMB). Histologic examination of cardiac tissue samples obtained by EMB remains the most effective method for surveillance of cardiac rejection in heart transplant patients. Electron microscopy is not very useful in the diagnosis of acute cardiac rejection, because longer processing time is required for this method. However, electron microscopy or immuno-electronmicroscopy may be useful in the research field, for better understanding the mechanisms of myocyte injury, and in identifying cells involved in the rejection process.

Histologic grading systems for diagnosing cardiac allograft rejection Two major systems were in everyday use to measure cardiac allograft rejection: qualitative, or Stanford system [11], introduced by M. Billingham, and quantitative or THI (Texas Heart Institute), system uses the terms mild, moderate and severe, in order to describe the degree of acute rejection. The THI system is based on numerical scale, ranging from 0 to 10, and describe the degree of rejection by assigning to it a numerical value[12]. A comparison of the THI and Stanford grading systems is illustrated on Table 2. The other important difference between the two systems is that Stanford system uses, in addition to the amount of mononuclear cells, myocyte necrosis as a criterion for distinguishing between moderate and severe rejection. The THI system is based on findings of intensity of myocyte degeneration as the criterion to evaluate the degree of rejection. True necrosis is rarely observed in rejection process except in high grades of rejection and degeneration is usually reversible if the patient is appropriately treated.

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Table 10. Comparison of the THI and Stanford grading system of rejection

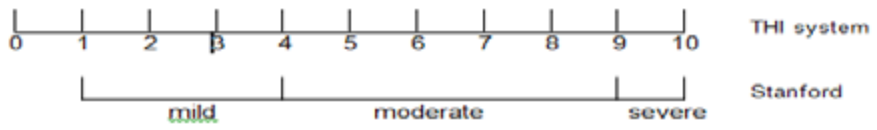

Table 2. Comparison of the THI and Stanford grading system of rejection

A modification of the Stanford classification was proposed by Kemnitz and colleagues in so-called "Hannover classification". The main difference is subdivision of the mild acute rejection and introduction of early and late resolution of rejection [13]. However, this combination of quantitative and qualitative grading system has not been widely accepted.

Grade	New nomenclature	Old nomenclature
0	No rejection	No rejection
1	A - Focal infiltrate without necrosis, Perivascular or intersticial B-Diffuse infiltrate without necrosis	mild rejection
2	One focus with infiltration and/or focal Miocene damage	"Focal", moderate rejection
3	A - Multifocal infiltration and/or myocyte damage B - Diffuse inflammatory process with necrosis	"mild" moderate "Border-line" severe
4	Diffuse polimorphonuclear infiltrate with oedema, haemorrhage and necrosis of cardiomyocytes	Severe acute rejection

Resolving - rejection in phase of diminution, presented with one smaller number
Resolved - finished rejection, presented with grade 0.
(Modified from: International Society for Heart Transplantation)
Table 3. Standardisation of classification of cardiac allograft rejection

Histologic findings corresponding to the numerical value of 0 (THI system) indicated that there is no evidence of cardiac rejection. A grade of 1 and 2 indicates only perivascular aggregates of mononuclear cells. Grade 3 indicates that mononuclear cells are extending into the interstitium. Grade 4 do 8 represent presence of interstitial mononuclear cells with cardiac myocyte degeneration of different, increasing severity cardiac myocyte degeneration of different, increasing severity. Grade 4 represents occasional myocyte degeneration; grades 5 and 6 – scattered myocyte degeneration and grades 7 and 8 signify multifocal degeneration (present in every piece and in all high-power fields).

Maintenance of the cardiac transplant patients

EMBs are performed in heart transplant patients with frequency depending on patient status, level of immunosuppressive agents, and time of previous biopsy. By correlation of the numerical values and date of the patients previous EMB, we can estimate: the degree of rejection, the direction of change (resolving or progressing), and the speed of change [14].

Using the THI grading system [12], we can avoid extensive immunosuppression treatment of hear transplant patients when impressive cellular infiltrate is present with no evidence of marked degeneration. Or, we can introduce higher levels of immunosuppressive agents in patients with multifocal myocyte degeneration and clinical signs of rejection with relatively few mononuclear cells. This change of levels of immunosuppressive treatment is practically impossible under diagnosis of "moderate" rejection.

Other factors of cardiac allograft damage can also be detected by EMB. These include: increased graft eosinophilia as sensitive indicator of severe graft rejection, eosinophilic coronary arteritis without classical lymphocytic allograft rejection, occlusive coronary arteritis as consequence of cyclosporine immunosuppression, and evidence of ischemic damage of myocyte in patients with longer survival and present coronary artery disease. Finally, by EMB is possible to identify toxoplasmosis, cytomegalic viral inclusions, fungi, leishmania, and coccidioidomycosis. It is difficult to clinically diagnose these affections of the myocardium. All these conditions are treatable, which support the use of EMB in maintenance of cardiac transplant patients.

