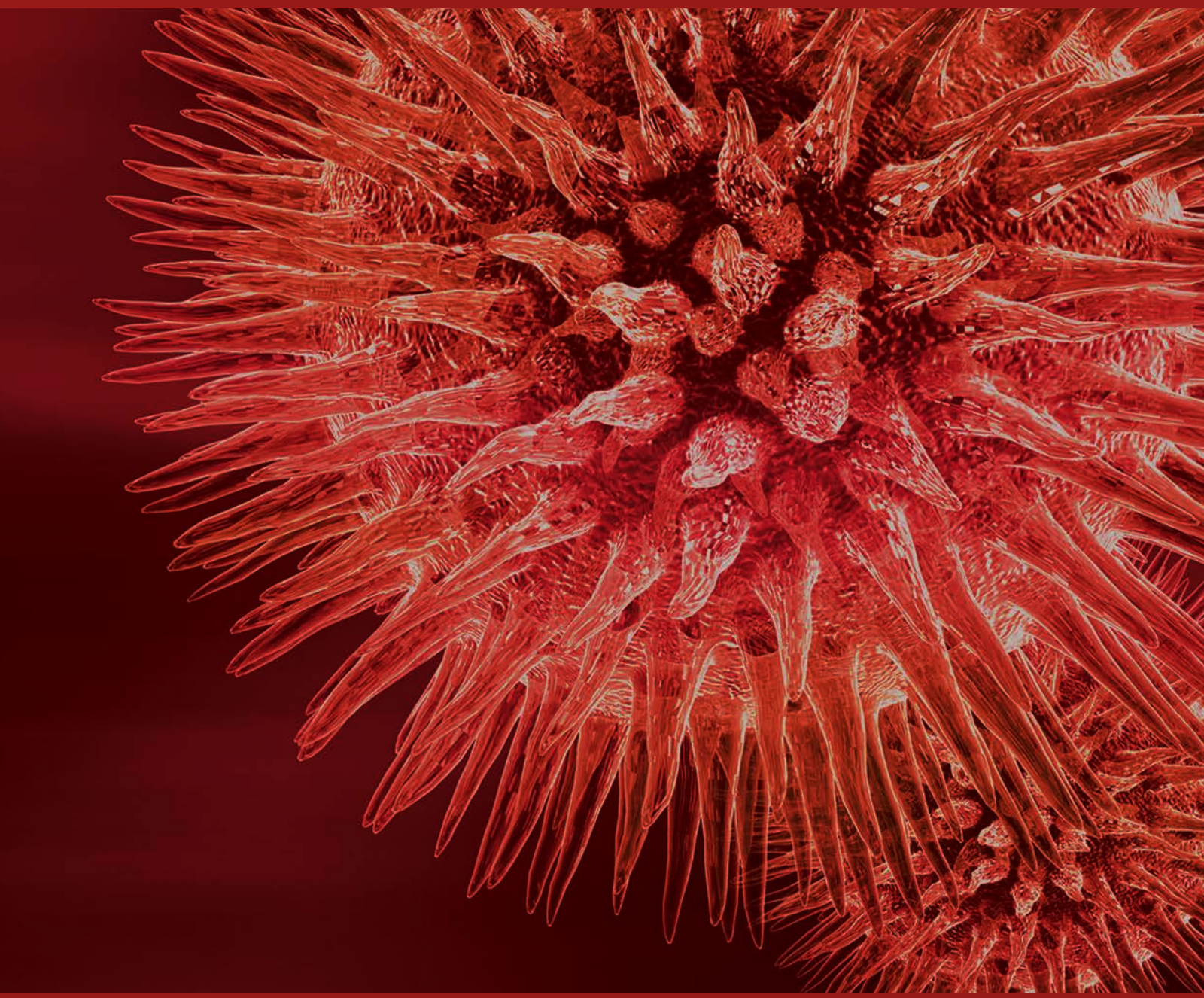
BioMed Research International

Myocardial Diseases: Current Views on Etiopathogenesis, Diagnostic Modalities, and Therapeutic Options

Guest Editors: Tomas Palecek, Javier Ganame, and Giovanni Di Salvo



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Editorial

Myocardial Diseases: Current Views on Etiopathogenesis, Diagnostic Modalities, and Therapeutic Options

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According to current European classification, cardiomyopathies are defined as myocardial disorders that cannot be explained by coronary artery disease or abnormal loading conditions including valvular and congenital diseases. Six specific morphological and functional phenotypes are distinguished: hypertrophic, dilated, restrictive, and arrhythmogenic right ventricular cardiomyopathy together with two unclassified subtypes: Tako-tsubo and left ventricular noncompaction cardiomyopathies. In almost all of these phenotypes, inherited forms may be found; moreover, in some of them—for example, in hypertrophic cardiomyopathy—a genetic origin is even a rule. Therefore, genetic counseling shall be an integral part of the state-of-the-art care of patients with heart muscle disorders. The affected individuals together with their relatives have to be informed about the genetic basis of their disease and the potential risk for other family members. A detailed cardiac evaluation, including ECG and echocardiography (in some cases also Holter ECG monitoring), of first-degree relatives is necessary with their further regular follow-up. If available, genetic testing shall be discussed with the patients as it may improve their management as well as enable effective preventive genetic testing in other relatives.

Because the evaluation of morphology and function of the heart, especially of the left ventricle, is a prerequisite for accurate phenotypic characterization of every myocardial disease, a multimodality imaging approach generally becomes necessary. Echocardiography is still a cornerstone of diagnostic imaging because it is accurate, widely available, safe, and without any contraindications. In recent years, advanced echo modalities gained a clinical role in the follow-up of chemotherapy-induced cardiomyopathy, and global longitudinal strain is recommended in the latest European guidelines.

However, the role of cardiac magnetic resonance is increasingly important. Not only is it the current gold standard for morphological and functional assessment of heart chambers, but it has a unique opportunity of noninvasive tissue characterization. Based on evaluation of the presence of myocardial edema, hyperemia, and necrosis using dedicated sequences, cardiac magnetic resonance is capable of diagnosing acute myocarditis with high accuracy. Late gadolinium enhancement technique is widely used to assess the presence and extent of replacement myocardial fibrosis, which appears to be strongly related to the prognosis of affected individuals. Presence of myocardial fibrosis predisposes to increased risk of ventricular arrhythmias, sudden cardiac death, and progression of left ventricular dysfunction associated with heart failure worsening. One also has to mention the significant contribution of various techniques of nuclear medicine for the diagnosis of cardiac sarcoidosis and transthyretin amyloid cardiomyopathy.

It is obvious that increasing knowledge on ethiopathogenesis of various myocardial disorders together with improved diagnostic possibilities shall result in novel therapeutic options. A complex immunohistochemical and PCR analysis

of endomyocardial biopsy specimens allows us to characterize in more detail the presence and type of myocardial inflammation as well as the presence of possible causative infectious agents and select better more targeted treatment of acute myocarditis and inflammatory cardiomyopathy with administration of immunosuppressive or immunomodulatory drugs or antiviral agents. Many therapeutic strategies are currently investigated in the field of transthyretin amyloidosis including stabilizers of transthyretin tetramers and gene therapies aimed at suppression of transthyretin expression. Another important topic is the development of new treatment possibilities that will be able to prevent late sequelae of cytostatic therapy associated with potential cardiotoxic effect.

In this special issue we are pleased to introduce several interesting original as well as review articles focused on different topics in the field of myocardial diseases.

(i) Two original articles deal with the topic of chemotherapy-induced cardiotoxicity. P. Robinson et al. investigated in their experimental study the role of substance P in chemotherapy-associated death of cardiomyocytes and chemoresistance and nicely showed that aprepitant, a substance P receptor antagonist, is able to decrease doxorubicin induced death of cardiomyocytes as well as to increase the sensitivity of cancer cells to this drug. A. F. Yu et al. evaluated the use of 2D speckle tracking echocardiography in early diagnosis of cardiotoxicity associated with anthracyclines and radiation therapy. Their results confirm that this novel imaging modality is more sensitive in detecting left ventricular systolic dysfunction than traditional indices of ventricular function such as ejection fraction and thus can be used for long-term cardiac surveillance of adult cancer survivors.

(ii) Three articles review several aspects of inflammation/infection related myocardial diseases. J. Krejci et al. largely summarize our current knowledge on pathophysiology, diagnosis, and treatment of inflammatory cardiomyopathy with emphasis on accurate evaluation of endomyocardial biopsy samples, which is prerequisite for the appropriate choice of subsequent treatment options. P. Kuchynka et al. nicely overview a difficult topic of eosinophilic myocarditis, a rare disorder necessitating quite rapid diagnosis, so early administration of immunosuppressive therapy that may improve the poor prognosis of affected individuals can be initiated. In the last article, R. H. Lumsden and G. S. Bloomfield present a brilliant review on HIV-associated cardiomyopathy which currently represents a significant cause of morbidity and mortality in this now chronic disease. The authors clearly demonstrate that there is a significant need to design clear guidelines for screening protocols and diagnostic criteria for HIV-related myocardial disease.

(iii) Several issues related to hypertrophic cardiomyopathy are discussed in three articles. R. Pudil et al. aimed to investigate the significance of vascular endothelial growth factor in hypertrophic cardiomyopathy and their results show that increased levels of this substance are associated with structural and functional parameters in patients with hypertrophic cardiomyopathy suggesting the possibility of its use for a more accurate diagnosis. L. K. Williams et al. focused on left atrial function in hypertrophic cardiomyopathy. Using magnetic resonance velocity vector imaging, they convincingly demonstrate a negative impact of left ventricular outflow tract obstruction on left atrial size and function that significantly improves after septal myectomy. Finally, P. P. Dimitrow and R. Rajtar-Salwa review several aspects related to left ventricular outflow tract obstruction with emphasis on invasive as well as noninvasive treatment possibilities.

If the readers will find the presented articles informative and stimulating their interest in myocardial disorders, the purpose of this special issue will be completely fulfilled.

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Review Article

Inflammatory Cardiomyopathy: A Current View on the Pathophysiology, Diagnosis, and Treatment

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Inflammatory cardiomyopathy is defined as inflammation of the heart muscle associated with impaired function of the myocardium. In our region, its etiology is most often viral. Viral infection is a possible trigger of immune and autoimmune mechanisms which contributed to the damage of myocardial function. Myocarditis is considered the most common cause of dilated cardiomyopathy. Typical manifestation of this disease is heart failure, chest pain, or arrhythmias. The most important noninvasive diagnostic method is magnetic resonance imaging, but the gold standard of diagnostics is invasive examination, endomyocardial biopsy. In a significant proportion of cases with impaired left ventricular systolic function, recovery occurs spontaneously in several weeks and therefore it is appropriate to postpone critical therapeutic decisions about 3–6 months after start of the treatment. Therapy is based on standard heart failure treatment; immunosuppressive or antimicrobial treatment may be considered in some cases depending on the results of endomyocardial biopsy. If severe dysfunction of the left ventricle persists, device therapy may be needed.

1. Introduction

Inflammatory cardiomyopathy (ICM) is defined as inflammation of the heart muscle associated with impaired function of the myocardium, which has most often the morphology of dilated cardiomyopathy. Inflammation of the heart muscle itself, that is, myocarditis, may have many infectious (viral, bacterial, and protozoal infections) and noninfectious causes (e.g., myocarditis accompanying autoimmune disease or hypersensitivity to certain noxious substances). According to the 1995 WHO/ISCF definition, myocarditis is an inflammation of the heart muscle and is diagnosed by using histological, immunological, and immunohistochemical criteria [1]. In 2013, the Position Statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases was published. It is stressed that histological and immunohistochemical evidence of myocardial inflammation is absolutely crucial, and therefore endomyocardial biopsy (EMB) is necessary for the final *in vivo* confirmation of myocarditis. Assessment of the bioptic samples of the myocardium allows beside the diagnosis of myocarditis itself also its accurate classification by typing of infiltrating cells or histological character of lesions (e.g., lymphocyte or eosinophilic infiltration, giant cell myocarditis (GCM) (see Figure 1), granulomatous or necrotizing process, and autoimmune features) with all important prognostic and therapeutic consequences. An integral and key part of EMB samples evaluation is the search for potential infectious agents in the myocardium, usually using reverse polymerase chain reaction (PCR) [2].

Clinical picture of myocarditis can vary, which may bring difficulties in the diagnosis of this disease, but it has been shown that the most frequent manifestation is heart failure [3].

It usually occurs due to a dysfunction of the left ventricle (LV), which is an integral part of the diagnosis of inflammatory cardiomyopathy.

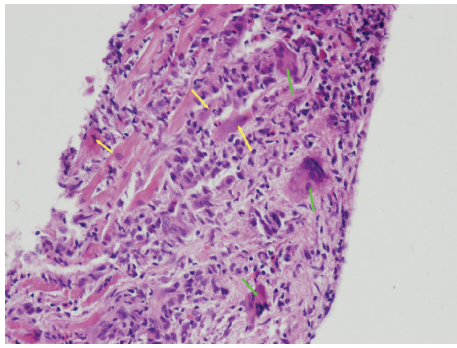

Figure 1: Giant cell myocarditis, hematoxylin eosin, magnification 200x. Massive inflammatory myocardial lesions with regressive cardiomyocytes (yellow arrows) and mixed reactive cellulisation with the giant multinuclear elements (green arrows) (from the archive of V. Zampachova, MD).

The most common etiological cause of myocarditis in Western civilization is considered to be viral infection. In recent decades there has been a shift in viral spectrum; previously dominating adenovirus and enteroviruses were currently replaced by parvovirus B19 (PVB19) and human herpes virus 6 (HHV-6) [4]. This has been also convincingly confirmed by the results coming from the Marburg Registry, the largest database of patients with suspected myocarditis who underwent EMB [5].

In Central and South America, Chagas disease is often found. It is caused by the protozoan *Trypanosoma cruzi* and one of the disease symptoms is myocarditis [6]. In some endemic regions, *Borrelia burgdorferi* is relatively frequently detected in patients with myocarditis [7, 8].

Contemporary view on the *pathophysiology* of myocarditis is based on animal models of enteroviral myocarditis and assumes the three-phase evolution of the disease [9–11]. The first acute phase is associated with viral entry into myocytes over the virus-specific receptor (CAR coxsackie-adenoviral receptor) with the participation of coreceptors (DAF, decay accelerating factor, for enteroviruses and integrins $\alpha v\beta$ 3 and 5 for adenoviruses) [12]. In this phase, which lasts several days to weeks, viral replication and inflammatory mediators production associated with nonspecific immunity are predominantly responsible for myocytes impairment (and thus the function of the myocardium). In clinical practice, this period may often be asymptomatic. The second phase starts usually 2–4 weeks after onset of the disease and is characterized by a specific immune reaction. This includes both cellular and antibody-mediated immune response which often could have autoimmune features. These autoimmune reactions are based on two main mechanisms: the first is the cross-reactivity of viral epitopes and some cardiac structures (molecular mimicry phenomenon); another option is the exposure of originally intracellular structures to the immune system that occurs after the virus-induced damage of myocytes. Such a situation is seen in the production of antibodies against alpha and beta myosin-heavy-chains, wherein the antibody against alpha chains is considered organ (heart) specific. Antibodies against myosin have a negative effect on myocyte contractility, which was confirmed *in vitro* and also in animal experiments. They also affect calcium channels, leading to calcium overload of myocytes. In patients with ICM, a number of other antibodies was captured, for example, antibodies against beta-adrenoceptors, against M2 muscarin-receptors, or against troponin [5, 13–16]. The third phase of the disease occurs after several weeks or months and may include either retreat of inflammation and improvement in LV function (in 50–70% of cases, usually after removal of viruses from myocardium) or persistent LV dysfunction associated with development of postinflammatory dilated cardiomyopathy (DCM). A number of factors play an important role in the disease course, for example, degree of initial damage of the myocardium, the intensity and duration of inflammation, or the persistence of viral replication [17, 18].

Whether the described course takes place in every case of myocarditis caused by various viruses (i.e., those that are primarily not invading myocytes, but endothelial cells of blood vessels as is the case of PVB19 or HHV6 infection) is not entirely clear [19]. It seems likely that a necessary condition for the creation of myocarditis is certain genetic predisposition; the vast majority of individuals will not develop myocarditis, even after meeting the so-called cardiotropic virus. This theory is also supported by more frequent occurrence of myocarditis in some families [2, 11].

Epidemiology. The real incidence of myocarditis is difficult to determine exactly due to the complex definitive diagnosis in routine clinical practice. In young adults who died suddenly, myocarditis was found *post mortem* in a wide range between 2 and 42%; other studies indicate up to 46% incidence of myocarditis in children with unexplained DCM [2]. Previous works using the Dallas Criteria reported incidence of biopsy-proven myocarditis in 9–16% of DCM cases [20]. More recent studies [21, 22] demonstrate that almost 50% of patients with clinical diagnosis of DCM have immunohistochemically detectable myocarditis and thus could be classified as ICM. Myocarditis (or ICM in particular) is considered as the most common cause of dilated cardiomyopathy [23].

Another interesting fact is the frequent detection of viral nucleic acids in the myocardium (up to 60–80% of cases) [21, 22, 24]. Given the fact that some viruses (e.g., PVB19) are often found even in individuals with normal LV function, their real significance is not elucidated with certainty, and this issue is the subject of intense research [24–27]. According to some authors, the presence of any virus in the myocardium is a negative prognostic factor [28, 29], while others have not confirmed that presence of a virus has negative effect on the prognosis and evolution of LV function [22, 30]. A lot of controversies are about the most frequently detected PVB19 or HHV6, respectively, because not only is their presence in the myocardium that plays an important role in the pathogenicity, but there are also other factors such as viral load, active virus replication, coinfection with other viruses, genetic background, sex differences, and others influencing their etiological role [31–35]. In Marburg Registry comprising data from almost 12,500 patients it was shown that prevalence of PVB19 in patients with myocardial inflammation and LV dysfunction was higher than in the group with inflammation

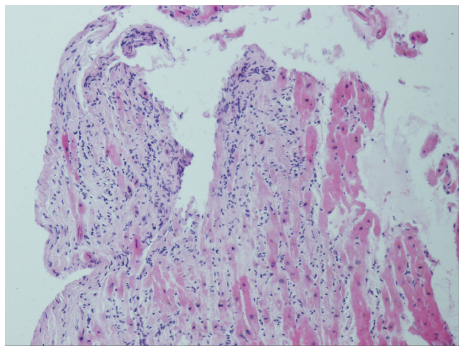

Figure 2: Fulminant myocarditis, hematoxylin eosin, magnification 100x. Residual cardiomyocytes with fibrotisation and dense lymphocytic cellulisation (from the archive of V. Zampachova, MD).