EMB DIAGNOSIS OF CARDIOMYOPATHIES

Idiopathic cardiomyopathies are heart muscle diseases of unknown aetiology (unaccompanied by any other disease process in the body [15]. Different classification have been proposed [16-19]. The so-called "secondary

cardiomyopathies" of known cause are often accompanied or preceded by disease process elsewhere in the body. We are calling them today - specific cardiomyopathies [16-19], besides familial group, how are also idiopathic [20].

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is another entity with many previous names, definitions, and clinical presentations [21, 22] . Hypertrophhic obstructive CMP, the so-called HOCM, implies that the obstructive element is an essential part of the manifestations. Typical clinical presentation is (1) abnormal stiffness of the hypertrophied ventricular muscle with impaired distensibility of the left ventricle; (2) a pressure gradient in systole between the outflow of the left ventricle and the aorta (usually over 60 mmHg and 70 –80 % of patients). Therefore, HCM without obstruction is well-recognised condition with signs confined to a powerful left ventricle and atria thrust and evidence of pulmonary hypertention. Asymmetrical apical hypertrophy is another condition recognised by ECG and echocardiography.

In classical HCM, macroscopically, there is bulging, extensive hypertrophy of the left ventricle, especially in the septum of the outflow tract, giving rise to the pressure gradient between the left ventricle and aorta, usually exuding 60 mmHg. This is the most common picture of HCM, also called "subaortic muscular stenosis" and once classified as congenital heart disease. A genetic basis (autosomal dominant trait with almost complete penetrance) has been established, with sequelae on catecholamin function and abnormal muscle fibre alignment (disarray).

Histological examination shows short myocardial fibres running in all directions (disarray), and often forming small whorls. Severe hypertrophy of individual myocardial fibres, which often measure 90 -100 μ in diameter is striking (normal range is 5 -12 μ, average cell diameter in hypertrophy is about 22-25μ). Additional histological features include: large bizarre-shaped nuclei, each surrounded by a clear zone, the so-called "perinuclear halo". Various amounts of interstitial fibrosis, often apparently interrupting short myocardial fibres, are seen.

Only the combination of the abnormal features (detailed above) is highly characteristic of HCM and permit diagnosis [23]. The so-called "histological HOCM index" is applied and values represent semiquantitative grading system (values 1-3 for each of the 5 histological features - hypertrophy, disarray, bizarre nuclei with perinuclear halo, short runs fibres and fibrosis). If the values over 50% of the maximal 15 are obtained, the PH diagnosis of HCM is confirmed [24, 25].

HCM without obstruction has PH, histochemical and EM changes similar to those already presented, and on EMB findings it is impossible to distinguish these to conditions. In all, the use of EMB in diagnosis of HCM is restricted by non-pathognomonic findings, absence of EM or enzymatic differences [(22)], and should be used only in combination with clinical data. Probably, the main value of EMB in HCM is to rollout other possible conditions with similar clinical impairment (amyloidosis, glicogenosis, small vessel diseases, etc.).

Dilated (congestive) cardiomyopathy

This world wide distributed cardiac condition have as main clinical sign congestive heart failure which may rapidly progress to end-stage heart disease. Today, among different heart transplant candidates, post-viral DCM is the leading condition [27-29].

Macroscopically non-specific features are found. The weight of the heart is around 600-700g with hypertrophy of myocardial wall, but all cardiac chambers are severely dilated and often masking the degree of hypertrophy. The parietal thrombus is often found. The myocardium is pale and flabby, and the coronary arteries are usually normal and patent [26].

Histologicaly, EMB findings in DCM shows changes that are non-specific, reflecting changes of hypertrophy with various degrees of degeneration and fibroses. The EMB diagnosis can be made only by exclusion of other specific histiological features (MC), and of other causes that may lead to heart failure. Although

a multifactorial aetiology was estimated likely, many experimental, epidemiological and new sophisticated pathological methods has shown the strong evidence of linking idiopathic viral myocarditis to DCM. Familial DCM is also a separate entity, with proof fo different gene involvement [20] .

Alcoholic CMP or alcohol induced heart disease, has no consistent features, and distinction from DCM is usually impossible on histologic level. Only enzymatic differences between two groups of patients have been observed by Richardson et al.[30] They found that thining and atrophy of the cardiomyocytes, with fatty infiltration, EM characteristics of dilatation of the sarcoplasmic reticulum, lipids, mitochondriosis and increased number of lysosome, followed by decreases in mitochondrial enzyme activity studied by enzyme histochemistry, represent the changes specific for alcoholic CMP.

Similarly, congestive heart failure associated with pregnancy, in so-called peripartum cardiomyopathy (PPCMP), cannot be distinguished pathologically from DCM [31]. PPCMP is defined as congestive heart failure in the last trimester of pregnancy or within the first six months postpartum. Although positive etiological connection with different factors have been established (viruses, toxoplasmosis, malnutrition, hypertension, immunological mechanism, race, number of pregnancies, etc.), unknown multifactorial aetiology is the most likely [32]. Higher incidence of MC in cases of PPCMP suggests that this condition is a separate entity, and similar relation with post-myocardial DCM [31,32].