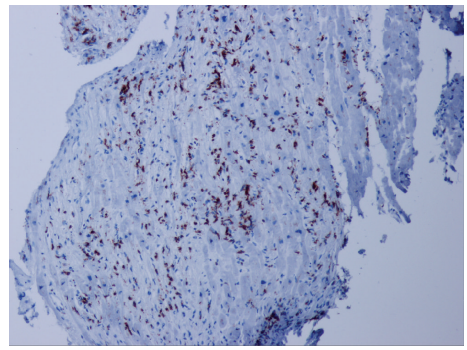

Figure 3: Fulminant myocarditis, detection of CD3+ T lymphocytes, immunohistochemistry, magnification 100x. Numerous positive elements (dark nuclei), focally detected 250 CD3+ T cells/mm^2 (from the archive of V. Zampachova, MD).

and preserved LV function. The same was true for comparison of patients with LVEF below 45% with and without myocarditis (PVB19 presence in 33.3% versus 17.6%; $p < 0.05$) [5].

2. Clinical Manifestation

Clinical manifestation of myocarditis/inflammatory cardiomyopathy ranges from virtually asymptomatic course of a mildly ongoing disease with slightly lesser extent of impaired myocardium on one hand and severe fulminant heart failure accompanied with malignant arrhythmias on the other. Sometimes, sudden cardiac death may be the first manifestation of the disease in a previously completely healthy individual. Often mentioned initial viral infection foregoing viral/postviral myocarditis can pass subclinically; therefore its absence in history of the patient certainly does not rule out possible evolution of inflammatory myocardial damage. In milder cases, of course, the symptoms during the active phase are less dramatic, but it does not automatically mean better long-term prognosis. However, extent of myocardial damage in acute phase is one of the important factors determining recovery of LV function in the later period [2, 5, 9–11, 17, 18].

The most common manifestation of myocarditis is *heart failure*. It may have a gradual onset and only mild symptoms, but there is no exception to see rapidly emerging cases terminating in cardiogenic shock, where only the implantation of mechanical circulatory support device or urgent heart transplantation can save the patient's life. This scenario is typical for fulminant myocarditis (see Figures 2 and 3); if the patient survives the acute phase, a significant improvement or even a complete normalization of LV systolic function with very good long-term prognosis may occur in a few weeks [36]. This is true to certain degrees for all types of myocarditis with initial systolic dysfunction, when LV function improves spontaneously or after standard heart failure treatment at least in half of the cases [17–20]. It is therefore appropriate to postpone crucial therapeutic decisions (such as implantation of a cardioverter/defibrillator with/without resynchronisation function or heart transplantation) for a period after the acute phase (when this is allowed by clinical situation), which means in clinical practice delay of about 3–6 months after disease onset or after the start of the treatment.

Our data from 6-month follow-up showed that the retreat of inflammatory infiltration in the myocardium is associated with improvement of a number of echocardiographic parameters, with decrease of NTproBNP and improvement of the functional status [37]. Tschöpe et al. published an interesting study describing a high incidence of PVB19 in patients with isolated diastolic dysfunction of LV (in 95% of patients), while in the group with normal diastolic function PVB19 occurred only in 24% of patients. So not only the systolic, but the diastolic dysfunction as well may be associated with viral heart disease or myocarditis. The basis of this fact seems to be the presence of endothelial dysfunction in individuals with endothelial cells infection, which may also be associated with a higher incidence of chest pain as a clinical manifestation of the disease [38]. The urgency of EMB in cases of isolated diastolic dysfunction is questionable, because there are no data for introduction of specific therapy according to the bioptic result regarding the presence of myocarditis. Thus, another clinical scenario is the manifestation of the disease by *chest pain* that can mimic angina pectoris or may have a pericarditis-like character, particularly if perimyocarditis is present. Patients are often brought to the catheterization laboratory to rule out acute coronary syndrome (especially if elevation of markers of myocardial damage is present). Normal findings on coronary arteries and the exclusion of other pathology (e.g., aortic dissection, atrial or ventricular tachycardia, but also noncardiac involvement in florid gastroduodenal ulcer disease or severe anemia) in such cases lead to consideration of possible myocarditis [39].

Third dominating complaint that brings a patient to the physician can be symptoms related to *arrhythmias*. Arrhythmias may be both supraventricular and ventricular; conduction disturbances or serious ventricular arrhythmias suggesting the possibility of giant cell myocarditis, cardiac sarcoidosis, or *Borrelia burgdorferi* associated myocarditis. Myocarditis can also be found incidentally at autopsy in patients who died suddenly, probably on the basis of malignant arrhythmia. Fortunately, we see more often less dramatic

course with the presence of palpitations, dizziness, or even syncope, which always have to alert attending physician to the possible presence of serious arrhythmias.

Of course, it is not unique that all described symptoms may be present in one patient, either simultaneously or at different time phases of the disease. In terms of prognosis, it has been reported that cases with symptoms of heart failure, namely, those which meet the criteria of inflammatory cardiomyopathy, have a poorer prognosis than cases manifesting by chest pain or arrhythmias [28].

Another disease that should be mentioned while speaking about ICM is peripartum cardiomyopathy (PPCM) [40–42]. PPCM is manifested by systolic heart failure in previously healthy women at the end of pregnancy or in the first months after the birth. The causes of the disease are not definitively clarified, but according to some of the authors myocarditis could play an important role in pathogenesis of this quite mysterious disease [43, 44]. It affects more often African-American women (relative risk is almost 16 times higher) [45] and has a relatively high frequency of recovery of LV function (on the contrary, particularly among white women) [46, 47]. Nevertheless, in about 10% of women, it progresses to severe heart failure, when the only solution may be urgent heart transplantation or LVAD implantation. In less developed countries where these treatment options are not available (and where PPCM is unfortunately relatively more frequent), the mortality is in comparison with European countries and the USA not negligible [47–49].

3. Diagnostics

In the past, the *diagnostics of myocarditis* was a difficult and challenging task. Even today, despite various imaging modalities that are available nowadays myocarditis often remains a diagnosis *per exclusionem*. The Position Statement of ESC Working Group on myocardial and pericardial diseases based clinical suspicion for myocarditis on the presence of typical clinical presentation (heart failure, chest pain, and arrhythmia) and noninvasive imagining techniques (see Diagnostic Criteria for Clinically Suspected Myocarditis) [2]. Endomyocardial biopsy is recommended for all patients who fulfil clinical diagnostic criteria and remains the standard tool for definitive confirmation of the diagnosis [2, 5, 10, 11, 17, 19]. However, this procedure is the method of first choice only in specialized centers with experience in performing EMB with advanced laboratory equipment needed for complex evaluation of EMB samples.

Diagnostic Criteria for Clinically Suspected Myocarditis. Diagnosis of myocarditis is suspected in presence of

(i) ≥1 clinical presentation and ≥1 diagnostic criterion,

(ii) ≥2 diagnostic criteria, if the patient is asymptomatic.

Clinical Presentation. Clinical presentation involves

(i) chest pain,

(ii) acute or chronic heart failure,

(iii) arrhythmic symptoms (palpitations, syncope, and sudden cardiac death).

Diagnostic Criteria. Diagnostic criteria are as follows:

(I) Electrocardiogram *(ECG)* test features (atrioventricular block, bundle branch block, ST/T-wave changes, supraventricular or ventricular arrhythmias, low voltage of QRS complex, and abnormal Q waves).

(II) Markers of myocardial necrosis (elevated cardiac troponins or CK-MB).

(III) Functional and structural abnormalities on echocardiography or CMR imaging (impaired left or right ventricle function, with or without left or right ventricle dilatation, increased ventricle wall thickness, pericardial effusion, and intracardiac thrombi).

(IV) Tissue characteristics by CMR (presence of at least two of three Lake Louise criteria, myocardial oedema and early and late gadolinium enhancement).

Similarly to diagnostics of other diseases in cardiology, the process starts with simply conventional examinations such as *ECG*, which can have very variable and also nonspecific findings (presence of arrhythmias, changes of PQ and ST interval, prolongation of QRS complex, and the presence of Q waves), although some findings (especially the presence of rhythm disorders, i.e., ventricular tachycardia or atrioventricular block of 2nd or 3rd degree) may be suggestive of special types of myocarditis (giant cell myocarditis or cardiac sarcoidosis).

Another basic diagnostic method is *echocardiography*. Here as well, there is not any typical finding allowing diagnosis with some of nonspecific echocardiographic features including both global and regional kinetic disorders of the left or right ventricle, diastolic dysfunction, left ventricle hypertrophy, and pericardial effusion. But even a normal finding does not rule the diagnosis out. The value of echocardiography lies rather in excluding other causes of the symptoms (valvular or pericardial disease, aortic dissection) and also in risk stratification based on evaluation of left ventricle systolic dysfunction [2, 9, 11, 17].

The most important noninvasive diagnostic method is *magnetic resonance imaging* (MRI) which is a routinely available technique in last years and is suited for the evaluation of both morphological and functional myocardial impairment and tissue characterization [50–53]. The clinical suspicion of myocarditis is one of the most common indications for MRI study in cardiology because it is an accurate modality for the assessment of a number of common features in myocarditis: myocardial oedema and hyperemia, capillary leak, necrosis and fibrosis, and contraction abnormalities or pericardial effusion [54]. The Lake Louise criteria have been proposed to standardize the evaluation of findings and to improve the diagnostic accuracy [50]. The criteria are based on the evaluation of myocardial oedema (T2-weighted sequences) frequently present in acute inflammation, early gadolinium enhancement (EGE) related to hyperemia, and in particular the assessment of the presence of the late gadolinium

enhancement (LGE) with the presence of a characteristic type of gadolinium accumulation in areas of myocardial necrosis or fibrotic reparative changes. If two of these three criteria are present, the MRI imaging demonstrates 67% sensitivity, 91% specificity, and 78% diagnostic accuracy [2, 50]. The LGE was shown as important for prognostic stratification; if not present, the outcome is very good; on the contrary the presence of LGE is considered to be a significant predictor of overall and cardiovascular mortality (OR 8.4 and 12.8, resp.) [55].

The diagnostic sensitivity of MRI is higher in acute scenarios than in chronic cases with less intensive inflammatory changes. The sensitivity is higher also in cases with clinical manifestation by chest pain ("infarction-like" symptoms) than in patients with arrhythmias or heart failure [56]. Despite several technical difficulties in evaluation of, for example, early gadolinium enhancement, MRI is definitely one of the leading diagnostic modalities if myocarditis is suspected. However, especially in fulminant forms, MRI should not delay EMB performance representing the gold standard with more significant additive information regarding treatment decision [2, 19, 34].

Some of the *laboratory tests* may be useful in myocarditis diagnostics; most useful is detection of myocardial damage in acute phase by troponin and CK-MB elevation; elevation of troponin was identified as a negative prognostic factor and may be also used for long-term monitoring of disease activity [57]. Elevated levels of natriuretic peptides are neither diagnostic nor specific but they can identify patients with worse prognosis [58]. Also the detection of certain antibodies against the myocardial structures (see above) related to autoimmune impairment showed to be contributive to diagnostics but standardized commercial kits are currently not available [15, 19]. The presence of antibodies could be one of the markers of positive response to immunosuppressive treatment [59]. If antibodies are detected in healthy relatives of patients with dilated cardiomyopathy, the risk of disease manifestation in these individuals is higher [2, 15]. Inflammatory markers can be elevated but this is not a rule. The diagnostic approach based on serological tests from peripheral blood often used in the past did not show significant correlation with EMB results [60].

Recently, the development of new sophisticated methods highlights the tendency for less invasive or even noninvasive diagnosis of myocarditis using modern approaches. One of these methods is detection of different gene transcription which seems to be promising due to high specificity and sensitivity to distinguish myocarditis and dilated cardiomyopathy [61, 62]. Another method is evaluation of the miRNA levels. MiRNAs are small noncoding RNAs regulating post-transcription gene expression. Their levels differ in various physiologic and pathologic conditions and the first studies based on animal models showed that some of them are upregulated (e.g., miRNA-155, miRNA-146b, and miRNA-21) in myocarditis and can distinguish inflammatory and non-inflammatory myocardial impairment [63]. Similar upregulation was also proved in patients with viral myocarditis for miRNA-155 and miRNA-148a [64]. Different expression of miRNAs [65] and different gene transcription [66] were recently published comparing individuals with replication active and latent myocardial PVB19 infection. The PVB19 replication activity seems to be the crucial factor in the understanding of PVB19 role in pathogenesis of myocarditis. The study by Kühl et al. including 415 patients with the PVB19 myocardial presence showed that only in 15,9% patients the virus was replicating and it was in relation to changes in cardiac gene expression, for example, INF- β1 (upregulation), FOXP3, ADIPOR2, and IL-10 (downregulation), and with elevated mRNA levels. These methods could be used for prognostic stratification and personalised treatment decisions [66].

Coronary angiography is indicated to exclude coronary artery disease (CAD) as one of the possible causes of the symptoms and should be done in all patients in risk of CAD regardless of the symptoms, which means also in patients without chest pain.

Endomyocardial biopsy is still considered as gold standard and the only method for definitive diagnosis *in vivo*. The sample can be obtained from left or right ventricle (or both); the diagnostic yield probably depends on the number of samples, not on the particular site of EMB [2, 67] despite the fact that some studies showed higher sensitivity in left ventricular and biventricular biopsy than only right ventricular one [68, 69]. Unlike the presence of infiltrating cells, which is comparable in both ventricles, some characteristics differ between the two chambers; for example, degree of fibrosis is more pronounced in LV [67].

The endomyocardial biopsy use in diagnosing of myocarditis is not a completely new trend. The beginning dates back to the 80s when Dallas criteria were set to standardize the histology evaluation of biopsy samples [70]. When these criteria were found to be of low sensitivity and high interobserver variability on histology assessment, it was necessary to set new, more sensitive and precise criteria that could be used in routine practice [71, 72]. There is also a noticeable difference in indication of the EMB between the US and European countries. In the US, it is recommended to use EMB only in specific clinical scenarios [73, 74]; the ESC recommended approach is, however, more aggressive and EMB should be performed in all cases when myocarditis is clinically suspected [2]. The consensus of European pathologists published in 2013 also came to the same conclusion [75]. The addition of immunohistochemistry used for typing of infiltrative leucocytes constitutes a breakthrough due to higher sensitivity of EMB for detection of myocarditis [72]. At the turn of the millennium, Marburg's criteria were set and were based on the presence of more than 14 mononuclear leucocytes/mm^2 of bioptic sample [5, 76]. The inclusion criteria of the TIMIC study added the alternative presence of more than 7 T-lymphocytes per mm^2 as a second criterion [77]. The current position statement requires the simultaneous presence of both and, moreover, excluded patients with the presence of more than 4 monocytes per mm^2 [2]. Recent studies from Berlin showed that setting novel cut-off values for the number of the infiltrating cells (e.g., more than 10 CD3+ cells per 10 mm^2 or more than 30 CD45+ per mm^2) could make the prognostic stratification even more precise.

Another new approach is evaluation of perforin-positive cells; the presence of more than 2,95 cells/mm^2 is related to a poor outcome [78]. The other immunohistochemical marker that can be also used is the assessment of HLA expression which upregulated during myocardial inflammation; this criterion was used for the selection of patients in Wojnicz et al.'s study [79]. We should be aware of a potential sampling error due to focal myocardial cellular infiltration which decreases the sensitivity of EMB [80, 81]. This could be sorted out by combination with the assessment of HLA antigen expression that is usually more diffuse. However, it is a semiquantitative method based on subjective assessment by the pathologist.

The evaluation of the samples should always include the evaluation of viral (or other agents) presence or more precisely the viral nucleic acid presence. PCR is the most common method used for viral detection in myocardium. Especially in PVB19 presence, the quantitative assessment of viral load (number of viral copies) should be done because low viral load might not be related to inflammation induction [31, 33]. Other authors consider the viral load as not so important and stress the need for replication activity evaluation (by detection of mRNA, miRNA profile, or gene transcription), which is of special interest in PVB19 where not replicating virus could be rather an "innocent bystander" than the direct cause of acute inflammation [65, 66].

From the foregoing facts can be concluded that the setting of diagnostic criteria is still in evolution and it can be assumed that it will lead to their further modifications in the future depending on new findings.

4. Therapy

The problem of therapeutic recommendations, or rather the reason why they are so cautiously formulated, is the fact that they are based more on results of small monocentric studies and institutional registries, while data from the randomized, multicenter, placebo-controlled trials are either very subtle or even completely absent [2, 5, 9–11, 17, 19, 22].

There is consensus on regime measures limiting physical activity for 6 months or till retreat of the inflammation in control EMB and/or till restitution of LV function [2]. Pharmacotherapy of inflammatory cardiomyopathy with the presence of LV dysfunction is based on administration of standard heart failure treatment according to current guidelines, consisting mainly of angiotensin converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs), beta-blockers, and aldosterone antagonists [82, 83]. For these drugs we also have some experimental and clinical data documenting the potential positive influence on inflammatory changes and the prognosis of patients [84–88]. Conversely administration of nonsteroid anti-inflammatory drugs (NSAIDs) and digoxin is not recommended as a result of animal experiments where these drugs have led to deterioration of LV function. Also, administration of positive inotropic agents may lead to further impairment of the myocardium already damaged by inflammation and should be reserved only for very exceptional situations [17].

In critical cases it is necessary to use a mechanical circulatory support, either as a "bridge to decision" or as a "bridge to transplantation" which may be in cases of persistent severe refractory heart failure the last therapeutic option. Approach to the treatment of arrhythmias and device therapy especially in primary prevention of sudden cardiac death should be preferably restrained in the acute phase because significant improvement in LV function and retreat of arrhythmias associated with regression of myocardial inflammation may be often seen in a few weeks. To overcome this critical acute phase, it is possible to use special external defibrillation equipment such Life-vest [2] in some countries. This can prevent the implantation of endovascular/intracardial devices for patients with only temporary need of antiarrhythmic nonpharmacologic treatment. Otherwise, hospitalization with monitoring of heart rhythm and evaluation of the arrhythmogenic risk with optional next therapeutic steps may be necessary.

In the specific treatment of myocarditis, the situation is ambiguous. For some specific subtypes of myocarditis, immunosuppression is associated with a distinct profit and is considered to be clearly indicated; this is especially the case of GCM [89–91], followed by eosinophilic myocarditis [92, 93]; immunosuppression should be started also in cardiac sarcoidosis [94]. Immunosuppressive schemes vary among different types of inflammation, in the case of GCM, immunosuppression should be far more aggressive, so this is why it is important to differentiate these types of myocarditis. In patients with chronic lymphocytic myocarditis with symptoms longer than six months, there are data from two randomized clinical trials showing the additive positive effect of combined immunosuppressive therapy (combination of prednisone and azathioprine) on echocardiographic parameters compared to standard care [77, 79].