Restrictive cardiomyopathy

Although a possible association between eosinophilia, endomyocardial disease and adherent thrombi had been suggested in the late 1800s, it was Loffler in 1936 who described two Swiss patients with hronic heart failure and marked eosinophilia [33]. Latter, this entity was recognised under variety of names: Loffler`s endocarditis parietalis fibroplastica, hypereosinophilic syndrome, endomyocardial fibrosis with eosinophilia, disseminated eosinophilic collagen disease, etc. and similarities with tropical endomyocardial fibrosis (EMF) was recognised.

These two conditions give very similar clinical picture that may resemble constrictive pericarditis or mitral insufficiency with pulmonary hypertension, but any hypothesis of common aetiology has to reconcile the following: myocardial damage has been reported in cases with increased eosinophilia other than Loffler (filariasis, trichinosis, acute leukaemias); some patients with long standing eosinophilia may have no evidence of EMF; a form of CMP similar to Loffler`s disease has been described in pts without eosinophilia. Brockington and Olsen have suggested that acquired eosinophilia (including the idiopathic hypereosinophilic syndrome and eosinophilic leukaemia) lead to Loffler's endocarditis, and finally endomyocardial fibrosis [34]. These two entities belong to the same disease process with evidence that a large proportion of circulating eosinophils are abnormal with reduced numbers of crystalloid granules. The degranulation of eosinophils is producing MC with nospecific fibrosis at the end of the process.

The unitarian hypothesis that "the presence of an eosinophilic leucocytosis, in a susceptible person, by some not completely explained mechanism, causes endomyocardial damage", is very likely. The cardiac damage is not dependent on the number of circulating eosinophils but on whether they are normal or they might be releasing their cationic proteins – potentially cardiotoxic agents. Degranulated eosinophils with individual immunological abnormalities are the basis for pathologycal changes in "eosinophilic heart disease" (EHD), clinically manifested as RCM. Finally, there are opinions that two form of RCM can be found: one without eosinophilia, with tipical findings of EMF and called idiopathic RCM, and the other form associated with eosinophilia and named EHD [35].

Macroscopically, the distribution of the thickened endocardium may vary and may involve the left, right or both ventricles. The endocardium may be several mm thick, and thrombus is superimposed in about 50% of patients. Fibrous septa, extending for a short distance into the underlying myocardium, are common. The cardiovascular involvement are shown on Table 4.

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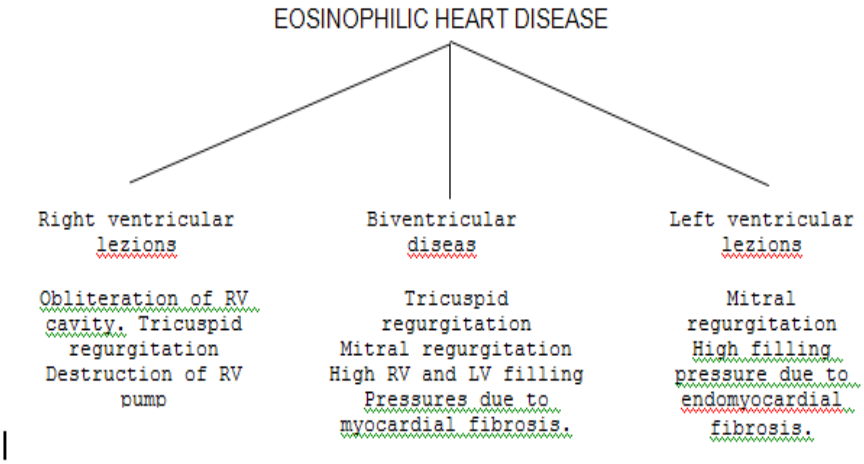

Table 3 : Cardiovascular findings in EHD

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Table 4. Cardiovascular findings in EHD

zone consisted of hyaline collagen. The middle layer is occupied by fibrous tissue and the deepest layer is the "granulation tissue layer", with variable number of eosinophils. The myocardial fibres between the septa may show degenerative changes, like in other CMPs.

The disease passes through 3 stages: first, acute, necrotic phase with inflammatory reaction and variable number of eosinophils. Interval between onset of symptoms and potential death are measured in weeks. The second stage is characterised by thrombus formation and represents the complication of eosinophilic endomyocarditis. There is prominent fibrous endocardial thickening, and arteritis may be still present. Interval between onset of symptoms and death are measured in months. The third, fibrotic stage represent the last phase with fibrosis and septa made of superficial layer of hyaline collagen, middle layer of fibrous tissue, and deepest layer of chronic inflammatory cell with varying number of eosinophils. These three pathological patterns of disease are one continious process connected with length of time between onset of disease and death.

EMB diagnosis of EHD is of great importance, especially in children [36]. Depending of the time of the biopsy, the possibility of founding degranulated eosinophils, thrombus, and MC, makes the EMB fundamental techniques in diagnosing this condition [37].

Arrhythmogenic Right Ventricular Cardiomyopathy

This entity is characterised by progressive fibrous-fatty replacement of right ventricular myocardium, mainly the free wall, followed by rhythm disturbances as major clinical problem. Initially, the changes are consistent with regional, and later global, right and some left ventricular involvement, and with relative sparing of the septum.

Although familial occurence has been documented and a gene defect was recently localized on chromosome 14q23-q24, the etiopathogenesis of the disease is still obscure. Familial form of arrhythmogenic right ventricular cardiomyopathy (ARVC) disease is very common, with autosomal dominant inheritance and

incomplete penetrance, although a recessive form is also described. Presentation with arrhythmia and sudden death is also very common, particularly in the young and athletes, as indicated by Thiene et al [38]. Contrary to the genetic form, some recent clinical and morphological findings demonstrated its acquired, progressive, frequently inflammatory character, and supports the notion of chronic, continuous process of injury and repair. Several hypotheses are made upon pathogenesis of ARVC, with idiopathic myocarditis as the most probable cause (Fontaine, Hofman, et al.).

The findings of adipose tissue in the heart is not allways the indication of pathologic process, and frequency, extent, and distribution of endomyocardial adipose tissue was analysed by morphomety from EMBs in 241 patients, indicating the particularities of adipose replacement in ARVC. It should be noted that pathologic evidence of extensive left ventricular involvement in ARVC was demonstrated. Finally, as possible causes of ARVC apoptosis was implicated, showing how normal and abnormal consequences of apoptosis can lead from postnatal morphogenesis to paroxysmal arrhythmias[39]. The frequent findings of MC with myocyte death lead to the consideration of the disease as chronic MC, with some immune factors involved.

The EMB diagnosis of ARVC should be performed with coution of perforation, and maneuver under echocardiographic guide is recommended. The usual site for EMB, the interventricular septum, is not the ideal location for biopsy. Unother problem is the fibrous-fatty atrophy and its pahtgnomonity for definitive diagnosis. Adipose and fibrous tissue in the myocardiun is not so specific findings, interstitial replacement is also often observed in many CMPs, so the issue is to quantify the components, rather then qualify them. As suggested by Angelini et al., the following diagnostic parameters should be obtained by morphometric analysis: myocardial atrophy with residual myocytes less then 45%; fobrous tissue less then 40%; and fatty tissue more then 3%. The sensitivity and specificity were 67% and)”%, respectively. This quantitative histologic criteria give better definitive diagnosis by tissue characterization then magnetic resonance imaging or other noninvasive procedures [38].

Unclassified Cardiomyopathies

Unclassified CMPs incised a few cases that do not fit readily into any group, like fibroelastosis, noncompacted myocardium, systolic dysfunction with minimal dilatation, mitochondrial disorders in myocardial involvement, etc.

Some of CMPs may present with features of more than one type of CMP (eg. amyloidosis, systemic hypertention), and overlapping morphology is even more common (end-stage HCM with dilatation, peripartum CMP, alcohol induced heart disease, and other entities similar with DCM).

It is recognised that arrhythmias and conduction tissue diseases may be primary myocardial disorders, but, at this time, they are not included as cardiomyopathies. They remain as one of the frequent indications for EMB (mainly to rule-out MC), but as many conditions may present with rrhythm disturbances, they are not a distinctive entity.

6. EMB DIAGNOSIS OF SPECIFIC CARDIOMYOPATHIES

Specific cardiomyopathies (SCMP) may be defined as “secondary CMPs” or heart muscle disease of known cause, associated with recognised general disorders. These conditions have been classified by many authorities in this field [(15-19)], but our approach to the classification of SCMP is mainly based on EMB findings, indicating three main groups of diseases: first, in which the pathognomonic EMB features are seen, that allows the morpho diagnosis of “specific entity”; second group of diseases in which “specific” morphological features can not be demonstrated, and the diagnosis is made by exclusion [40] .

In spite of this luck of “morphological specificity”, many general condition in which the heart can be affected are classified under SCMP. We strongly believe that division of these two groups, based on possibility of EMB diagnosis, is the most appropriate way to classify, diagnose and treat SCMP.

Entities with pathognomonic PH findings
1. Infective diseases (MC)
2. Metabolic (familial storage and endocrine diseases)
3. Infiltrations, granulomas and neoplasia
4. Sensibility and toxic reactions (adriamycin)
Entities with non-pathognomonic PH findings
5. Connective tissue disorders
6. Heredofamilial neuromuscular diseases
7. Deficiencies and avitaminoses
8. Rare syndromes

Table 5. Classification of Specific CMPs (specific heart muscle diseases).

Besides infective diseases (viral, bacterial, fungal, protozoal, etc.), with specific or pathognomonic findings, many storage diseases (glicogenoses, haemochromatosis, amyloidosis, etc.) can be diagnosed by EMB. All otter specific CMPs have less pathogno-monic findings and only clinical characteristics makes them "specific" heart disease [33].