In each of these studies a different dose was used and the duration of treatment was also different, although the same drugs were administered. In Wojnicz et al.'s study enhanced expression of HLA antigens was used as an inclusion criterion and, moreover, the presence of microbial agents in the myocardium was not ruled out [79]. Frustaci et al. included patients into the study according to the number of infiltrating cells and the absence of an infectious agent in the myocardium [77, 95]. Thus, these studies are not entirely consistent in methodology and therefore results cannot be simply "added up." In addition, because both are single-center trials it would be required to verify the results in a multicenter study. The results of one older meta-analysis suggest that immunomodulatory treatment improves LV function in patients with symptoms longer than 6 months [96]. More recent meta-analysis of Lu et al. from 2014 evaluated the results of nine studies (covering a total of 342 patients treated with immunosuppression and 267 treated with conventional therapy) and showed that immunosuppressive therapy does not affect mortality or the need for heart transplantation, but favorable effect on improvement of LV systolic function was apparent. Conclusion of this study was that immunosuppressive therapy may be considered as an adjunct to conventional treatment, if this is not effective [97].

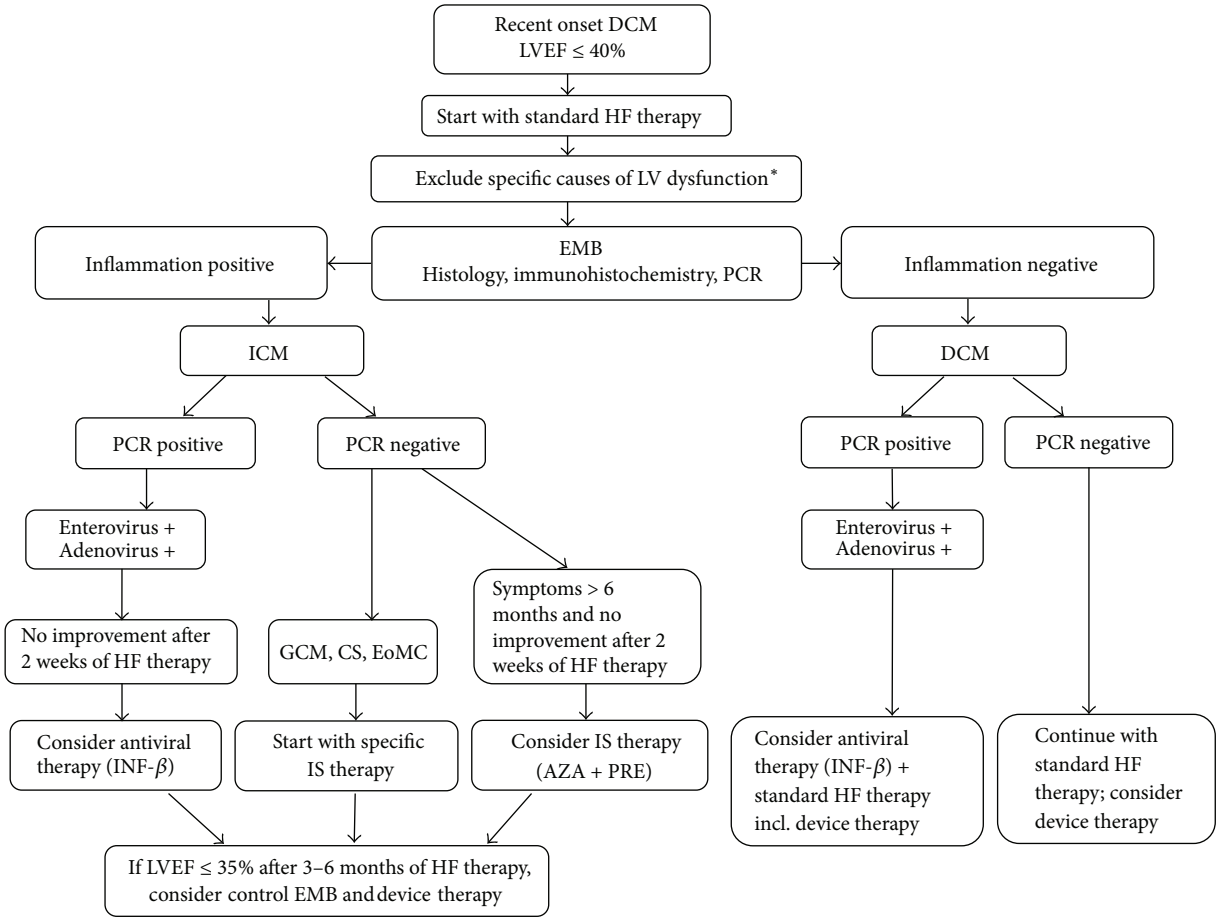

Figure 4: Diagnostic and therapeutic algorithm in suspected inflammatory cardiomyopathy. DCM: dilated cardiomyopathy, LVEF: left ventricle ejection fraction, LV: left ventricle, HF: heart failure, EMB: endomyocardial biopsy, ICM: inflammatory cardiomyopathy, PCR: polymerase chain reaction, GCM: giant cell myocarditis, CS: cardiac sarcoidosis, EoMC: eosinophilic myocarditis, INF-β: interferon-beta, and IS: immunosuppressive. *Specific causes of LV dysfunction: coronary artery disease, valvular disease, toxic causes (alcohol, drugs, and chemotherapy), tachycardia-induced cardiomyopathy, and endocrine disorders.

According to a majority of experts, but based on data from a single study, viral presence in the myocardium is associated with the absence of a positive response to immunosuppression (data from 17 patients, but only one was positive for PVB19) [69, 95]. Viral presence in the myocardium has not been determined in Myocarditis Treatment Trial (with neutral effect of immunosuppression) nor in the Polish study (with positive effect of immunosuppression on echocardiographic parameters), which makes the situation in this regard even more confusing [79, 98]. In the TIMIC study [77], worsening of echocardiographic parameters was observed in patients in the placebo group. Indeed, this is in contradiction with the results of other studies, including our own experience [22, 37, 79]. Because of this, CZECH-ICIT study was initiated with the ambition to bring more light to the uncertainties in the use of immunosuppressive therapy in myocarditis [99]. Recruitment of patients in the study is still in progress and the results are expected in coming years.

Treatment with intravenous immunoglobulins has a logical theoretical basis, which was confirmed in several small studies with quite favorable results [100, 101], but the largest multicenter trial by McNamara et al. showed no profit versus placebo [102]. Therefore, the administration of immunoglobulins is not currently considered as routinely indicated [2]. Similarly unclear is the position of immunoadsorption, where some studies have shown little effect on improvement of LV function, reduction of biomarkers levels, and retreat of inflammatory changes in the myocardium [5, 103, 104]. However, until these subtle data are confirmed by other studies, neither treatment could be recommended [2].

In the field of antiviral treatment, the published data are somewhat controversial as well. Administration of common antiviral drugs is possible, but there is no evidence about their actual effect. Theoretically, this treatment could be justified in the first phase of a disease associated with viral replication, but in clinical practice myocarditis is usually detected later in the second phase, when the administration is likely to have little benefit. It was proven that interferon-beta treatment removed enteroviruses and adenoviruses of the myocardium and in some studies was shown as beneficial [105]. In other more common types of viruses such treatment is unfortunately less efficient. That is probably the reason why

the results of other studies with higher proportion of PVB19 were not so optimistic [106]. However, according to German authors, interferon-beta therapy may be at least in the case of enteroviruses associated with long-term prognostic benefit [29]. In PVB19 infection, telbivudine therapy is currently tested and we have to wait for the results. For some other rare agents, such *Borrelia burgdorferi*, antibiotic treatment is considered to be indicated, although the data from placebo-controlled studies are missing and also the results are not unequivocal [7, 8]. Algorithm with the proposal of therapeutic decisions based on knowledge of EMB result is shown in Figure 4.

5. Conclusion

The diagnosis of myocarditis and inflammatory cardiomyopathy remains highly complex and challenging despite the great expansion in diagnostic methods. Beside careful anamnestic data and physical examination, a comprehensive diagnostic approach using a range of noninvasive as well as invasive methods is required, together with highly sophisticated laboratory facilities. The most important noninvasive diagnostic method is cardiac magnetic resonance imaging, but endomyocardial biopsy still remains the gold standard. Standard therapy of inflammatory cardiomyopathy is based on the recommendations for the treatment of heart failure or arrhythmias; specific therapies may be indicated only with known results of EMB. Evidence for the therapeutic recommendation is not entirely convincing, and therefore individual assessment of each specific case and experience of the attending physician plays an important role in treatment decision. It is obvious that without carrying out large multicenter randomized prospective trial our therapeutic decisions will fall short of the requirements of evidence based medicine. A considerable effort is still ahead to reach comparable level of knowledge in the field of myocarditis and inflammatory cardiomyopathy to other areas of cardiology, where we have both clear and proven diagnostic criteria and also clear and robust data-based therapeutic recommendations.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Obstructive Form of Hypertrophic Cardiomyopathy-Left Ventricular Outflow Tract Gradient: Novel Methods of Provocation, Monitoring of Biomarkers, and Recent Advances in the Treatment

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Dynamic (latent or/and labile) obstruction of left ventricular outflow (LVOT) was recognized from the earliest clinical descriptions of hypertrophic cardiomyopathy (HCM) and has proved to be a complex phenomenon, as well as arguably the most audible ("visible") pathophysiological hallmark of this heterogeneous disease. The aim of the current review is focused on two novel issues in a subgroup of obstructive HCM. Firstly, the important methodological problem in HCM is the examination of a subgroup of patients with nonobstructive hypertrophy in resting conditions and hard, but possible provoking obstruction. Recently, investigators have proposed physiological stress test (with double combined stimuli) to disclose such type of patients. The upright exercise is described in the ESC guideline on hypertrophic cardiomyopathy from 2014 and may appear as a candidate for gold standard provocation test. The second novel area of interest is associated with elevated level of signaling biomarkers: hypercoagulation, hemolysis, acquired von Willebrand 2A disease, and enhanced oxidative stress. The accelerated and turbulent flow within narrow LVOT may be responsible for these biochemical disturbances. The most recent advances in the treatment of obstructive HCM are related to nonpharmacological methods of LVOT gradient reduction. This report extensively discusses novel methods.

1. Introduction

History of recognition of hypertrophic cardiomyopathy (HCM) and definition of frequency of obstructive form is associated also with methodology of stress test to provoke LVOT gradient >30 mmHg. Maximized and (semi)physiological stress tests were introduced only several years ago.

For more than 50 years, in the period of first description among alive patients (in preechocardiographic era, Figure 1), HCM was only exclusive as obstruction, where systolic murmur in auscultation was verified as turbulent flow with gradient in obstructive LVOT measured invasively [1–3]. In fact, in the early preechocardiographic era (1957 to 1969), an outflow gradient was a virtual prerequisite for the diagnosis of HCM. Dynamic obstruction to LVOT rapidly achieved distinction as the most represented feature of HCM, dominating the initial comprehensive description of the disease.

Thus, predominantly, but not solely [4, 5], obstructive HCM has been diagnosed in early reports. After introduction of echocardiography [6–8] (Figure 1), during family screening for sudden cardiac death, a large number of nonobstructive patients were recognized. In this period researchers postulated that, in the whole population of HCM, the ratio of the obstructive: nonobstructive is 1/3 : 2/3. In 2003 it was reported that, among older patients, female's left ventricular contractility was higher and LVOT gradient occurred more frequently than in males [9]. Recent observation that the vast majority of patients with HCM have the propensity to develop outflow obstruction (either at rest or with exercise) underscores a return to the characterization of HCM in early period of exploration as a predominantly obstructive disease [10].

To sum up the history, our perception of obstructive : nonobstructive ratio has changed during the last 55 years.

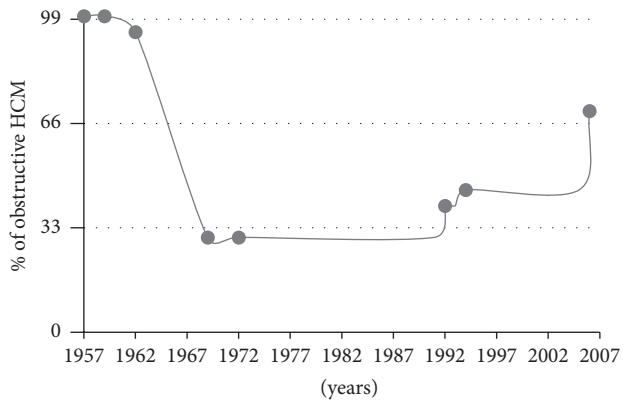

Figure 1: Probable timeline displaying major events concerning the history of left ventricular outflow tract obstruction in hypertrophic cardiomyopathy. Point 1: first description of obstructive HCM by Brock. Point 2: Morrow and Braunwald (1959) and Braunwald et al. (1960): all patients were defined as obstructive HCM by systolic murmur in auscultation and LVOT gradient in invasive catheterization. Point 3: Braunwald et al. (1962, 1963), description of first HCM patient without obstruction. Point 4: first echocardiographic image of SAM by Shah et al. Point 5: echocardiographic description of asymmetrical left ventricular hypertrophy by Henry et al. Point 6: description of using of DDD pacing. Point 7: description of intervention defined as alcohol septal ablation. Point 8: publication by Maron et al. describing exercise test for LVOT gradient provocation.

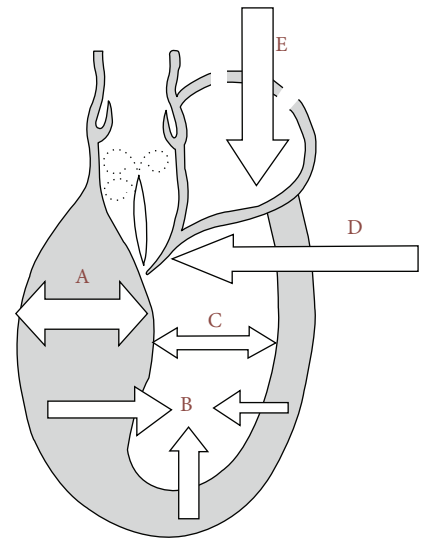

Figure 2: Mechanisms predisposing to LVOTG induction. A: left ventricular hypertrophy, particularly basal septal segment (HCM, hypertension, and storage disease). B: LV hypercontractility (moderate tachycardia). C: small size LV cavity (HCM, children, women, dehydration). D: prolonged/thickened mitral leaflet(s). E: reduced LV preload (dehydration, diuretics, vasodilators, hemodialysis, fever, and septic shock).

In Figure 1 we can recognize early period of obstructive predominance, midperiod with nonobstructive advantage due to broad availability of echocardiography, and later period with increasing frequency of obstructive form up to value 70% [10]. The low percent of obstruction proportions started to reverse during the nineties. With the introduction of less invasive than myotomy-myectomy, but nonpharmacological therapies, DDD pacing [11] and alcohol septal ablation [12] were associated with more selected groups of patients (referral bias) and increasing types and numbers of provocative maneuvers. More varied stimuli were needed to more adequately qualify patients with obstruction.

2. History of LVOT Provoking Stimuli

Obstruction in HCM has been featured by dynamic nature [13, 14], in which pressure gradients can vary considerably with a variety (nonstandardized methodology) of pharmacological and physiological provocations that reduce peripheral arterial pressure or ventricular volume or enhance myocardial contractility and may change even after heavy meals or alcohol intake or spontaneously on a day-to-day or hour-to-hour basis [10, 13–21]. Mechanisms predisposing to LVOT gradient induction are presented in Figure 2. It was recognized that dynamic outflow gradients could be provoked by physiological exercise or a variety of nonphysiological maneuvers, including sympathomimetic agents, such as infused isoproterenol or dobutamine, or by introducing premature ventricular beats, amyl nitrite inhalation or nitroglycerin, and the Valsalva maneuver [10, 13–21]. The ground breaking point occurred in 2006, when Maron et al. proposed exercise as a provocation and therefore the obstructive group was as numerous as 70% [10].

Importantly, this study included a large number of patients. However, there are some disadvantages of the proposed method. The gradient provoked by exercised stress test was measured with delay in postexercise recovery and in semisupine position, instead of at peak exercise in upright position.

Earlier, in small selected group of patients, German [22] and Portuguese [23] investigators proposed pioneering full-upright exercise test. Researchers began to consider that we need natural, strong but physiological stimuli. The exercise test to provoke gradient has been postulated as a gold standard. Thus, the latest step of development was to combine two (perfectly physiological) stimuli, that is, upright position (reduced LV preload) and exercise by ergometer or treadmill [18, 20–31] (Figure 3). Standing position, both at rest and during exercise, is a normal and fundamental activity of daily life but may precipitate an unexpected fall in cardiac patients predisposed to syncope, especially in patients with unsuspected LVOT obstruction. Recently, a novel interesting combination was use of exercise test with postprandial status which additionally reduces LV preload [32].

In the most recent report, other types of stimuli have been combined—nitroglycerine and Valsalva to maximize provocation [33]. Proposed procedure is less time-consuming and does not require special equipment [33]. Looking for

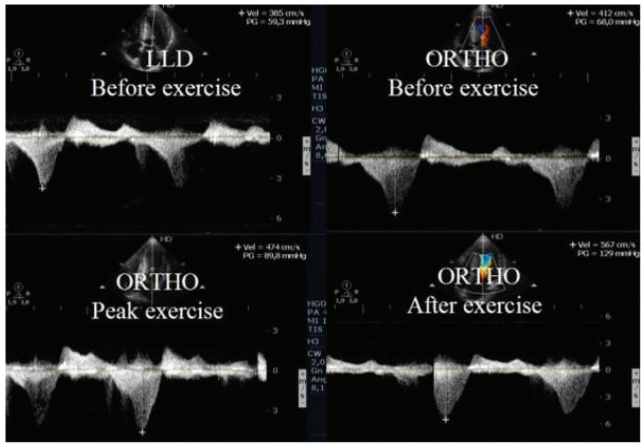

Figure 3: Intraventricular gradient present in all phases of the study in a HCM patient with increase also after exercise in orthostatic position.

new clinical applications, it has been proposed to use exercise stress echocardiography in the upright position with LVOT gradient monitoring both during and after exercise, as a marker of genotype-positive/phenotype negative hypertrophic cardiomyopathy (prehypertrophic status of HCM) [34]. Upright exercise to provoke (sub)valvular gradient has been used in many cardiac diseases [18].

3. Turbulent Flow and Activated Various Biomarkers

Apart from hemodynamic disturbances, obstructive HCM may induce several hematological abnormalities. Abnormalities in blood parameters associated with hemolysis and hypercoagulability status have been recently recognized.