Glycogen storage disease

Of the twelve types of glycogen storage disease, the heart is involved in three: type II (Pompe's disease), deficiency of alpha-4,4-glucosidase (acid maltase); type III (Cori's disease), a deficiency of the debranching enzyme amylo-1,6-glucosidae; and type IV (Andersen's disease), caused by a deficiency of the branching enzyme alpha-1,4-glucan-6 glucosyltransferase. These diseases are transmitted as autosomal recessives and are manifest by accumulation of glycogen in various tissues [41]. The diagnosis should be based not only on the demonstration of increased glycogen, but also by demonstration of the enzyme defect. Most cases of glycogen storage disease, causing cardiomegaly, are due to type II glycogenosis. The heart is enlarged, and all chambers have thickened walls and small cavities. Progressive impairment of myocardial function ensues, and Pompe,s disease is fatal within the first year of life. Death is due to cardiac failure or respiratory complications. Grossly, the heart appears rubbery and pale pink. There may be fibroelastotic thickening of the endocardium. In histologic sections there will be severe vacuolisation within the central areas of the myocytes, giving a lacework appearance to the tissue, due to massive deposits of glycogen which displace myofibrils to the periphery. Myofibrillar loss related to cardiac failure can also be demonstrated ultrastructurally. If a glycogen storage disease is suspected, the endomyocardial biopsy can be fixed in absolute alcohol tryed in preserving the glycogen, and electron microscopy is usually required as well [(42)]. The characteristic ultrastructural alteration is large collections of glycogen (either free in the cytoplasm in Pompe's disease, or within lysosomes). The glycogen may be in a morpholo-gical form, granules (type II and III) or in an abnormal form (fibriles, in type IV glycogenosis).

Fabry's Disease

Fabry's disease (angiokeratoma corporis diffusum universale) is an X-linked recessive disorder caused by deficiency of lysosomal alpha-galactosidase A, resulting in excessive deposits of ceramide trihexoside, particularly in the skin, cornea, kidneys, and heart. It is manifest by angiokeratomas, pain and paresthesias of the extremities, and progressive renal and cardiovascular disease [43]. Symptoms relative to the heart include cardiac hypertrophy and dilatation, congestive heart failure, angina, and hypertension, all of which are due to

deposits of ceramide trihexoside in lysosomes in endothelial cells, smooth muscle and pericytes throughout the vascular system. Because of these deposits, patients may have myocardial infarction at an early age, microaneurysms, cystic medial necrosis of the aorta, and valvular lesions (mitral stenosis, aortic regurgitation, pulmonic regurgitation). Death is often from congestive heart failure.

Endomyocardial biopsy from patients with Fabry›s disease will demonstrate a lacework appearance of the myocardial fibres on routine light microscopy, with marked perinuclear vacuolisation and displacement of the contractile elements to the periphery. In frozen sections, the deposits of ceramide trihexoside appear as vacuoles, which are sudanophilic and PAS-positive. By electron microscopy, these deposits appear as intralysosomal aggregates of concentric or parallel lamellae, which stain positively by ultrastructural techniques that demonstrate cardohydrates [44].

Endomyocardial biopsy may be helpful in making the diagnosis of Fabry›s disease, particularly in cases not recognised in childhood, or in patients who lack the usual signs and symptoms (i.e., proteinuria, corneal apacities).

Cardiac Amyloidosis

Amyloidosis describes disease processes, which are characterised by extracellular deposits of proteins, which have a beta-pleated sheet conformation. Current classification is based on the biochemistry of the amyloid fibril, and who major groups are recognised: AL, in which fibrils consist of light chains of immunoglobulin, and AA, in which the fibrils consist of fragments of serum amyloid A protein. A third group, in which the fibrils are comprised predominantly of prealbumin and which occurs in senile cardiac amyloidosis and familial polyneuropathy. The AL form is associated with plasma cell dyscrasias or can occur as localised deposits without evidence of generalised involvement, while AA often occurs with inflammatory processes, such as rheumatoid arthritis or chronic infection. Amyloid deposits can be found in the heart in elderly people or as part of generalised amyloidosis. Senile cardiac amyloid has been reported in 30-69% of patients older than 60 years, while 54-90% of patients with generalised amyloidosis (usually AL associated with multiple myeloma, or less often AA) have cardiac involvement [45].

Depending upon its origin and extent, cardiac amyloidosis may be asymptomatic, or it may cause progressive heart failure and refractory arrhythmia. Clinically significant cardiac amyloidosis must be differentiated from constrictive pericarditis, hypertrophic cardiomyopathy, storage diseases, or other infiltrative myocardial diseases. It can also manifest as ischemic heart disease, with typical or atypical angina and a “pseudoinfarct” pattern on ECG, and it may produce arrhythmias or conduction defects. Cardiac amyloidosis produces a stiff myocardium, which impedes diastolic ventricular filling and creates restrictive hemodynamics.