Accordingly, in two Japanese articles [35, 36], the interesting biochemical phenomenon of hemolysis in hypertrophic cardiomyopathy (HCM) patients has been clearly documented, concerning only patients with the obstructive form. In the first case report [35], mechanical intracardiac hemolysis due to LVOT obstruction was advanced and hemolytic anemia recurred. Implanted DDD pacing decreased the LVOT gradient and reduced hemolysis. In the next article, stabile and provoked obstruction was associated with hemolysis [36]. Importantly, for everyday practice, this hemolysis appears to be associated also with LVOT obstruction, only provoked by daily physical activities. Although there was no relation between erythrocyte creatine levels and LVOT gradient in the subgroup of patients without obstruction at rest (before provoked stress test), a correlation between erythrocyte creatine and LVOT gradient provoked by Valsalva became significant in the subgroup of nonobstructive patients. Summing up, patients with higher erythrocyte creatine levels exhibited greater LVOT gradient with Valsalva provocation.

These results suggest that intravascular hemolysis is correlated with severity of LVOT obstruction not at rest, but with daily activities. Importantly, as a clinical implication, this biomarker may be useful for the identification of a subgroup of HCM patients with latent (none easily identified) obstruction. In perspective, we may postulate that measurement of this biomarker appears to be valuable for monitoring and management of HCM with not only stable, but especially interesting labile obstruction.

Hematological abnormalities were described in other studies, which clearly documented increased levels of biomarkers heralding hypercoagulation [37]. Moreover, acquired type of von Willebrand disease (type 2A) [38] has been recognized. It was postulated that rapidly turbulent blood flow within LVOT (narrow, irregular canal) obstruction plays a stimulating role in the activation of various biomarkers and hematological processes.

LVOT obstruction in HCM may provoke procoagulant mechanisms due to high shear stress. Both anatomical (systolic anterior motion of mitral leaflet with or without septal contact) and hemodynamical (high pressure gradient) abnormalities generating turbulent flow might play a role in the prothrombotic state [37]. The precise role of long and thickened mitral leaflets disturbing flow to nonlaminar pattern is an important question for further exploration of hematological disturbances.

Next, pathology in obstructive HCM [38] is the evidence of von Willebrand factor (vWF) impairment as frequent deficiency and closely related to the magnitude of LVOT obstruction. The problem is large in scale because resting LVOT gradient as low as 15 mmHg (common definition of obstruction is >30 mmHg) is absolutely sufficient to impair vWF. The proposed [38] interpretation of hemodynamic-hematologic phenomenon is attractive. Given the unique shear stress characteristics of vWF, it has been postulated that accelerated LVOT velocity at rest or during exercise might increase proteolysis of vWF multimers and impair primary hemostasis in the obstructive HCM. The biological effect of LVOT obstruction on vWF function is detectable in patients with either baseline or latent (exercise-provoked) obstruction. Primary hemostasis assays that assess vWF function impairment strongly correlate with the maximal LVOT gradient and improve with therapeutic intervention reducing gradient. Hemostasis impairment might be responsible for spontaneous bleeding in patients with an obstructive form of HCM.

In conclusion, the authors [38] have demonstrated that high shear forces could induce structural changes in the shape of the vWF molecule, facilitating the action of the specific vWF protease ADAMTS 13, which would lead to the loss of the high-molecular-weight multimer of vWF. Importantly, this process may be reversed by alcohol septal ablation [39] and septal myectomy [40] reducing LVOT gradient. Thus, we are able to correct hematological abnormalities by reduction of LVOT obstruction. In gastrointestinal bleeding as complication of Heyde's syndrome, thalidomide and octreotide therapy can be used to bridge to surgical or alcohol septal ablation [41].

According to other biochemical abnormalities, enhanced oxidative stress [42] and endothelial dysfunction [43] are also characteristics features for obstructive HCM.

4. Role of High Troponin

As regards biomarker of ischemic injury of myocardium, levels of serum cTnI are elevated in a significant proportion of obstructive HCM patients [44]. Serum cTnI is associated with multiple parameters of disease severity, suggesting its great significance in assessing cardiac remodeling in patients with obstructive HCM. In obstructive HCM left ventricular hypertrophy is the major determinant of serum cTnI levels [44] and high level of serum cTnT is associated with the presence of AF [45].

Serum cTnI is an independent predictor useful for identifying myocardial fibrosis visualized by late hyperenhancement of gadolinium in cardiac magnetic resonance [46].

5. Pharmacological Treatment: Negative Inotropic Effect

According to eminent expert Sherrid [47], inotropic negative effectiveness (power) is categorized as follows: beta-blocker > disopyramide > verapamil. Disopyramide possesses additional beneficial value, that is, vagolytic effect, acting as antibradycardia agent. This positive chronotropic effect of disopyramide may beneficially counterbalance the negative chronotropic effect of beta-blocker and together provide opportunity to safety combination of both drugs for aggressive, but only pharmacological reduction of LVOT gradient.

6. Nonpharmacological Option for Treatment

Generally, two invasive methods are strong competitors in current armamentarium. The comparison between surgical myotomy-myectomy and alcohol septal ablation (ASA) is most interesting, whereas DDD pacing is marginalized for rare indication. However in Spanish center [48] beneficial effect has been observed in 18-year-long follow-up study on DDD pacing. Sequential pacing in selected patients with obstructive HCM improves functional class and reduces dynamic gradient and mitral regurgitation immediately after pacemaker implantation and at final follow-up [48]. Prolonged ventricular pacing has no negative effects on systolic or diastolic function in these patients. Both surgical and transcoronary methods have dynamically developed. The long-term survival and clinical outcome of the ASA and surgical method are extensively debated in both USA and Europe. The crucial problem is safety of both invasive methods. Very recently, an important document has appeared [49], analyzing systematic review and meta-analysis of long-term outcomes after invasive septal reduction intervention therapy in obstructive HCM. In large-scale analysis, the authors have compared sixteen cohorts, including 2791 patients after myectomy (mean follow-up: 7.4 years) versus 11 ASA cohorts with 2013 patients (mean follow-up: 6.2 years). Importantly, long-term mortality and (aborted) SCD rates after ASA and myectomy are similar and importantly low. Patients who undergo ASA have more than twice the risk of permanent pacemaker implantation and 5 times higher risk of the need for additional septal reduction therapy, compared to those who undergo myectomy. Also, other systematic analysis [50] confirmed no significant difference in symptom relief between the two approaches. ASA was as safe as myectomy with regard to SCD and short-term and long-term mortality. The benefit from ASA has been very recently confirmed by Veselka et al. [51] studies, suggesting that, in patients with obstructive HCM and important symptoms who underwent ASA, long-term survival after the procedure did not differ significantly from that of the general population. Additionally, Veselka et al. [52] have documented that results of the first European multicenter study demonstrated that real-world early outcomes of ASA patients were better than had been reported in earlier observations from the first decade after ASA introduction. Finally, in the newest, large-scale multinational Euro-ASA registry with 1275 patients, Veselka et al. [53] have demonstrated low periprocedural and long-term mortality after ASA. This intervention provided significant relief of symptoms and a reduction of LVOT obstruction.

The American College of Cardiology Foundation/American Heart Association guidelines reserve ASA for elderly patients and patients with serious comorbidities but recent study [54] changes the clinical perspective. Accordingly, ASA is similarly effective for reduction of symptoms in young and elderly patients; however, younger patients have a lower risk of procedure-related atrioventricular conduction disturbances. The long-term mortality rate and risk of adverse arrhythmic events following ASA are low, in both young and elderly patients, and are comparable to age-matched nonobstructive HCM patients.

From pathophysiological point of view, LVOT obstruction and subsequent mitral regurgitation appear to further impair left atrial functional mechanics [55]. Importantly, septal myectomy has significantly reduced left atrial volumes, paralleled by an improvement in hemodynamic function [55].

7. From Technical Point of View in Both Surgical and ASA Procedures Novel Invasive Interventions Have Been Very Recently Developed

Myectomy cannot be performed in 5–15%, due to technical difficulties. Also ASA methodology has several limitations, which are currently overcome. The newest 10 innovative procedures have been discussed.

7.1. 1st Innovation. Modification of surgical approach: exposure of the basal and midventricular septum through the aortic root to relieve obstruction in HCM can be challenging. Inadequate myectomy will lead to persistent symptoms and disability. Adequate exposure of the obstructive left ventricular septum is of paramount importance in primary and secondary corrected myectomy. In selected patients, either at primary myectomy or at remyectomy, septal excision can be approached through a left ventricular transapical incision when transaortic exposure is inadequate [56]. Transapical

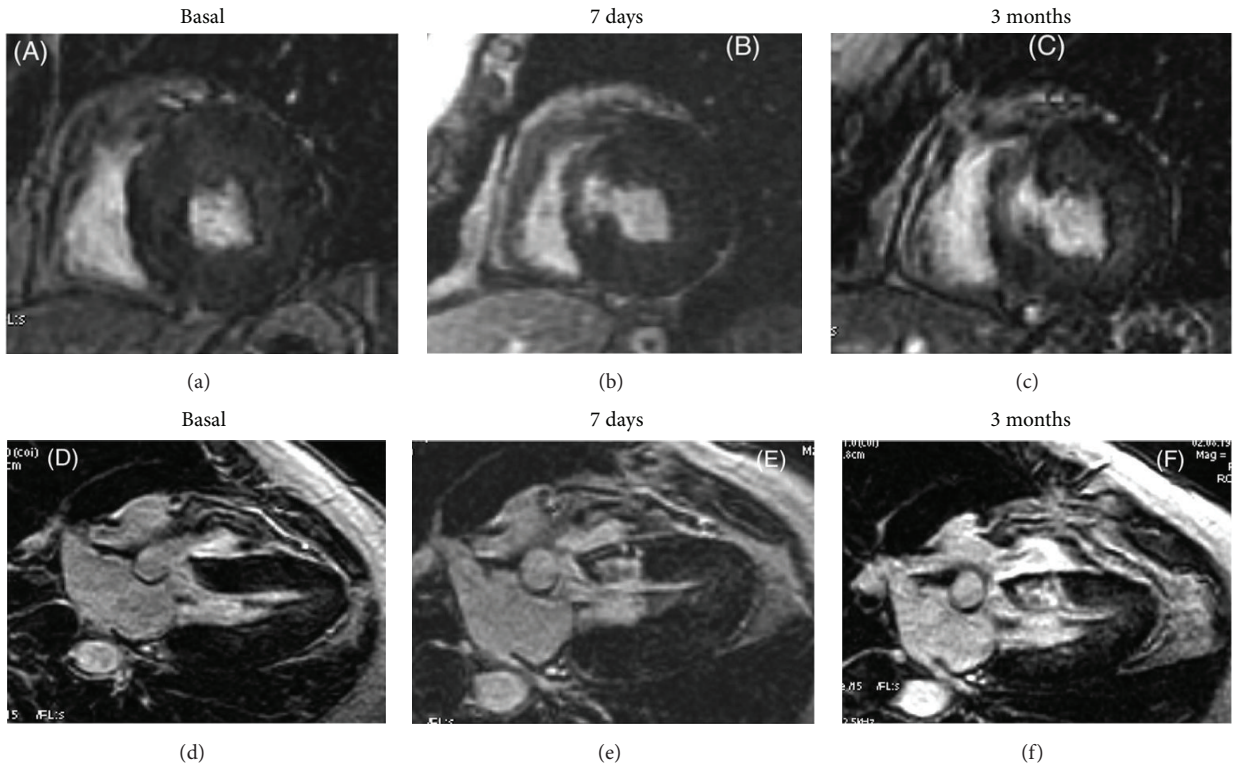

Figure 4: Cardiac magnetic resonance imaging before and after septal reduction therapy (myocardial scar after blockage of septal perforator artery by coil). Example of extension of delayed contrast-enhanced images of patchy areas of hyperenhanced myocardial in the interventricular septum 7 days (b, e) and 3 months (c, f) when compared with baseline (a, d). (a–c) Shot axis view. (d–f) Three-chamber view (reprinted with permission from [65]).

myectomy may be suitable approach for severe midventricular obstructive HCM [57].

7.2. 2nd Innovation. In surgical approach a very novel intervention is transaortic chordal cutting with mitral valve repair for obstructive HCM with mild septal hypertrophy [58]. This procedure relieves heart failure symptoms, abolishes LVOT gradient, and avoids mitral valve replacement in patients with obstructive HCM and mild septal thickness.

7.3. 3rd Innovation. There is strong evidence that treating the mitral valve abnormalities is a key feature of obstructive HCM. Investigators [59] successfully corrected both the anterior and posterior leaflet size and geometry. The proposed technique is double-stage procedure: first septal resection through the aortic valve and the detached anterior leaflet of the mitral valve and second mitral valve repair by reducing posterior leaflet height (leaflet resection or artificial neochordae) and increasing anterior leaflet height with pericardial patch.

7.4. 4th Innovation. The new concept has been also developed on percutaneous technique [60–62]. It was reported on catheter-based treatment of LVOT obstruction, targeting primarily a systolic anterior motion of the anterior mitral leaflet in obstructive HOCM. A patient was successfully treated with the MitraClip [60] two years after septal myectomy, in conjunction with mitral valve repair. The results prove the concept that systolic anterior motion (SAM) is clearly involved in gradient formation and is more than an epiphenomenon in obstructive HCM. Thus, SAM-induced subaortic obstruction might be a target for MitraClip implantation.

7.5. 5th Innovation. The transradial approach using a sheathless guiding catheter has appeared feasible and safe for ASA [63].

7.6. 6th Innovation. Peri-intervention monitoring of labile LVOT gradient is very practical. The intravenous nitroglycerin test during ASA is a useful method for rapidly confirming acute reduction of the latent gradient after the ASA procedure, and the outcome of ASA for labile obstruction was favorable [64].

7.7. 7th Innovation. It consists in nonsurgical septal myocardial reduction (ischemic scar by coil embolization into septal perforator artery) [65] (Figure 4).

7.8. 8th Innovation. Glue septal ablation is a promising technique for the reducing LVOT obstruction [66]. Glue seems to be superior to alcohol due to some intrinsic advantageous

properties of glue such as immediate polymerization which prevents the leak into the left anterior descending coronary artery. Glue septal ablation technique is particularly useful in patients with collaterals to the right coronary artery in whom alcohol ablation is risky and contraindicated.

7.9. 9th Innovation. Improved renal function in a patient with obstructive HCM after multidetector computed tomography-guided ASA was witnessed [67].

7.10. 10th Innovation. Cooper et al. [68] have proposed that method of delivering percutaneous tissue damage to the septum that is not reliant on coronary anatomy is desirable. To directly ablate the interventricular septum at the mitral valve systolic, anterior motion-septal contact point using radiofrequency energy guided by CARTOSound was shown. The authors have postulated that radiofrequency ablation using CARTOSound guidance is accurate and effective in treating LVOT gradients in HCM.

8. Phenocopies with LVOT Gradient with Successful ASA Treatment

Useful for everyday practice are reports of invasive reduction of LVOT gradient in diseases mimicking HCM as phenocopies. This problem is associated with storage and infiltrative disease. Using ASA method, LVOT gradient was effectively reduced in Fabry disease [69] and amyloidosis [70]. Surgical correction was successfully performed in Noonan disease [71]. Septal myectomy may be a viable option to relieve symptoms and interrupt progression of heart disease also in selected Friedreich's ataxia patients [72].

9. Conclusion

From a practical point of view, the effective monitoring, including biochemical biomarkers indicating reduction of LVOT gradient, is important because this parameter has become a risk factor for sudden death in the 2014 ESC guideline [73]. In this context, the maximized physiological LVOT gradient provocation stimulus seems to be an important diagnostic element. Novel invasive therapeutic techniques have been recently dynamically developed, providing attractive treatment opportunities.

Competing Interests

The authors declare that they have no competing interests.

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Research Article

Substance P Receptor Signaling Mediates Doxorubicin-Induced Cardiomyocyte Apoptosis and Triple-Negative Breast Cancer Chemoresistance

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Doxorubicin (DOX), an anthracycline, is broadly considered the most active single agent available for treating breast cancer but has been known to induce cardiotoxicity. Although DOX is highly effective in treating triple-negative breast cancer (TNBC), DOX can have poor outcomes owing to induction of chemoresistance. There is an urgent need to develop new therapies for TNBC aimed at improving DOX outcome and DOX-induced cardiotoxicity. Substance P (SP), a neuropeptide involved in pain transmission is known to stimulate production of reactive oxygen species (ROS). Elevated cardiac ROS is linked with heart injury and failure. We investigated the role of SP in chemotherapy-associated death of cardiomyocytes and chemoresistance. We showed that pretreating a cardiomyocyte cell line (H9C2) and a TNBC cell line (MDA-MB 231) with aprepitant, a SP receptor antagonist that is routinely used to treat chemotherapy-associated associated nausea, decreased DOX-induced reduction of cell viability, apoptotic cell death, and ROS production in cardiomyocytes and increased DOX-induced reduction of cell viability, apoptotic cell death, and ROS production in TNBC cells compared with cells treated with DOX alone. Our findings demonstrate the ability of aprepitant to decrease DOX-induced killing of cardiomyocytes and to increase cancer cell sensitivity to DOX, which has tremendous clinical significance.

1. Introduction

Doxorubicin (DOX), an anthracycline, is broadly considered the most active single agent available for treating breast cancer [1–3]. However, DOX has been known to induce cardiotoxic side effects such as electrocardiographic changes, decreased left ventricular ejection fraction values, and life-threatening heart failure or acute coronary syndromes in some patients [4–6]. There is an urgent need to develop new noncardiotoxic therapies. Importantly, DOX often also induces chemoresistance. For example, though DOX is highly effective in treating triple-negative breast cancer (TNBC; lacking 3 of the hormone/molecular receptors/markers, ER2, PR2, Her-22), DOX can have poor outcomes owing to induction of chemoresistance [7, 8]. Breast cancer is one of the leading causes of cancer-associated death in women. In the United States alone, each year, more than 260,000 new cases of breast cancer are diagnosed, and more than 40,000 breast cancer-associated deaths occur [9, 10]. Given the lack of validated molecular targets and the poor outcomes in TNBC, there is an urgent need for new therapies to prevent chemoresistance.

Substance P (SP), an 11-amino acid neuropeptide involved in pain transmission, is made by and elicits response from nerves, endothelial cells, and cells of the immune system [11–17]. SP mediates pain, neurogenic inflammation, and

mitogenesis via interaction with its high-affinity receptor NK-1R, which is widely distributed throughout the body. SP stimulates production of reactive oxygen species (ROS) [18, 19]. Elevated cardiac ROS is linked with heart, injury, and failure in other cardiac settings [20, 21]. We have previously demonstrated that SP was elevated in hearts of mice infected with the encephalomyocarditis virus, which causes viral myocarditis [22]. We also have previously shown that, in mice infected with a parasite that causes cysticercosis and in those infected with encephalomyocarditis virus, the mortality, heart weight to body weight ratios, cardiomyocyte, hypertrophy, and apoptosis were significantly higher than that of uninfected mice. In contrast, SP-deficient mice and/or NK-1R antagonist-treated animals were protected against all these effects [22]. Other groups have shown that SP is involved in inducing chronic volume overload-induced heart failure and that deletion of the SP gene protected mice from developing left ventricular hypertrophy in the form of ventricular dilatation [23]. Furthermore, studies have shown that animals with magnesium deficiency had higher SP levels in their cardiac lesions than did normal animals [24] and that blockade of NK-1R significantly reduced ROS production in cardiac cells and improved diastolic and systolic function in these animals [24, 25]. These findings suggest that elevated SP can be detrimental to the heart and that NK-1R antagonism can be used to treat SP-induced cardiac manifestations.

SP and NK-1R have been detected in tumor cells and in intra- and peritumoral blood vessels [26–28]; furthermore, SP has been shown to protect tumor cells from apoptosis [29]. The relevance of the SP/NK-1 receptor system has been specifically shown in pancreatic cancer, where SP is involved in pancreatic cancer proliferation, neoangiogenesis, and migration of pancreatic cancer cells and SP receptor antagonism has been shown to reverse these alterations [26, 29–31]. These findings suggest that elevated SP can be detrimental in cancer and suggest that NK-1R antagonism can be beneficial in cancer treatment.

We hypothesized that SP plays a role in chemotherapy-associated death of cardiomyocytes and in chemoresistance. In order to test this hypothesis, we determined the effects of aprepitant, an antagonist of SP receptor (neurokinin 1 receptor [NK-1R]) that is routinely used to treat chemotherapy-associated nausea, on DOX-induced reduction of cell viability, apoptotic cell death, and ROS production by cardiomyocytes and TNBC cells. We showed that pretreating a cardiomyocyte cell line (H9C2) and a TNBC cell line (MDA-MB 231) with aprepitant decreased DOX-induced reduction of cell viability in the cardiomyocytes and increased DOX-induced reduction of cell viability in TNBC cells compared to cells treated with DOX alone. Also, the levels of apoptotic cell death and ROS in response to DOX were decreased in aprepitant pretreated cardiomyocyte cells and were increased in aprepitant pretreated TNBC cells compared to the untreated cells.

Currently, no studies have investigated SP as a mediator of chemotherapy-induced cardiotoxicity or the role of SP antagonism as a synergistic mechanism to enhance chemotherapy's ability to kill resistant TNBC cells. These studies show that SP plays a dual detrimental role in DOX-associated killing of cardiomyocytes and induction of chemoresistance in TNBC and has implications for tremendous future clinical translational relevance. These studies may lead to use of SP receptor antagonism, for prevention of DOX-mediated toxicity and at the same time for increment of antitumor effects of DOX for TNBC and probably other cancers.

2. Material and Methods

2.1. Cell Culture and Chemicals. Rat cardiomyocyte cell line, H9C2, and human TNBC breast cancer cell line, MDA-MB-231, were purchased from the American Type Culture Collection (Manassas, VA). Culture media, antibiotics, fetal bovine serum, and other supplements were bought from Invitrogen (Carlsbad, CA). Cells were maintained in complete media with 10% fetal bovine serum, antibiotics (streptomycin and penicillin), an antifungal agent (amphotericin B), GlutaMAX (Thermo Fisher Scientific, Waltham, MA), and pyruvate and were not passed continuously more than 4 weeks. Doxorubicin and aprepitant (resp., 15007 and 4867) were purchased from Cayman Chemicals, Ann Arbor, Michigan.

2.2. MTT Assay. To determine the effect of aprepitant on cell proliferation, we measured cell viability using the MTT assay. Following treatment with the indicated concentrations of DOX with and without aprepitant or with aprepitant or media or vehicle alone, cells were dispersed by trypsinization and seeded at 8,000–10,000 cells/well in a 96-well plate overnight before being treated. Subsequently, MTT (1 mg/mL) in medium with 1% serum was added to each well, and the wells were incubated for 2 h at 37°C. An extraction buffer (20% sodium dodecyl sulfate and 50% dimethylformamide) was added, and the cells were incubated overnight at 37°C. The optical density was measured at 590 nm using a 96-well multiscanner (Molecular Devices, Sunnyvale, CA). The proliferating capacity of the cell was measured by dividing the viability at a certain experimental condition by the viability of corresponding controls (media or vehicle control). Data are presented as percentage viability related to untreated cells ± SEM for each group.

2.3. TUNEL Assay. The effect of aprepitant on cell death was determined by measuring levels of apoptotic cells using the TUNEL assay. The ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (EMD Millipore, Billerica, MA) was used to detect apoptotic cells, according to the manufacturer's instructions [32]. Briefly, following treatment with the indicated concentrations of DOX with and without aprepitant or with aprepitant or media or vehicle alone, cells on tissue culture chamber slides were fixed in 1% paraformaldehyde in phosphate-buffered saline solution (PBS, pH 7.4) for 10 min at room temperature, followed by 2 washes with 1x PBS for 5 min each wash. Samples were postfixed in precooled ethanol : acetic acid (2 : 1) for 5 min at −20°C to subject the cells to permeabilization. Cells were then quenched in 3.0% hydrogen peroxide in PBS for 5 min at room temperature, were rinsed twice with PBS or distilled water for 5 min each time, and were treated with equilibration buffer for 1 min. The equilibration buffer was then drained, and the

cells were treated with terminal deoxynucleotidyl transferase enzyme for 1 h at 37°C. The cells were then treated with stop-wash buffer and were washed with PBS 3 times (1 min each wash) followed by treatment with antidigoxigenin conjugate (30 min, room temperature (RT)), 1 wash with PBS (1 min, RT), treatment with peroxidase substrate (3–6 min, RT), and 3 washes with distilled water (1 min each). The samples were counterstained with hematoxylin, were mounted, and were viewed under a light microscope. The number of TUNEL-positive nuclei was counted in 10 randomly chosen high-power fields (400x) of each slide by an experienced microscopist blinded to the study design. The percentage of positive cells was calculated. Data are presented as percentage of positive cells ± SEM for each group.

2.4. ROS Measurement. The effect of aprepitant on ROS production was determined by measuring ROS levels by the dichlorofluorescein diacetate (DCFDA) assay. Following treatment with the indicated concentrations of DOX with and without aprepitant or with aprepitant or media or vehicle alone, cells were stained with DCFDA (5 μM) for 30 min at 37°C in the dark. ROS production was determined by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm, respectively. Data are presented as fluorescence intensity ± SEM for each group.

2.5. Quantitation of SP Protein. To determine whether DOX treatment increased SP levels in cardiomyocytes and cancer cells, we treated H9C2 and MDA-MB 231 cells with their respective median inhibitory concentration (IC_{50}) doses of DOX and then determined SP levels in cell lysates. Quantitation of SP protein was performed as described previously [33]. Briefly, cells with and without DOX treatment were washed once and then reconstituted with cold 1x PBS containing protease inhibitor cocktail (04 693 132 001, Roche, Indianapolis, IN). Cells were scraped, were spun at 1500 rpm for 10 min, were reconstituted in lysis buffer (43-040, Cell Signaling, Danvers, MA), and were incubated on ice for 15 min. The lysed cells were then spun at 12,000 rpm for 15 min, and the supernatant was used for SP quantitation by using an enzyme-linked immunosorbent assay kit from Enzo Life Sciences (ADI-900-018, Farmingdale, NY). Total protein was quantified using the Bradford method (500-0006, Bio-Rad, Hercules, CA). Results are expressed as picogram of SP per milligram of total protein ± SEM for each group.

2.6. Statistical Analyses. Data presented are mean ± SEM of a minimum of 2 experiments. Statistical differences were determined using analysis of variance (ANOVA), followed by Tukey's or Dunn's posttest as appropriate or by Student's unpaired t-test. Statistical significance was set at $p \leq 0.05$. Data and statistical analysis were performed using Graph Pad Prism version 6.04 for Windows, Graph Pad Software (San Diego, CA).

3. Results

3.1. SP Receptor Antagonism Prevents DOX-Induced Reduction in Cardiomyocyte Viability. In order to determine if SP receptor antagonism prevents DOX-induced reduction in cardiomyocyte viability, H9C2 cells were seeded in a 96-well plate (10,000 cells per well); after 24 hrs, duplicate wells were treated with different concentrations of DOX (ranging from 0.03 μm to 100 μm) with and without aprepitant pretreatment (0.03 μm, 2 hrs before DOX treatment). Control wells included treatment with the corresponding concentrations of vehicle (DMSO) used for reconstituting the aprepitant (0.0005% DMSO in water). Also included was a group of wells treated with aprepitant alone. All experiments were performed at least twice and results are expressed as mean ± SEM, unless otherwise indicated. We determined that aprepitant pretreatment decreased the DOX-induced loss of cell viability compared with DOX alone. The median inhibitory concentration (IC_{50}) of DOX was 1.46 μm ± 0.4 μm; aprepitant pretreatment led to a 3-fold increase in the IC_{50} levels to 4.23 μm ± 0.8 μm (Figures 1(a) and 1(b); $p < 0.05$, ANOVA, $n = 2$).

3.2. SP Receptor Antagonism Reverses Chemoresistance of MDA-MB 231 TNBC Cells. To determine if SP receptor antagonism is beneficial in decreasing chemoresistance of TNBC cells, we determined whether aprepitant is beneficial in increasing the DOX-mediated killing of MDA-MB 231 TNBC cells. DOX and aprepitant treatment were as above. All results are expressed as mean ± SEM, unless otherwise indicated. We determined that aprepitant pretreatment increased the DOX-induced killing compared with DOX alone. The IC_{50} of DOX was 2.74 μm ± 0.05 μm; aprepitant pretreatment led to a 3.14-fold decrease in the IC_{50} levels to 0.87 μm ± 0.10 μm (Figures 1(c) and 1(d); $p < 0.05$, ANOVA, $n = 2$).

3.3. SP Receptor Antagonism Led to Decreased Levels of Apoptosis of H9C2 Cardiomyocytes. To determine whether the protective effects of SP antagonist pretreatment on reduction of DOX-induced reduction of viability of cardiomyocytes was accompanied by decreased levels of apoptosis, we determined the levels of apoptotic cells in response to DOX in aprepitant pretreated versus untreated H9C2 cardiomyocytes. We determined that aprepitant pretreatment reduced the DOX-induced level of apoptotic TUNEL-positive cardiomyocytes by 7-fold compared to DOX alone (Figures 2(a)–2(c)). The percentage of positive apoptotic cells in the DOX alone group was 24%± 1%; aprepitant pretreatment reduced the percentage of positive apoptotic cells to 3.5%± 0.5% (Figure 2(d), $p < 0.05$, ANOVA, $n = 2$). Control groups (media, vehicle, and aprepitant alone groups) did not have any positive apoptotic cells.

3.4. SP Receptor Antagonism Led to Increased Levels of Apoptosis of MDA-MB 231 TNBC Cells. To determine whether the beneficial effects of SP antagonist pretreatment on increment of DOX-induced reduction of viability of MDA-MB 231 TNBC cells were accompanied by increased levels of apoptosis, we determined the levels of apoptotic cells in response to DOX in aprepitant pretreated versus untreated MDA-MB 231 TNBC cells. We determined that aprepitant pretreatment increased the DOX-induced level of apoptotic TUNEL-positive cells by 3-fold compared to DOX alone

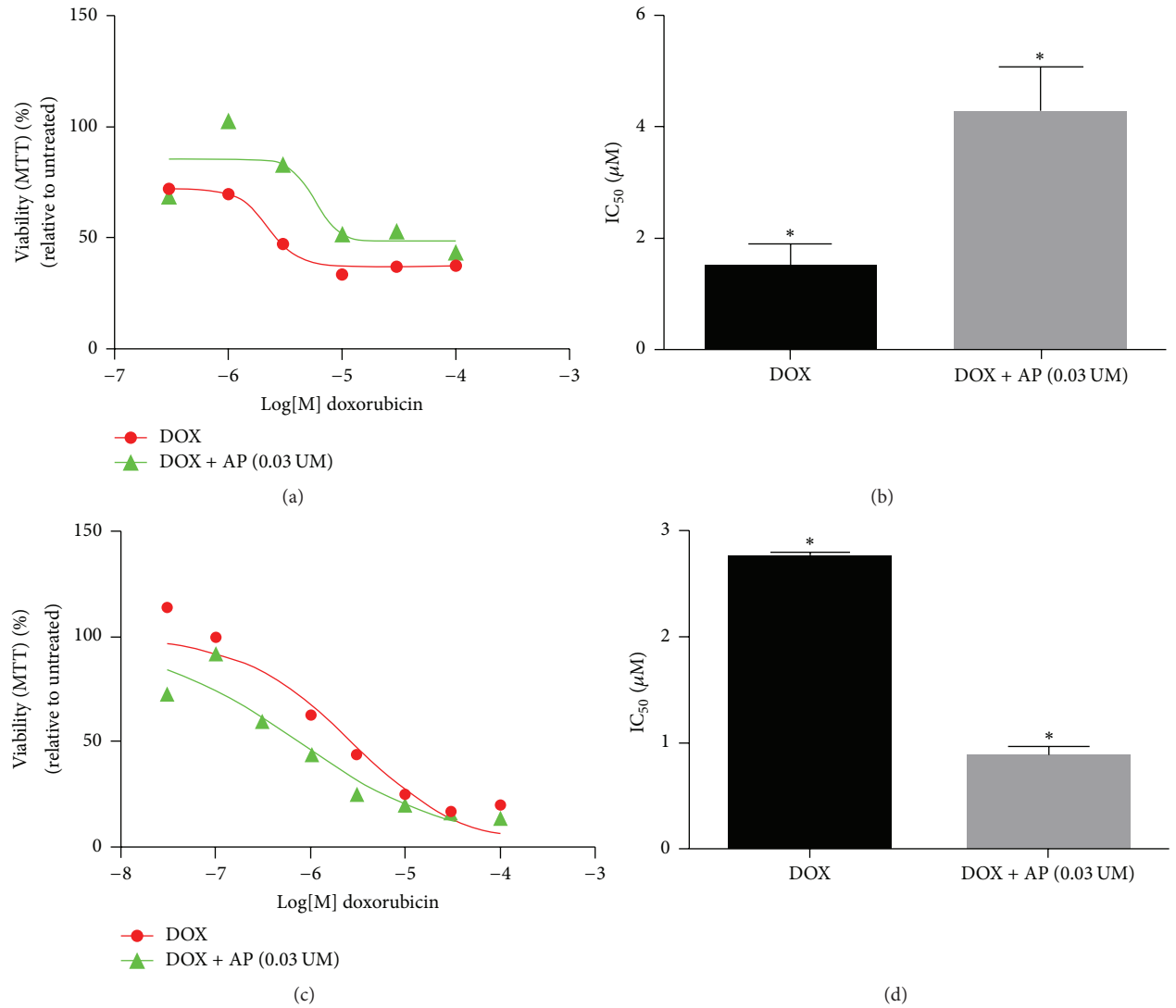

Figure 1: Effect of substance P receptor antagonist pretreatment on doxorubicin-induced cardiomyocyte growth inhibition and triple-negative breast cancer cell chemoresistance. Levels of viability as determined by the MTT assay, in response to DOX in aprepitant pretreated versus untreated rat H9C2 cardiomyocytes (a and b) and MDA-MB 231 TNBC cells (c and d) ($^*p \leq 0.05$, ANOVA, $n = 2$, for both).

(Figures 2(e)–2(g)). The percentage of positive apoptotic cells in the DOX alone group was 17% ± 7%; aprepitant pretreatment increased the percentage of positive apoptotic cells to 49 ± 3 (Figure 2(h), $p < 0.05$, ANOVA, $n = 2$). Control groups (media, vehicle, and aprepitant alone groups) did not have any positive apoptotic cells.

3.5. SP Receptor Antagonism Inhibits DOX-Induced ROS Production by H9C2 Cardiomyocytes. To determine whether the protective effects of SP antagonism on DOX-induced killing of cardiomyocytes were accompanied by decreased ROS levels, we determined the levels of ROS in response to DOX in pretreated versus untreated H9C2 cardiomyocytes. We determined that aprepitant pretreatment decreased DOX-induced ROS production compared with DOX alone. The level of ROS as seen by fluorescence intensity produced by 24,000 cells in response to DOX alone was 2804 ± 601.5 units, whereas aprepitant pretreatment led to a 4.3-fold decrease in levels of ROS to 651.5 ± 259.5 units (Figure 3(a), $p < 0.05$, ANOVA, $n = 2$).