In clinically significant cardiac amyloidosis, the heart may be heavy and the walls are thickened, firm, and have a pale colour and rubbery consistency. Amyloid deposits occur in the interstitium, conduction tissue, valves, endocardium, pericardium, and in small intramural arteries, veins and capillaries. In the myocardium, amyloid may be found surrounding individual myocytes, or in the form of focal nodular interstitial deposits that push aside and replace fibres, or both. Myocardial fibrosis may be assesed by different methods [46] In coronary vessels, it may involve all vascular layers and even cause luminal occlusion. The presence of amyloid can be confirmed by the apple green birefringence it gives under polarised light after Congo red staining, or by metachromasis with methyl violet, or ultraviolet fluorescence with thioflavin T (the latter may yield false positives). Amyloid fibrils can also be identified by their characteristic ultrastructural appearance as 7.5 nm diameter nonbranching fibrils. If clinically indicated, immunohistochemical typing of amyloid can be done using paraffin embedded sections with antisera against purified amyloid fibril protein and monoclonal antibodies against protein AA.

In patients suspected of having cardiac amyloidosis, an endomyocardial biopsy cannot be substituted by biopsy of another site, such as the rectum, as only 60-80% of patients with idiopathic (primary) amyloidosis will have a positive rectal biopsy. At least four biopsy specimens should be obtained to minimise the possibility of a false negative result. While patients with known amyloidosis who have typical echoradiographic

features do not necessarily need to undergo endomyocardial biopsy, cardiac amyloidosis cannot be excluded on the basis of negative biopsies of extracardiac tissues. In patients who have clinically unexplained cardiac disease and suspected amyloidosis, endomyocardial biopsy is a reliable diagnostic procedure.

Cardiac hemochromatosis

Iron deposits occur in the myocardium in idiopathic (familial) hemo-chromatosis as well as in hemosiderosis secondary to iron overload (e.g., multiple transfusions, dietary intake). The clinical manifestations vary, depending on the extent of myocardial involvement, but patients usually have a dilated, or rarely restrictive, cardiomyopathy. Some patients are asymptomatic but may have echocardiographic evidence of myocardial infiltration (increased left ventricular wall thickness). Other abnormalities include ECG changes (ST-segment and T-wave changes), supraventricular arrhythmias, atrioventricular conduction disturbances, and ventricular arhythmias. The severity of myocardial dysfunction may be proportional to the amount of iron present, and extensive deposits are usually associated with congestive heart failure (occurring in one third of patients) which is usually the cause of death [47].

The heart appears brown and may be hypertrophied and/or dilated. Normally, there is no stainable iron within the myocardium. In hemochromatosis/hemosiderosis, iron deposits are more extensive in the epicardial third, intermediate in the inner (subendocardial) third, and least extensive in the middle third of the ventricular wall. They are typically perinuclear in location initially, but eventually occupy most of the cells. Involvement of the conduction system, coronary arteries, and valves in limited. There may be associated fibrosis, in which case restrictive hemodynamics may be present. As hemochromatosis in usually associated with involvement of other organs, EMB is not required for diagnosis. If a biopsy is performed, however, multiple specimens should be obtained to minimise sampling error, since iron deposition may be focal [48].

Cardiac Sarcoidosis

Most patients with cardiac sarcoidosis have clinically apparent systemic sarcoidal involvement, but in some patients the heart may be the primary site, without clinical evidence of other organ involvement. The clinical manifestations are determined by the extent and location of process and may include atrioventricular conduction defects, ventricular arrhythmias, sudden death, congestive heart failure (due to widespread myocardial involvement, ventricular aneurysms, arrhythmias, or cor pulmonale due to pulmonary hypertension), chest pain with or without ECG changes of ischemia or dysfunction of papillary muscles and mitral regurgitation. Pericardial abnormalities (effusion, constrictive pericarditis, tamponade) may also occur.

Cardiac sarcoidosis is a focal disease. Therefore, when endomyocardial biopsy is performed in patients with suspected cardiac involvement, multiple specimens from several sites should be obtained. The predominant sites of myocardial involvement are, in decreasing order of frequency, left ventricular free wall, base on the interventricular septum, right ventricular free wall, and atrial walls. A negative biopsy does not rule out the diagnosis of cardiac sarcoidosis [49]. The lesions in the heart are identical to those described in the lungs, consisting of histiocytes, giant cells, lymphocytes and plasma cells. Patchy fibrosis and lymphocytic myocarditis can also be observed but are nonspecific. Other conditions associated with giant cells in the heart that may need to be distinguished from sarcoid include idiopathic giant cell myocarditis, infective endocarditis, rheumatoid arthritis, Takayasu,s arteritis, and Wegener,s granulomatosis [50].

In summary, sarcoidosis should be suspected in young adults, especially in blacks, who have cardiomyopathy, conduction disturbances or other ECG abnormalities. Although sarcoid involves the heart in only 20% of autopsy-proven cases of sarcoidosis, endomyocardial biopsy may aid in the diagnosis.