3.6. SP Receptor Antagonism Led to Increased Levels of ROS in Response to DOX in MDA-MB 231 TNBC Cells. To determine whether SP antagonist pretreatment induced increased DOX-induced killing of TNBC's was accompanied by increased ROS levels, we determined the levels of ROS in response to DOX in aprepitant pretreated versus untreated MDA-MB 231 TNBC cells. We determined that aprepitant pretreatment increased DOX-induced ROS production in the MDA-MB 231 TNBC cells compared with DOX alone. The level of ROS as seen by fluorescence intensity produced by 24000 cells in response to DOX alone was 5856 ± 372 units, whereas

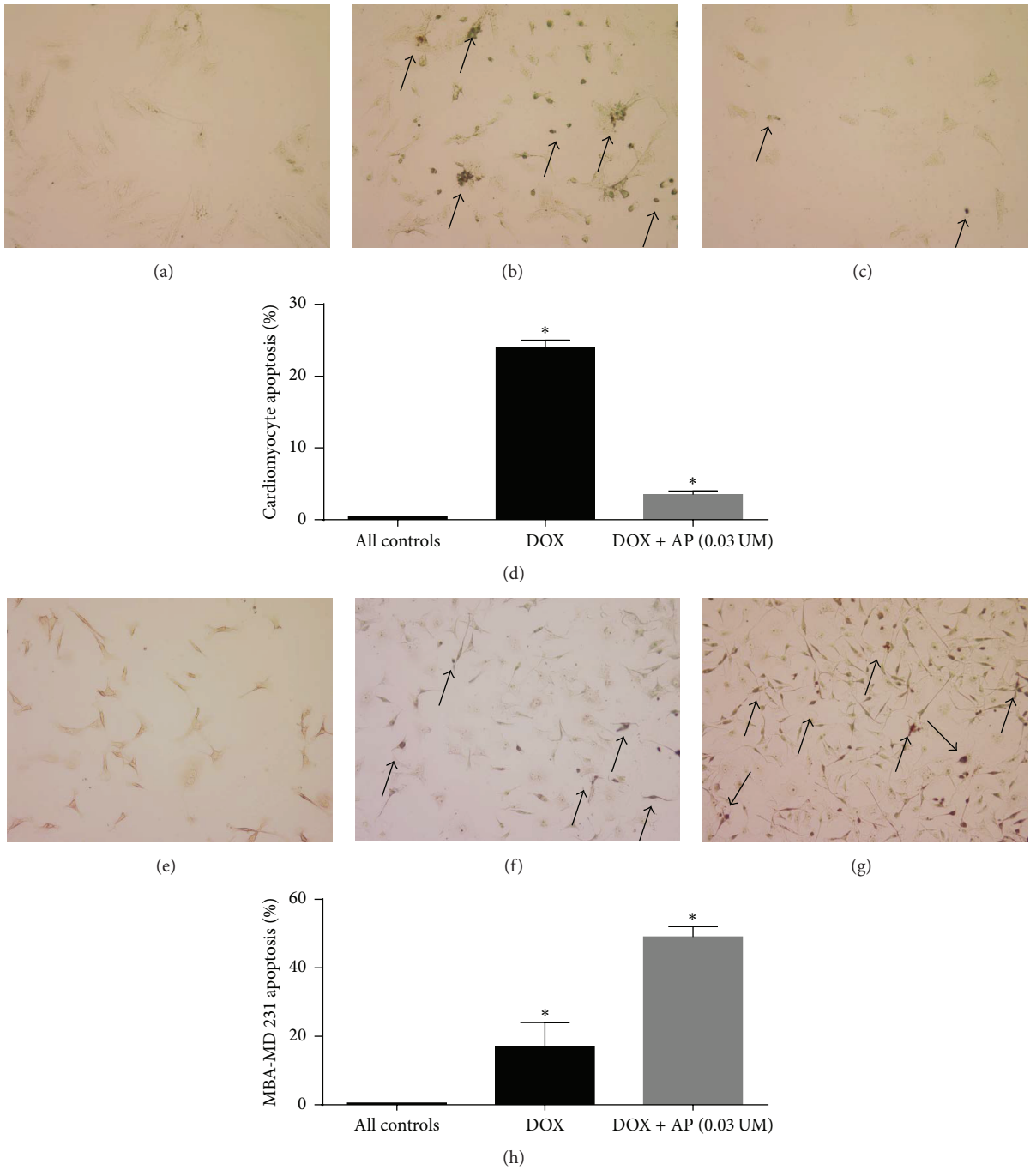

Figure 2: Effect of substance P receptor antagonist pretreatment on doxorubicin-induced apoptosis of cardiomyocytes and triple-negative breast cancer cells. Levels of apoptosis as determined by the TUNEL assay, in response to DOX in aprepitant pretreated versus untreated rat H9C2 cardiomyocytes and MDA-MB 231 TNBC cells. A photomicrograph of H9C2 cells from (a) control aprepitant treated, (b) DOX-treated, and (c) DOX + aprepitant pretreated cells showing numerous strongly positive brown apoptotic nuclei in the DOX group and very few faintly positive nuclei in the DOX + aprepitant group (arrows depict positive cells, original magnification 200x). (d) Number of apoptotic H9C2 cells in the 2 experimental groups and all control groups (media, vehicle, and aprepitant alone groups, all showing no positive apoptotic cells) ($^{*}p \leq 0.05$, ANOVA, $n = 2$). Only statistical comparisons between DOX and DOX + AP are shown. A photomicrograph of MBA-MD 231 cells from (e) control aprepitant treated, (f) DOX-treated, and (g) DOX + aprepitant pretreated cells showing numerous strongly positive brown apoptotic nuclei in the DOX + aprepitant group versus the group treated with DOX alone (arrows depict positive cells, original magnification 200x). (h) Number of apoptotic MBA-MD 231 cells in the 2 experimental groups and all control groups (media, vehicle, and aprepitant alone groups, all showing no positive apoptotic cells) ($^{*}p \leq 0.05$, ANOVA, $n = 2$). Only statistical comparisons between DOX and DOX + AP are shown.

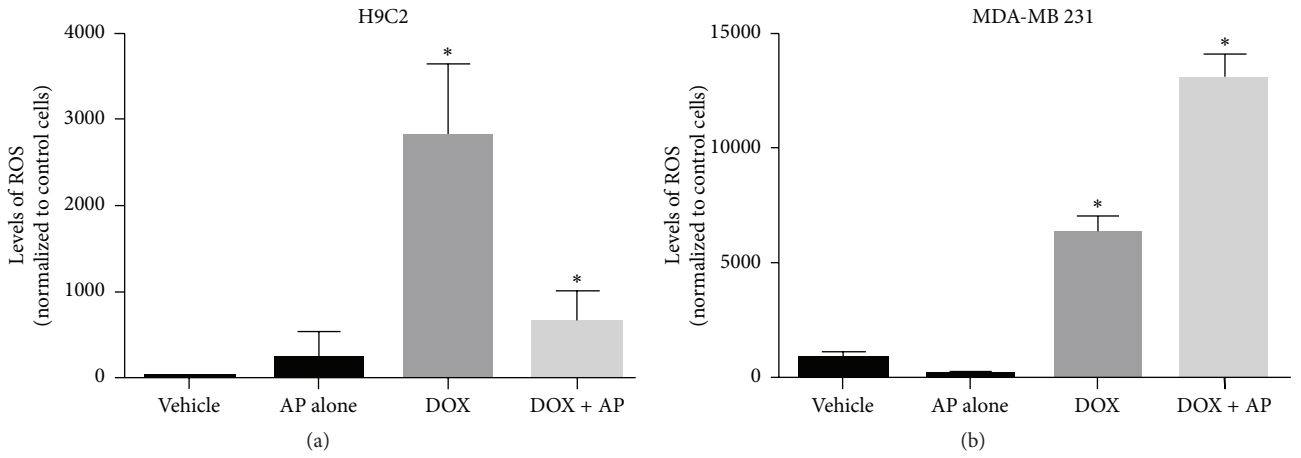

Figure 3: Effect of substance P receptor antagonist pretreatment on doxorubicin-induced, reactive oxygen species (ROS) production in cardiomyocytes and triple-negative breast cancer cells. Levels of ROS as determined by the DCFDA assay, in response to DOX in aprepitant pretreated versus untreated rat H9C2 cardiomyocytes (a) and MDA-MB 231 TNBC cells (b) ($^{*}p \leq 0.05$, ANOVA, $n = 2$, for both). Only statistical comparisons between DOX and DOX + AP are shown.

aprepitant pretreatment led to a 2.7-fold increase in levels of ROS to 13828 ± 137.5 units (Figure 3(b), $p < 0.05$, ANOVA, $n = 2$).

3.7. DOX Increases SP Levels in Both H9C2 Cardiomyocytes and MDA-MB 231 TNBC Cells. To confirm that aprepitant-mediated effects were mediated via SP, we investigated whether DOX treatment increased SP levels in cardiomyocytes and cancer cells. We treated H9C2 and MDA-MB 231 cells with their respective IC_{50} dose of DOX (i.e., 1.5 μm for H9C2 and 2.74 μm for MDA-MB 231 cells) and then determined SP levels by ELISA in the cell lysates. We determined that DOX-treated H9C2 cells and MDA-MB 231 cells, respectively, had a 2.2-fold and a 4-fold increase in the level of SP compared to that of untreated cells (H9C2: DOX-treated, 156 ± 7 pg; untreated, 39 ± 5 pg; $p \leq 0.05$) (MDA-MB 231: DOX-treated, 293 ± 88 pg; untreated, 149 ± 19 pg, $p \leq 0.05$, t-test for both, $n = 2$ for both, Figure 4).

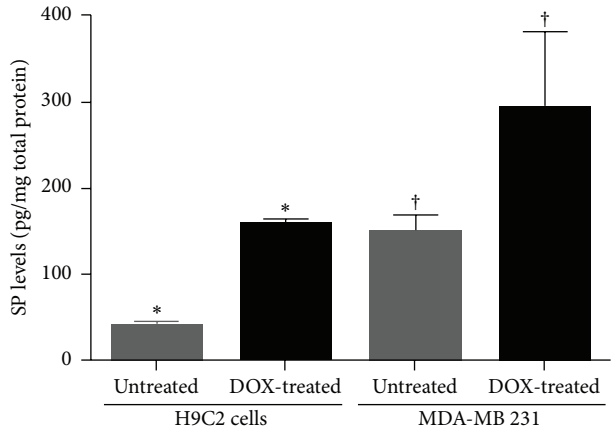

Figure 4: Levels of SP are increased in response to DOX in cardiomyocytes and TNBC cells. Levels of SP as determined by ELISA, in H9C2 and MDA-MB 231 cells with and without DOX treatment ($^{*,\dagger}p \leq 0.05$, t-test, $n = 2$).

4. Discussion

Breast cancer is one of the leading causes of cancer-associated death in women [2]. Although DOX has shown to be highly effective in treating TNBC, it has a poor outcome owing to induction of resistance. Most importantly, DOX is associated with induction of cardiotoxicity in many patients. There is an urgent need to develop new noncardiotoxic therapies for cancer or prevent DOX-mediated toxicity without reducing its antitumor effects.

In the current paper, we determined the role of SP in chemotherapy-associated death of cardiomyocytes and chemoresistance of TNBC cells. We showed that pretreating a cardiomyocyte cell line (H9C2) and a TNBC cell line (MDA-MB 231) with aprepitant, a SP receptor antagonist that is routinely used to treat chemotherapy-associated nausea, decreased DOX-induced reduction of viability, apoptotic cell death, and ROS production in cardiomyocytes and increased DOX-induced reduction of viability, apoptotic cell death, and ROS production in TNBC cells compared with cells treated with DOX alone. These studies show that SP plays a dual detrimental role in induction of DOX-associated killing of cardiomyocytes and induction of chemoresistance in TNBC.

We do not know the mechanism by which SP enhances chemotherapy-associated killing of cardiomyocytes or induces chemoresistance. DOX is known to induce cardiotoxicity via induction of DNA double-strand breaks. Studies have shown that inhibiting the enzyme topoisomerase IIB (TOP-IIB), which is responsible for unwinding supercoiled DNA strands during replication, prevents DOX-induced cardiotoxicity [34]. We speculate that the mechanism by which SP mediates DOX-induced cardiotoxicity may be via the SP/NK-1R pathway-associated molecules Rac1 (Ras-related C3 botulinum toxin substrate 1) and Nur77 (nerve growth factor IB). Both molecules are associated with apoptotic cell death linked to TOPIIB [35] and/or nonapoptotic cell death

[32, 36–39]. We speculate that the mechanism by which SP mediates DOX-induced chemoresistance in TNBC may be via activation of Forkhead box protein M1 (FOXM1, a transcription factor that is linked to increased survival of tumor cells) and programmed cell death 1 (PD-1, an immune surveillance escape factor) [33, 40, 41]. In future studies, we aim to determine the exact mechanisms involved in both SP-induced events. As part of future studies, we will determine levels of TOPIIB, Rac1, and Nur77 in H9C2 cells and levels of FOXM1 and PD-1 in TNBC cells, treated with and without DOX and/or aprepitant. Also, as part of our future studies we will determine whether a new synergistic therapy consisting of aprepitant + DOX will prevent chemotherapy-associated cardiotoxicity and improve the outcome of TNBC using in vivo murine models of cardiotoxicity and TNBC.

Although ours and other studies using murine viral, parasitic, and other heart failure models have shown that elevated SP can be detrimental to the heart and can cause cardiac manifestations such as dilated cardiomyopathy and chronic volume overload-induced heart failure [22–25], there are no studies that demonstrate increased cardiac SP and its high-affinity receptor, NK1, in malignancy or doxorubicin therapy. Most importantly, currently no studies have investigated SP as a mediator of chemotherapy-induced cardiotoxicity.

Similarly, other studies using murine, pancreatic cancer models, human breast cancer tissues, and in vitro pancreatic cell lines and TNBC cell lines have shown elevated SP and or NK-1R expression, linked to proliferation, migration, and cancer metastases [26–31, 42–48]. Furthermore, very important studies by Dr. Munoz's group have shown that SP receptor antagonist aprepitant has antitumor action against breast cancer cell lines and also that doxorubicin has synergic effect with aprepitant against human hepatoblastoma cell lines [48, 49]. Although the above studies demonstrate the importance of SP/NK-1R pathway in cancers, and also demonstrate the role of SP antagonism as a synergistic therapy with DOX in hepatoblastoma cells, there are no studies that have investigated the role of SP antagonism as a synergistic mechanism to enhance chemotherapy's ability to kill resistant TNBC cells. Most importantly, there are no studies that have investigated if SP receptor antagonism will play a dual protective role to prevent chemoresistance of TNBC and at the same time prevent cardiotoxicity. Our findings show that SP plays a dual detrimental role in induction of DOX-associated killing of cardiomyocytes and induction of chemoresistance in TNBC. These studies, showing SP receptor antagonism to decrease DOX-induced killing of cardiomyocytes and to increase cancer cell sensitivity to DOX, have the potential for development into tremendous future clinical translation. These studies may lead to use of SP receptor antagonism, for prevention of DOX-mediated cardiotoxicity and enhancement of antitumor effects of DOX for TNBC and probably other cancers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Two-Dimensional Speckle Tracking Echocardiography Detects Subclinical Left Ventricular Systolic Dysfunction among Adult Survivors of Childhood, Adolescent, and Young Adult Cancer

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Two-dimensional speckle tracking echocardiography (2DSTE) provides a sensitive measure of left ventricular (LV) systolic function and may aid in the diagnosis of cardiotoxicity. 2DSTE was performed in a cross-sectional study of 134 patients (mean age: 31.4 ± 8.8 years; 55% male; mean time since diagnosis: 15.4 ± 9.4 years) previously treated with anthracyclines (mean cumulative dose: 320 ± 124 mg/m^2), with ($n = 52$) or without ($n = 82$) mediastinal radiotherapy. The prevalence of LV systolic dysfunction, defined as fractional shortening < 27%, LV ejection fraction (LVEF) < 55%, and global longitudinal strain (GLS) ≤ 16%, was 5.2%, 6.0%, and 23.1%, respectively. Abnormal GLS was observed in 24 (18%) patients despite a normal LVEF. Indices of LV systolic function were similar regardless of anthracycline dose. However, GLS was worse (18.0 versus 19.0, $p = 0.003$) and prevalence of abnormal GLS was higher (36.5% versus 14.6%, $p = 0.004$) in patients treated with mediastinal radiotherapy. Mediastinal radiotherapy was associated with reduced GLS ($p = 0.040$) after adjusting for sex, age, and cumulative anthracycline dose. In adult survivors of childhood, adolescent, and young adult cancer, 2DSTE frequently detects LV systolic dysfunction despite a normal LVEF and may be useful for the long-term cardiac surveillance of adult cancer survivors.

1. Introduction

Strategies in cancer diagnostics and therapeutics have improved dramatically over the last several decades, leading to a growing number of adult survivors of childhood, adolescent, and young adult cancer. The 5-year survival rate for all cancers diagnosed before age of 45 exceeds 80% [1]. As of 2008, there were 619,000 survivors of cancer under the age of 40 years [2]. Despite this progress, the long-term noncancer mortality rate in this population remains substantially higher than age- and gender-matched controls [3], with cardiovascular disease as one of the leading causes of death behind disease recurrence and secondary malignancy [4, 5]. Survivors of childhood (age of 0–14 years) and adolescent (age of 15–19 years) cancer are 7 times more likely to die from cardiac-related events compared to age- and sex- specific rates in the US population [3, 6] and 15.1 times more likely than their siblings to develop heart failure [7]. Less is known about the cardiac outcomes in survivors of young adult (age of 20–39 years) cancer.

Late cardiac effects due to anthracycline chemotherapy and mediastinal radiotherapy in adult cancer survivors are well recognized [8], and subclinical changes in left ventricular (LV) structure and/or function may be seen in more than half of survivors exposed to cardiotoxic therapy [9–11]. Anthracycline chemotherapy can lead to dilated cardiomyopathy and heart failure [12]. Mediastinal radiotherapy is associated with heart failure, premature coronary artery disease, pericardial injury, arrhythmias, and valvular abnormalities [13, 14]. Determination of left ventricular ejection fraction (LVEF) by two-dimensional (2D) transthoracic echocardiography is the primary modality used to screen for LV systolic dysfunction. The Children's Oncology Group (COG) and the National Comprehensive Cancer Network (NCCN) recommend screening echocardiography for all survivors of childhood, adolescent, and young adult cancer, with a frequency based on age at first cardiotoxic treatment, exposure to cardiac-directed radiotherapy, and cumulative anthracycline dose [15, 16]. However, due to the inherent interobserver and intraobserver variability of LVEF assessment by echocardiography, a change in LVEF of 9–11% is the minimum that can be recognized with 95% confidence [17]. In addition, a decline in LVEF is considered to be a late finding of cardiotoxicity [18, 19].

Two-dimensional speckle tracking echocardiography (2DSTE) is a novel method that has potential advantages over LVEF or FS measurement in the early detection of LV systolic dysfunction among both children [20, 21] and adults [22, 23] treated with cardiotoxic therapies and is recommended by the American Society of Echocardiography for the evaluation of patients during and after cancer therapy [24]. However, less is known regarding the use of 2DSTE for the detection of LV systolic dysfunction among adult survivors of childhood, adolescent, and young adult cancer. We performed a single institution cross-sectional study to investigate the use of 2DSTE to detect LV systolic dysfunction among adult survivors of childhood, adolescent, and young adult cancer treated with prior anthracycline chemotherapy, with or without mediastinal radiotherapy.

2. Methods

2.1. Study Population. A total of 134 consecutively referred adult survivors of childhood, adolescent, and young adult cancer followed up in the Adult Long-Term Follow-Up Program at Memorial Sloan Kettering Cancer Center, a program for high-risk cancer survivors, were included in this study. Screening echocardiograms were performed between July 1, 2010, and December 31, 2012. As per COG and NCCN recommendations, screening echocardiograms were performed annually or biennially [15, 16]. All patients were previously treated with anthracycline chemotherapy, with or without mediastinal radiotherapy, and did not have any known history of symptomatic heart failure. The following data was extracted retrospectively from medical records for each patient: age at diagnosis, cancer diagnosis, date of diagnosis, cardiac risk factors (hypertension, diabetes mellitus, and dyslipidemia), cumulative anthracycline dose, total mediastinal radiotherapy dose, and use of cardiac medications (including aspirin, beta blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, or statins). As previously described [15], the cumulative isotoxic anthracycline dose was calculated as the sum of the following: doxorubicin + (daunorubicin × 0.833) + (epirubicin × 0.57) + (idarubicin × 5) + (mitoxantrone × 4). Mediastinal radiotherapy was defined as any form of radiotherapy in which the myocardium was within the prespecified radiation field.

2.2. LV Function by 2D Echocardiography. Conventional 2D and Doppler echocardiography was performed using a commercially available standard ultrasound scanner (Vivid E9, General Electric Medical Systems, Milwaukee, WI), according to the standardized American Society Echocardiography (ASE) protocol [25]. LVEF was calculated using the modified Simpson's method. Fractional shortening (FS) was calculated using the standard equation. Abnormal LV systolic function was defined as LVEF < 55% or FS < 27%. Mitral inflow velocity pattern was recorded from the apical 4-chamber view with the pulsed-wave Doppler sample volume positioned at the tips of the leaflets during diastole. Peak early (E-wave) and late (A-wave) diastolic filling velocities were measured and their ratio (mitral E/A) was calculated. Doppler tissue imaging of the mitral annulus was performed with measurement of the early (e') diastolic velocity at the septal annulus. Diastolic function assessment was based on mitral E/A ratio and tissue Doppler (e') velocity only.

2.3. 2D Speckle Tracking Echocardiography. Three apical views were used to measure peak systolic global longitudinal strain (GLS) and strain rate using dedicated automated software (EchoPAC 12.0, GE Healthcare, Milwaukee, WI). Three points were manually placed at the endocardial border (one in the apex and two at the mitral valve annulus) in each of the apical views, allowing the software to automatically track myocardial movement throughout the cardiac cycle. After careful inspection, manual correction was performed if the automatic detection was suboptimal. Each view was divided into 6 segments, for a total of 18 segments representing the entire left ventricle. In the case of unsatisfactory tracking due to inadequate image quality, that segment was eliminated from analysis. The timing of aortic valve closure in the apical 3-chamber view was used to define end-systole. Longitudinal strain and strain rate curves were generated for each segment. Strain data were expressed in absolute values (|%|). Global radial (GRS) and circumferential (GCS) strain were calculated by averaging the peak systolic strain values in all 6 segments of the parasternal short-axis view at the papillary muscle. Abnormal LV systolic function was defined as GLS ≤ |16|%. This is a conservative estimate of the lower limit of normal that was derived from studies performed in several healthy cohorts using the mean GLS minus two standard deviations [23, 26, 27].

Interobserver variability was assessed by comparing the original GLS, GRS, and GCS calculation with that calculated by a blinded second observer in 20 randomly selected patients. Intraobserver variability was calculated by repeated

measurements in 20 patients by the primary reviewer 3 weeks after the initial measurement.

2.4. Statistical Analysis. Continuous measures were summarized as median and interquartile range (IQR) whereas categorical measures were summarized as frequency and percent. Patients were stratified based on cumulative anthracycline dose (<250, 250–399, and ≥400 mg/m^2) and by mediastinal radiotherapy (yes versus no) for the comparison of the 2D echocardiographic and strain indices, and by-group comparisons were tested using Fisher's exact test when categorical and Kruskal-Wallis test when continuous. The prevalence of abnormal GLS and abnormal FS and LVEF was compared using an exact McNemar's test. Dependent variables considered in multivariable linear regression analysis included FS, LVEF, LV mass/BSA, GLS, mitral E/A ratio, and septal e' velocity. GLS $\leq$ |16|% was considered the dependent variable in a multivariable logistic regression model. There were too few events to consider FS < 27% or EF < 55% as outcomes in multivariable analysis. Each multivariable regression model incorporated sex, age at echocardiogram, mediastinal radiotherapy (yes or no), and cumulative anthracycline dose (continuous) as independent variables. Interobserver and intraobserver variability were assessed using the intraclass correlation coefficient (ICC). Bland-Altman plots were constructed by plotting the average of the two readings on the x-axis versus the difference between the two readings on the y-axis. The mean and standard deviation of the differences were calculated. Because of the small sample size, the t-distribution was used as the reference distribution to calculate the 95% limits of agreement. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using either SAS software version 9.2 (SAS Institute, Cary, NC) or R software version 2.13.1 (R Core Development Team, Vienna, Austria).

3. Results

3.1. Patient Characteristics. A total of 134 survivors of childhood, adolescent, and young adult cancer were included in this study. Demographic and treatment characteristics are provided in Table 1. The median age at echocardiographic follow-up was 31 years (range, 18 to 62 years), and the median interval since diagnosis was 15 years (range, 2 to 39 years). Sarcoma (n = 54), Hodgkin lymphoma (n = 29), and acute leukemia (n = 31) were the most common diagnoses. All patients were treated with anthracycline chemotherapy, and the median cumulative anthracycline dose was 300 mg/m^2 (range, 27 to 660 mg/m^2). Less than half of patients (39%) received mediastinal radiotherapy as part of their cancer treatment, with a median dose of 35 Gy (range, 2 to 56 Gy). The overall prevalence of cardiac risk factors such as hypertension and diabetes was low (9.0% and 5.2%, resp.). Patients who received mediastinal radiotherapy as part of their cancer treatment regimen (n = 52) had a lower cumulative anthracycline dose exposure (median dose, 279 versus 375 mg/m^2, $p < 0.001$) compared to those who were treated with anthracycline chemotherapy alone.

3.2. LV Structure and Function. Overall, the mean FS, LVEF, and GLS were 33.3% (range, 23.8% to 44.1%), 61.1% (range, 46.9% to 75.6%), and 18.0% (range, 12% to 26%). LV systolic dysfunction was detected by FS < 27% in 5.2% and LVEF < 55% in 6.0%. However, the prevalence of abnormal GLS $\leq$ |16|% was 23.1%, significantly higher than abnormal FS ($p < 0.001$) or LVEF ($p < 0.001$) (Figure 1). In the 31 patients with abnormal GLS, only 7 had an abnormal LVEF. There was no significant association between echocardiographic parameters of LV systolic or diastolic function (by 2D echocardiography or 2DSTE) and cumulative anthracycline dose (data not shown).

Echocardiographic parameters of LV systolic function, stratified by mediastinal radiotherapy exposure, are shown in Table 2. GLS was worse in the mediastinal radiotherapy group as compared to the nonradiotherapy group (18% versus 19%, $p = 0.003$) and the prevalence of patients with GLS $\leq$ |16|% was more than two times greater in the radiotherapy group (36.5% versus 14.6%, $p = 0.004$). However, there was no difference in FS (33.3% versus 33.1%, $p = 0.686$) or LVEF (60.7 versus 61.2%, $p = 0.301$) between the groups with and without mediastinal radiotherapy. Although GCS for the whole study group was significantly lower than the normative reference value (mean 23.3%; 95% confidence interval [CI] 22.1% to 24.6%) based on a recent meta-analysis of normal ranges [28], there was no difference in GCS (16.1% versus 17.6%, $p = 0.203$) or GRS (42.1% versus 42.0%, $p = 0.843$) between the groups with and without mediastinal radiotherapy.

In multivariable linear regression analysis, mediastinal radiotherapy was associated with reduced GLS (beta = 0.923, standard error = 0.444, $p = 0.040$) after adjusting for sex, age at echocardiogram, and cumulative anthracycline dose. Mediastinal radiotherapy was also associated with decreased indices of diastolic function, including lower transmitral E/A ratio (beta = −0.250, standard error = 0.087; $p = 0.005$) and medial septal tissue Doppler e' velocity (beta = −1.221, standard error = 0.404; $p = 0.003$). There was a trend towards increased LV mass index with higher doses of anthracycline chemotherapy (beta = 0.702, standard error = 0.404; $p = 0.085$) and decreased LV mass index with mediastinal radiotherapy (beta = −3.459, standard error = 2.085; $p = 0.099$), but neither association reached statistical significance.

3.3. Intraobserver and Interobserver Variability. For GLS, the ICC for interobserver agreement was 0.837 (95% CI 0.639 to 0.932) and the ICC for intraobserver agreement was 0.822 (95% CI 0.610 to 0.925), reflecting substantial agreement for measurement of GLS. The Bland-Altman plots indicate that, for interobserver agreement, we expect 95% of measurements from two different readers to differ by between −1.86% and 2.28% whereas, for intraobserver agreement, we expect 95% of measurements taken by the same reader at two times to differ by between −0.84% and 2.35% (Figure 2). For GCS, the ICC for interobserver agreement was 0.74 (95% CI 0.45 to 0.89) and the ICC for intraobserver agreement was 0.93 (95% CI 0.82 to 0.97). For GRS, the ICC for interobserver

Table 1: Demographics and treatment characteristics ($n = 134$)*.

Characteristic	No.	%	Mean	SD
Current age, years			31.4	8.8
Median	30.5			
Range	18.1–62.6			
Age at diagnosis, years			16.0	9.0
Median	15.8			
Range	0–48.7			
Interval since diagnosis, years			15.4	9.4
Median	15			
Range	2.4–39.6			
Sex				
Male	73	54.5		
Female	61	45.5		
Race				
Non-Hispanic white	114	85.1		
Black	9	6.7		
Other	11	8.2		
Diagnosis				
Sarcoma	54	40.2		
Hodgkin lymphoma	29	21.6		
Acute lymphoblastic leukemia	17	12.7		
Acute myeloid leukemia	14	10.4		
Non-Hodgkin lymphoma	9	6.7		
Other†	11	8.2		
Anthracycline cumulative dose exposure			320	124
Median	300			
Range	27–660			
>350	57	42.5		
150–350	58	43.3		
1–150	19	14.2		
Mediastinal RT dose, Gy				
None	82	61.2		
1–30	38	28.4		
>30	14	10.4		
Cardiovascular risk factors				
Hypertension	12	9.0		
Diabetes	7	5.2		
Dyslipidemia	42	31.3		
Treatment with beta-blockers or ACE-I	14	10.4		
Body mass index			24.8	4.7
Median	23.9			
Range	18.0–47.0			
Overweight (BMI 25–<30)	39	29.1		
Obese (BMI ≥ 30)	17	12.7		

*RT, radiotherapy; ACE-I, angiotensin converting enzyme inhibitor; BMI, body mass index; SD, standard deviation.
†Other: neuroblastoma ($n = 3$), chronic myeloid leukemia ($n = 2$), teratoma ($n = 2$), ependymoma ($n = 1$), nasopharyngeal carcinoma ($n = 1$), retinoblastoma ($n = 1$), and Wilms' tumor ($n = 1$).

agreement was 0.83 (95% CI 0.63 to 0.93) and the ICC for intraobserver agreement was 0.77 (95% CI 0.51 to 0.90).

4. Discussion

The principle finding of this study is that a significant proportion of adult survivors of childhood, adolescent, and young adult cancer have abnormal LV systolic function detected by 2DSTE despite having a normal LVEF. While an abnormal GLS (≤|16|%) was observed in nearly one-quarter of study participants, a corresponding reduction in FS of <27% or LVEF of <55% was observed in only 5.2% and 6.0% of participants, respectively. These observations suggest that the prevalence of LV systolic dysfunction among long-term cancer survivors may be significantly underestimated using LVEF alone as compared to GLS by 2DSTE.

Table 2: LV size and function by receipt of mediastinal radiotherapy*.

	Total (overall)	Mediastinal radiotherapy		p value†
		Yes (n = 52)	No (n = 82)	
Fractional shortening, %	33.3 (30.4, 36.1)	33.3 (30.6, 36.0)	33.1 (29.9, 36.1)	0.948
Fractional shortening < 27%	7 (5.2)	5 (9.6)	2 (2.4)	NA
Ejection fraction, %	61.1 (58.0, 63.6)	60.7 (57.6, 63.4)	61.2 (58.1, 64.0)	0.457
Ejection fraction < 55%	8 (6.0)	5 (9.6)	3 (3.7)	NA
GLS, %	18.0 (17.0, 20.0)	18.0 (16.0, 19.5)	19.0 (17.0, 20.0)	0.040
GLS ≤ 16%	31 (23.1)	19 (36.5)	12 (14.6)	0.036
GLS (APLAX), %	18.0 (16.0, 20.0)	17.0 (15.0, 19.0)	19.0 (17.0, 21.0)	0.023
GLS (A4C), %	18.0 (16.0, 20.0)	17.0 (15.0, 19.0)	18.0 (17.0, 20.0)	0.010
GLS (A2C), %	19.0 (17.0, 21.0)	19.0 (16.5, 20.0)	19.0 (17.0, 21.0)	0.279
GLS rate, 1/s	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	0.995
GRS, %**	42.1 (31.1, 53.7)	42.1 (26.5, 55.2)	42.0 (31.9, 51.4)	0.843
GRS rate, 1/s**	2.2 (1.9, 2.7)	2.3 (1.9, 2.9)	2.2 (1.9, 2.7)	0.086
GCS, %**	17.3 (15.2, 19.7)	16.1 (14.5, 19.7)	17.6 (16.0, 19.7)	0.086
GCS rate, 1/s**	1.5 (1.3, 1.8)	1.5 (1.3, 1.9)	1.4 (1.3, 1.8)	0.058
LV mass/BSA, g/m^2	64.3 (55.9, 72.1)	63.1 (56.9, 69.2)	65.4 (55.6, 74.3)	0.099
Mitral E/A ratio	1.5 (1.2, 1.8)	1.3 (1.0, 1.6)	1.6 (1.3, 1.9)	0.005
Septal e', cm/s	10.4 (8.8, 12.3)	9.3 (8.3, 11.2)	11.6 (9.8, 12.6)	0.003

*Numbers are median (interquartile range) for continuous variables and N (%) for categorical variables.
GLS, global longitudinal strain; APLAX, apical long axis; A4C, apical 4-chamber; A2C, apical 2 -hamber; LV, left ventricle; BSA, body surface area.
†p value from multivariable linear or logistic regression adjusted for continuous cumulative anthracycline dose, sex, and age at echocardiogram.
**Based on only n = 130 patients.

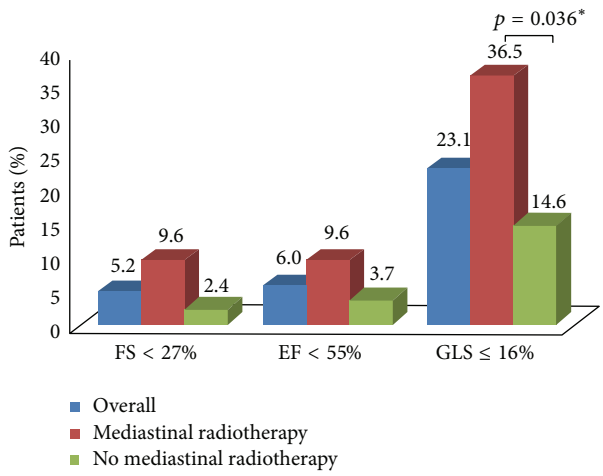

Figure 1: Prevalence of left ventricular systolic dysfunction as measured by left ventricular ejection fraction (LVEF), fractional shortening (FS), and global longitudinal strain (GLS), with or without mediastinal radiotherapy. *p value from multivariable linear or logistic regression adjusted for continuous cumulative anthracycline dose, sex, and age at echocardiogram.

In the current study, there was no association between cumulative anthracycline dose and conventional or strain indices of LV systolic function. However, this may be attributable to the heterogeneity of the study population and the limited sample size. Indices of diastolic function were significantly lower among patients treated with mediastinal radiotherapy (median dose 35 Gy). This is consistent with a study by Heidenreich et al. in which a high prevalence of diastolic dysfunction was observed among Hodgkin lymphoma survivors treated with at least 35 Gy of mediastinal radiotherapy [29]. Interestingly, the presence of diastolic dysfunction was associated with a poorer cardiac event-free survival.

Although LVEF by 2D echocardiography is the current standard method for monitoring LV systolic function in cancer survivors, myocardial strain by 2DSTE has several important advantages as a screening tool for cardiotoxicity. First, myocardial strain can identify subclinical LV systolic dysfunction prior to a decrement of LVEF. Poterucha et al. found that a reduction in GLS preceded a decrease in LVEF among adolescents receiving anthracycline chemotherapy [21]. Similar results were reported in a study of women with HER2-positive breast cancer in which GLS < |19|% was predictive of subsequent cardiotoxicity and decreased LVEF [30]. In addition to being a more sensitive marker of LV systolic function, GLS has also been shown to be a predictor of all-cause mortality that may be superior to LVEF or wall motion score index [31]. And, consistent with previous reports, the current study shows excellent reproducibility for measures of peak GLS, as represented by interobserver and intraobserver ICCs of ≥0.80.

Findings from several prior studies support the utility of myocardial strain assessment in cancer survivors [32–35]. Cheung et al. demonstrated that GLS was reduced among asymptomatic children previously treated for acute lymphoblastic leukemia with anthracycline chemotherapy despite having normal fractional shortening [20]. Tsai et al. also showed that GLS was lower among long-term survivors

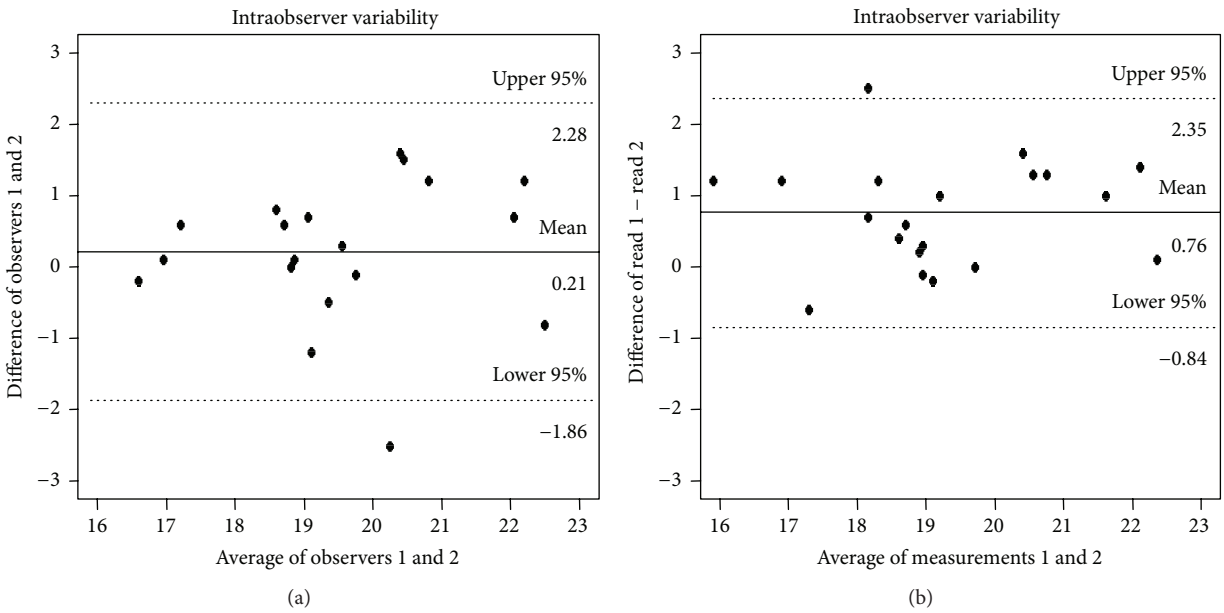

Figure 2: Bland-Altman analysis for interobserver (a) and intraobserver variability (b) for global longitudinal strain measurements in 20 randomly selected patients.

of Hodgkin lymphoma after treatment with anthracycline chemotherapy and mediastinal radiotherapy compared to mediastinal radiotherapy alone [36]. In the current study, GLS was significantly lower and the prevalence of LV systolic dysfunction by 2DSTE was more than two times greater among patients who received both mediastinal radiotherapy and anthracycline treatment compared to those with anthracycline treatment alone. This is consistent with the general finding that longitudinal LV mechanics, which are primarily governed by the subendocardial layer, are the most sensitive and vulnerable to myocardial disease. The circumferential function, which is predominantly governed by the midwall and subepicardial regions, may vary depending on the severity of myocardial involvement. The lack of difference in circumferential strain between the groups with and without radiotherapy may be contributed by the lower reproducibility and accuracy of the circumferential strain measurements.