Cardiac Tumors

Primary cardiac tumors are rare, ranging in incidence at autopsy from 0.0017 to 0.28%, compared to 1.22% for metastatic cardiac tumors. The most common primary cardiac tumor is myxoma (40%), followed by angiosarcoma and rhabdomyosarcoma, with these three comprising over half of all cardiac tumors. Other primary cardiac tumors include papillary fibroelastoma, fibroma and fibrosarcoma, hemangioma, teratoma, and mesothelioma of the atrioventricular node. Metastatic tumors to the heart include lung carcinoma, hematopoietic-lymphoid neoplasms, and melanomas, with cardiac involvement occurring in about 20% of patients with malignancies. Endomyocardial biopsy has the potential to aid in the diagnosis of primary and secondary cardiac tumors, and there have been several reports in which the diagnosis of an intracardiac tumor was made by left or right ventricular transvenous biopsy [40]. Some patients may benefit from the procedure, which has less discomfort, risk and expense than an open thoracotomy, particularly those in whom the benefit of surgery might be questionable. The risk of tumor embolization is unknown, but could be a potential problem, particularly if the tumor is friable (i.e., myxomas).

Sensitivity and toxic reactions

Anthracycline cardiotoxicity

Anthracyclines, antineoplastic drugs that include doxorubicin (Adriamycin) and daunomycin, are effective chemotherapeutic agents for the treatment of numerous solid and hematopoietic malignancies. Cardiac toxicity is a well-recognised complication and is the doselimiting factor in the use of these drugs. While the usual practice is to administer these agents up to a maximum total dosage of 500-550 mg/m2, some patients may suffer cardiotoxic effects at lower cumulative doses, particularly if they have pre-existing cardiac disease (including hypertension), are greater than 70 years in age, or have received prior irradiation. Prior cyclophosphamide therapy may also potential the cardiotoxic effects of anthracyclines. There is significant individual variation in a patients susceptibility to anthracycline-induced cardiac damage, with the subsequent development of heart failure. Radionuclide ejection fraction is sensitive but cannot differentiate heart failure due to other causes from that due to anthracycline alone, and the drug may be withdrawn prematurely in some patients. For these reasons, serial EMBs have become a reliable method to evaluate anthracycline cardiotoxicity [51]. The degree of myocardial injury can be estimated by histologic grading of biopsy specimens, and the EMB is an effective means to monitor patients.

Anthracycline produces both early and late cardiotoxic effects. The early effects, which can occur after one dose, include a pericarditis-myocarditis syndrome, drug-induced cardiovascular dysfunction, and arrhythmias. Dose-related myocyte damage and heart failure is directly related to the amount of myocyte damage. The cardiomyopathy appears 1 to 6 weeks after the last dose is given and prognosis is poor, with death occurring in 79% of patients. It is not possible to accurately evaluate anthracycline cardiotoxicity by light microscopy. Therefore all EMBs must be submitted for EM [15,52] . Two characteristic lesions can be seen ultrastructurally: sarcotubular dilatation and loss of myofibrils. The extent of these changes are the basis for the grading system. Sarcotubular dilatation is due to coalescence of dilated sarcoplasmic reticulum and, when severe, can be seen by light microscopy, but this must be confirmed by electron microscopy. Myofibrillar loss may be detected by light microscopy by the appearance of small, shrunken cells with homogenous, pale cytoplasm but, again, this must be confirmed ultrastructurally. If cardiotoxicity is severe, the non-specific finding of interstitial fibrosis may also be seen.The pathogenesis underlying anthracycline cardiotoxicity is not clear. One postulated mechanism is via generation of free radicals, which has considerable corroborating experimental evidence. Other possibilities include inhibition of coenzyme Q 10 (ubiquinone) which is involved in oxidative phosphorylation.

7. SPECIAL VALUE OF EMB IN DIAGNOSIS AND CHOICE OF TREATMENT MODALITIES OF IDIOPATHIC MYOCARDITIS

Among all SHMD, the most common and important is viral MC, which diagnosis is based mainly on "Dallas criteria". By consensus, the authors defined acute MC as "a process characterised by an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes which was not typical of the ischaemic damage associated with coronary artery disease". Idiopathic (presumed viral) MC would be termed "primary MC", whereas acute MC due to other causes (toxoplasmosis or Chagas disease) would be termed secondary MC. As mentioned previously, the Dallas criteria were defined for the MC trial, therefore, separate terminology was adopted for the first diagnostic biopsy and for the subsequent biopsies [53].

On the first EMB the following diagnoses could be made: (1) active MC, with or without fibrosis: both, an infiltrate and damage of the adjacent myocytes were required for this diagnosis. (2) Borderline MC: (not diagnostic findings, requiring a repeat biopsy). This term implies that the inflammatory infiltrate is too sparse or that the myocyte damage is not seen on PH. Additional sections of the original biopsy might demonstrate diagnostic changes in which active MC can be diagnosed. (3) No evidence of MC: a biopsy in which there is no inflammatory infiltrate or myocyte damage.

All subsequent biopsies were divided also into three categories. (1) Persistent or ongoing MC: this diagnosis is made when the degree of interstitial infiltrate is the same or worse than on the first biopsy. (2) A resolving (healing) MC: this diagnosis is made when the inflammatory infiltrate is less expressed than in the previous biopsy, and reparative changes are evident. (3) Resolved (healed) MC: where there is now no inflammatory infiltrate remaining and no evidence of ongoing cellular necrosis. Scar tissue (fibrous replacement or collagen fibrosis) may be present with adjacent compensatory hypertrophy.

It is becoming increasingly apparent that following an acute or subacute episode of MC due to a virus infection, the virus might persist in the cardiac tissues causing a DCM. It is not clear how RNA viruses switch from acute to persistent infections, but it seems that viral transcription and translation are reduced. The treatment of viral MC may be different and depend on therapeutic approach of the clinician and can be defined as 3 main modalities: conventional therapy, immunosuppressive and immunomodulatory treatment [53-56].

The conventional therapy is mainly based on bed rest, aspirin, and all other necessary cardiac medicaments (including diuretics, ACE-inhibitors, β-blockers, etc). There is no attempt to eliminate the virus or to interfere with patients' immune system. From the very "popular" immunosuppressive treatment there was too many expectations, and unfortunately not very promising results. Introduced and recommended by many, the final trial had no definitive proof for recommendation of these medicaments.

Finally, the immunomodulatory therapy was introduced. If the viruses are involved in MC (and consequently with DCM), therapeutic use of interferons or its inducers seems to be appropriate, either due to its direct action on the target cell or indirect effects (activation of cytotoxycity, modulation of immune response, and interaction with other mediators of immune response).

Presuming that heart in MC and DCM may represent a site of low-grade persistent infection, intermediate doses of interferon (IFN) should be considered as a treatment schedule. Administration of IFN in doses over a certain level does not increase antiviral activity, and too frequent administration may prolong the refractory state of the cells.

We enrolled 180 patients (clinical study by Dr M.Mirić at "Dedinje" Institute) to receive α-interferon (IFN) with doses of 3-5 million units per day, for 3 months, and thymomodulin 10 mg 3 times per week, for 2 months. Some patients received conventional therapy alone (depending on EMB analysis). Patients were followed up for 7 years after the end of treatment. Left ventricular function, exercise tolerance and survival rate were significantly better at long-term follow-up in patients treated with IFN or thymomodulin, than in conventionally treated patients. These results implicate that immune modulating therapy might represent important contribution for treatment of MC and DCM, and suggest that adding immunomodulators to the conventional therapy improved the functional capacity and survival of these patients [55].

To improve the treatment, Figulla et al. presented a new classification of MC/DCM patients[21]. The term idiopathic DCM should be used if myocardial dysfunction of unknown cause is detectable by hemodynamic

measurements. According to EMB findings, 4 subsets of MC/DCM can be differentiated: patients without any infectious agent present on EMB and without inflammatory infiltrate; patients without infectious agent but with cellular infiltrate; the third group are patients with an infectious agent and with an infiltrate; and the last group are patients with infectious agent without infiltrate. Those patients without any infectious agents and without cellular infiltrates should receive unspecific heart failure therapy (conventional). If a virus is detectable, heart failure therapy in combination with a virus-suppressive agent may be more appropriate. If a cellular infiltrate is present in combination with an infectious agent and severe left ventricular dysfunction is found, unspecific (conventional) therapy is recommended in combination with a virus-suppressive agent and cytokine blockers. In the case of an infectious agent without cellular infiltrates, a combination of unspecific therapy and virus-suppressive agent should be used.

OVERALL DIAGNOSTIC VALUE OF ENDOMYOCARDIAL BIOPSY

During the past three decades, the technique for performing EMBs has been improved substantially. However, this alone does not explain the increasing clinical use of this procedure. Probably, the combination of experience, diagnostic accuracy, new technical aspects [57], and sophisticated technique in analysing fresh myocardial tissue revealed such interest for enlarged EMB applications. The main value of EMB in diagnosis and treatment of primary heart diseases is the possibility of giving the precise diagnosis (with severity and extent of the pathologic process), followed by the choice of treatment modalities [55,56)].

EMB analyses not only aid diagnosis, but also help in the differential diagnosis of endomyocardial fibrosis from other causes of heart failure[57]. Further, it has helped to evaluate prognosis, particularly in patients with congestive CMP. The personal experience of the author extends to biopsies obtained from over 1100 patients. It has been found that in 82 % of patients, morphological information that is helpful to the referring physician can be obtained. Additional 13% of analysed material present non-specific findings with no PH diagnosis. Only the remaining 5% of EMB were to small or inadequate material for appropriate diagnosis. If we consider that in non-specific findings we did not found any other new entity or changed the clinical diagnosis, by elimination these results can also be considered as helpful. In that case, we can estimate clinical merit in over 80% of the cases. With special references to the choice of the treatment, EMB will play even more important role in the future, for the management of patients with CMPs and SHMD.

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