Less than 10% of the patients were treated with cardioprotective medications, which may confound the association between mediastinal radiotherapy and GLS. However the results of our analysis were not substantively different when these patients were excluded. While radiation increases the cardiotoxicity of anthracyclines via different mechanisms of injury, it is unclear whether this relationship is additive or synergistic.

Overall, 24 of 134 patients (18%) in the current study had abnormal GLS despite a normal LVEF. This is consistent with findings from the St. Jude Lifetime Cohort Study, in which abnormal GLS was more prevalent than a reduction in LVEF among adult survivors of childhood cancer and was associated with both chest-directed radiotherapy and anthracycline exposure [37]. Although GLS has been identified as a robust parameter for early detection and prediction of cardiotoxicity during cancer therapy, the prognostic significance of abnormal GLS among survivors after completion of cancer therapy remains unknown [38]. A recent expert consensus statement from the American Society of Echocardiography highlights the utility of 2DSTE to detect early subclinical LV systolic dysfunction related to cancer treatment, which may allow for better cardiac risk stratification and facilitate timely intervention [24]. Furthermore, the International Late Effects of Childhood Cancer Guideline Harmonization Group recently performed a comprehensive review of the evidence for cardiomyopathy surveillance in survivors of childhood cancer and concluded that echocardiography-based imaging is the preferred modality for surveillance among survivors treated with cardiotoxic therapies [39]. Additional studies are needed to evaluate if early detection of subclinical LV systolic dysfunction using 2DSTE can lead to improved cardiovascular outcomes among cancer survivors.

The findings of this study reinforce the importance of continued routine cardiac surveillance for adult survivors of childhood, adolescent, and young adult cancer many years beyond their initial treatment. Consistent with prior reports, LV systolic dysfunction was detected 15 years after successful treatment in this study, despite only modest doses of anthracycline (median 300 mg/m^2) exposure. Lipshultz et al. also demonstrated that parameters of LV function progressively declined as many as 15 years after diagnosis among childhood cancer survivors treated with doxorubicin [11]. Given the high prevalence of subclinical LV systolic dysfunction in our study and the potential associated late cardiovascular consequences, continued periodic cardiac surveillance among adult cancer survivors should be an essential component of their long-term care.

This study has several limitations. The prognostic significance of abnormal GLS was not evaluated, and long-term follow-up is currently underway to determine whether

abnormal GLS is predictive of subsequent LVEF decline or symptomatic heart failure in this population. Given the limitations of a retrospective cross-sectional study, we assessed LV function at one point in time and cannot comment on longitudinal changes that may have occurred. An analysis of serial echocardiograms is currently underway to investigate the natural history of GLS and LVEF impairment in this at-risk patient population. Although the study did not include a healthy control group, we used reference normative strain data that have been published in several healthy cohorts. Blood pressures were not measured at the time of the echocardiogram which can cause fluctuation in the GLS, but the majority of the patients were normotensive. Finally, our study pools together a heterogeneous study population with multiple cancer types from a single cancer referral center, which may limit the generalizability of the results.

LV systolic dysfunction is significantly more prevalent when assessed by GLS than by 2D LVEF among adult survivors of childhood, adolescent, and young adult cancer. 2DSTE may be useful for the long-term follow-up in this high risk population to identify patients with subclinical LV systolic dysfunction despite a normal LVEF. Mediastinal radiotherapy appears to be an independent risk factor for the development of LV systolic dysfunction in patients treated with anthracycline chemotherapy, and additional studies are needed to further explore the effect of possible confounders. Whether early identification of subclinical LV systolic dysfunction using 2DSTE will translate into long-term cardiovascular benefits warrants further investigation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Current Diagnostic and Therapeutic Aspects of Eosinophilic Myocarditis

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Eosinophilic myocarditis (EM) represents a rare form of myocardial inflammation with very heterogeneous aetiology. In developed countries, the most prevalent causes of EM are hypersensitivity or allergic reactions, as well as hematological diseases leading to eosinophilia. The disease may have a variable clinical presentation, ranging from asymptomatic forms to life-threatening conditions. Most patients with EM have marked eosinophilia in peripheral blood. Endomyocardial biopsy needs to be performed in most cases in order to establish a definitive diagnosis of EM. The therapy depends on the underlying aetiology. Immunosuppressive therapy represents the treatment mainstay in the majority of EM forms.

1. Introduction

The association between blood eosinophilia and related cardiac pathology was first documented in 1936 by Löffler, who described two cases of endocarditis parietalis fibroplastica [1]. Since that time much research has focused on eosinophilic heart disease. Eosinophilic myocarditis (EM) represents the initial stage of cardiac disorder that can disappear with or without any sequelae or may lead to advanced heart disease characterized by endomyocardial fibrosis.

2. Eosinophils

Eosinophils, along with other polymorphonuclear leukocytes, are produced by the bone marrow. They gradually differentiate into mature eosinophils under the influence of several cytokines. This maturation process takes approximately eight days. The main cytokines responsible for increases in eosinophil numbers are granulocyte macrophage colony-stimulating factor, interleukin- (IL-) 3, and IL-5 [2]. Among these cytokines, IL-5 produced by T helper 2 T lymphocytes is considered to be the major eosinophil growth factor. Moreover, this cytokine is also involved in survival, chemotaxis, and degranulation of eosinophils. These cells usually remain in the peripheral blood for only 8–12 hours before migrating to certain tissues. Extravasation of eosinophils from the bloodstream is considered to be a dynamic multistep process that involves capture, rolling, activation, adhesion, and transendothelial and subendothelial migration of the cells. In this process preactivation of eosinophils mediated by P-selectin and IL-5 seems to very important. In healthy subjects, eosinophils are normally found in the blood and in certain tissues (e.g., all portions of gastrointestinal tract with the exception of the oesophagus) [3]. The upper normal limit of eosinophils in the peripheral blood is 3–5% with a corresponding absolute eosinophil count of 350–500/mm^3. The severity of eosinophilia has been arbitrarily divided into mild (<1500/mm^3), moderate (1500–5000/mm^3), and severe (>5000/mm^3) [4]. Eosinophils measure 12–15 μm in diameter and are characterized by a bilobed

nucleus and numerous eosin-staining granules in their cytoplasm. These granules contain high concentrations of hydrolases, cationic and basic proteins. The most important cationic proteins are major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin, and eosinophil peroxidase. These proteins can lead to production of free radicals and induction of cell apoptosis or necrosis. Eosinophils are involved in the process of inflammation, as well as innate and adaptive immunity. Their specific granules are capable of inducing tissue damage and dysfunction by degranulation following activation by an immune stimulus [3].

3. Prevalence of Eosinophilic Myocarditis

Eosinophilic myocarditis is a rare myocardial disease. This form of myocarditis has been identified in 0.5% of cases in an unselected autopsy series [5] and in 0.1% of cases among a cohort of patients biopsied for suspected myocarditis [6]. The prevalence of EM in patients undergoing heart transplantation differs among published studies; usually it is reported between 3 and 7% [7, 8].

4. Pathophysiology of Eosinophilic Myocarditis

Eosinophilic heart disease includes several types of cardiac damage from acute myocarditis to endomyocardial fibrosis. The degree of heart involvement associated with eosinophilic infiltration of cardiac tissue depends on the stimulus attracting the eosinophils, the duration of eosinophilia, and the degree of eosinophil activation. Deleterious effects are more common in subjects with profound blood eosinophilia (>5000/mm^3) [1]. Three phases of eosinophilic heart disease are classically described. The first stage represented by EM is due to initial eosinophilic infiltration of the heart and subsequent myocardial necrosis associated with the degranulation of eosinophils. When endomyocardial biopsies are performed, deposits of ECP, MBP, and eosinophil peroxidase have been consistently detected [1]. The second phase, known as the thrombotic stage, is mainly associated with a hypercoagulable state associated with increased levels of circulating thrombin. Because eosinophil cationic proteins normally bind to an anionic exosite on thrombomodulin, higher numbers of circulating eosinophils bind available thrombomodulin, causing impaired formation of the thrombomodulin-thrombin complex. Moreover, eosinophils store tissue factor, the main initiator of blood coagulation, in their specific granules. Furthermore, it has been recently shown that tissue factor expression is higher in subjects with hypereosinophilia [9]. The third and final phase of EM is represented by fibrotic scarring. Eosinophil-associated fibrosis is observed specifically in the endocardium because endothelial cells are very sensitive to eosinophil granule constituents, especially to ECP and MBP. Eosinophils have the potential to promote fibroblast activation, proliferation, and extracellular matrix production, likely through secretion of transforming growth factor- (TGF-) β and IL-1 [2, 3].

5. Aetiology of Eosinophilic Myocarditis

The principal aetiologic factors associated with EM are hypersensitivity or allergic reactions, infections, malignancies, vasculitis, and hypereosinophilic syndromes. In developed countries, EM seems to be predominantly connected with hypersensitivity or allergic reactions due to various stimuli including drug reactions. Drugs that are most frequently associated with EM are listed as follows [10].

Principal Drugs Associated with Eosinophilic Myocarditis (Based on Table 3 in [10]). Consider the following:

Antimicrobial drugs (amphotericin B, ampicillin, chloramphenicol, penicillin, tetracycline, streptomycin, cephalosporin, sulfonamides, and antituberculous drugs).

Antipsychotics (clozapine).

Anti-inflammatory drugs (indomethacin, oxyphenbutazone, and phenylbutazone).

Diuretics (acetazolamide, chlorthalidone, hydrochlorothiazide, and spironolactone).

Angiotensin converting enzyme inhibitors (captopril, enalapril).

Inotropes (dobutamine, digoxin).

Others (tetanus toxoid, methyldopa, amitriptyline, lenalidomide, and sulfonylurea).

In patients undergoing heart transplantation, EM is occasionally observed as an incidental histological finding in endomyocardial biopsy (EMB) specimens before heart transplantation, as well as in explanted heart specimens obtained at the time of transplantation. There may be an association between EM and dobutamine use, particularly prolonged intravenous administration [11].

Eosinophilia may be associated with a number of neoplastic disorders. It is considered to be reactive in some solid lung, GIT, and urogenital tumors as well as in certain types of hematologic disorders such as T-cell and Hodgkin lymphomas, acute lymphoblastic leukemia, or mastocytosis. Eosinophilia can also be part of the neoplastic clone in hematologic disorders, such as in acute and chronic myeloid leukemia, myelodysplastic syndrome, or other myeloproliferative diseases including polycythemia vera or essential thrombocythemia [12]. Reactive eosinophilia can be associated with various microbial agents but it usually represents a sequela of parasitic infections. Protozoal infections caused by *Trypanosoma*, *Toxoplasma*, *Trichinella*, *Entamoeba*, or *Echinococcus* are usually among the reported infectious causes of EM [10].

Eosinophilic myocarditis may develop in individuals suffering from certain types of vasculitis, namely, Churg-Strauss syndrome (CSS). This rare entity is also known as eosinophilic granulomatosis with polyangiitis. The syndrome was first described by Churg and Strauss as a disease characterized by disseminated necrotizing vasculitis with extravascular granulomas occurring among patients with bronchial asthma and tissue eosinophilia. Currently,

diagnosis of CSS is based on criteria described by the American College of Rheumatology [13]. According to this classification at least four of the following criteria must be met for diagnosis of CSS: (1) marked peripheral eosinophilia >10%, (2) paranasal sinus abnormality, (3) bronchial asthma, (4) nonfixed pulmonary infiltrates, (5) mononeuropathy or polyneuropathy, and (6) extravascular eosinophil infiltration on biopsy findings. Heart involvement is more common in the subgroup of CSS patients with absence of ANCA (antineutrophil cytoplasmic antibody) [13]. Symptomatic cardiac manifestations occur in 27–47% of CSS cases and represent the major cause of death and poor long-term prognosis in these individuals [14].

Eosinophilic myocarditis can be also caused by a heterogeneous group of hematologic disorders called idiopathic hypereosinophilic syndrome (HES). This rare condition is defined as unexplained peripheral blood eosinophilia (>1500/mm^3) persisting for at least six months associated with tissue damage [3]. If tissue damage is absent, idiopathic hypereosinophilia is the preferred diagnosis. Dermatologic involvement followed by pulmonary and gastrointestinal manifestations is most common in HES. Heart involvement occurs in approximately 20% of patients with HES and only a minority has cardiac manifestations at the time of initial presentation [15].

6. Clinical Manifestation

Eosinophilic myocarditis may present in many different ways, ranging from asymptomatic cases to life-threatening conditions such as cardiogenic shock or sudden cardiac death due to malignant ventricular arrhythmias. The diversity of clinical scenarios depends also on the underlying cause of eosinophilia. Prior to the onset of EM, approximately two-thirds of patients have symptoms of the common cold and one-third of cases suffer from allergic diseases such as bronchial asthma, rhinitis, or urticaria [16]. The manifestation of EM, similarly as in other types of myocarditis, may be in the form of chest pain, dyspnoea, fatigue, palpitations, or syncope.

7. Laboratory Markers

Eosinophilia in peripheral blood samples is present in the vast majority of patients with EM and is very useful in the diagnosis of EM. Essentially, the finding of hypereosinophilia in a patient presenting with cardiac symptoms should always raise high suspicion for EM. However, peripheral eosinophilia may be absent in the early stage of EM and may not develop during the course of the illness in a small subgroup of affected individuals.

Inflammatory markers like C reactive protein levels and erythrocyte sedimentation rate as well as levels of markers of myocardial injury such as creatine kinase or troponins are often raised in EM, but their absence does not exclude myocarditis. Moreover, their elevation is not specific for myocarditis. This also applies to brain natriuretic peptides, circulating cytokines, markers related to extracellular matrix degradation, and new biomarkers such as pentraxin, galectin, and growth differentiation factor [17].

8. Electrocardiogram (ECG)

An electrocardiogram is one of the first-line tests for suspected myocarditis. Although the ECG is often abnormal in EM, mostly demonstrating ST-T segment abnormalities, ECG signs are neither sufficiently specific nor sensitive for myocarditis [17]. Nevertheless, some ECG features like QRS complex prolongation are known to be associated with poor clinical outcome [17].

9. Echocardiography

Echocardiography is a very useful first-line method in diagnosis of EM. It helps not only to rule out other causes of patients' complaints but also to assess and monitor changes in cardiac chamber size, wall thickness, and ventricular systolic and diastolic function and to detect the presence of pericardial effusion and observe its dynamics. As mentioned above, cardiac involvement associated with hypereosinophilia is classified into three stages based on the degree of eosinophil-mediated heart injury. The first stage, known as necrotic stage, corresponds typically to EM and there are no pathognomonic echocardiographic signs that reliably distinguish between EM and other types of myocarditis [18]. In fulminant cases of myocarditis, a nondilated, thickened, and hypocontractile left ventricle (LV) is usually observed. However, a wide range of echocardiographic features may be present in patients with nonfulminant EM, ranging from severe global LV systolic dysfunction to almost normal echocardiographic findings. During the next thrombotic stage, endomyocardial and valvular involvement occurs, with the possibility of thrombus formation in the apical parts of the ventricles. Finally, at the last fibrotic stage, endomyocardial scarring progresses and restrictive cardiomyopathy develops. In an echocardiographic study by Ommen et al. which included 51 patients with idiopathic HES and assessed the degree of cardiac involvement, endocardial thickening was present in 12% of the subjects, posterior mitral valve leaflet involvement in 20%, tricuspid involvement in 10%, LV hypertrophy in 10%, LV dilatation in 14%, LV apical thrombus in 24%, and right ventricle (RV) apical thrombus in 20% [19].

10. Cardiac Magnetic Resonance (CMR)

Cardiac magnetic resonance is currently the gold standard in noninvasive diagnosis of myocarditis. Its main advantage with respect to diagnosis of myocardial inflammation is its availability to characterize myocardial tissue. Based on the generally accepted Lake Louise criteria [20], a CMR study is consistent with the presence of myocarditis if at least two of the three following criteria are present: (1) regional or global myocardial signal increase in T2-weighted images, (2) increased global myocardial early gadolinium enhancement ratio between myocardium and skeletal muscle in gadolinium enhanced T1-weighted images, and (3) at least one focal

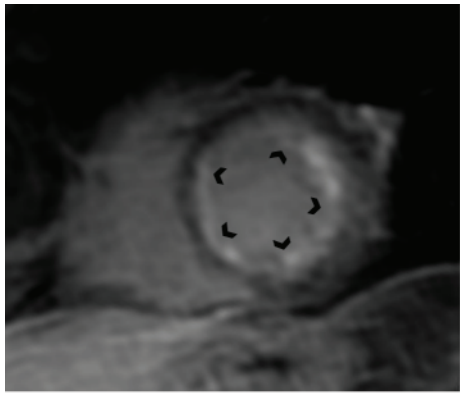

Figure 1: Cardiac magnetic resonance (short-axis) showing global subendocardial late gadolinium enhancement of the left ventricle in a patient with histologically proven eosinophilic myocarditis.

lesion with nonischemic regional distribution in inversion recovery-prepared late gadolinium enhanced T1-weighted images. The presence of LV systolic dysfunction or pericardial effusion provides supportive evidence for myocarditis. In contrast to other types of myocarditis, EM is often associated with subendocardial late gadolinium enhancement, which can be patchy or diffuse (Figure 1). As opposed to ischemic heart disease, these subendocardial regions of late gadolinium enhancement in EM are not restricted to the territory of one of the main coronary arteries [21]. Moreover, in more advanced stages of heart involvement associated with eosinophilia, endomyocardial fibrosis with typical apical LV or RV involvement can be easily detected by CMR.

11. Cardiac CT and PET-CT

Cardiac CT can be useful mainly to exclude significant coronary artery disease in patients with EM presenting with chest pain. Moreover, in those who are unable to undergo CMR it might represent an alternative method for noninvasive detection of myocarditis (Figure 2). PET-CT plays an important role in assessing the activity of the underlying disorder causing hypereosinophilia. In CSS, PET-CT examination is able not only to detect myocardial involvement but also to distinguish between myocardial fibrotic and inflammatory lesions [22].

12. Endomyocardial Biopsy

Endomyocardial biopsy is currently the only method which can make the definite diagnosis of EM by confirming eosinophilic infiltration of the myocardium (Figure 3). In cases of focal myocarditis and less profound eosinophilic myocardial involvement, negative biopsy results may occur due to sampling error. However, if there is a strong clinical suspicion for EM, endomyocardial biopsy should be repeated.

13. Treatment

Generally, strict restriction of physical activity is recommended in all patients during the acute phase of EM with

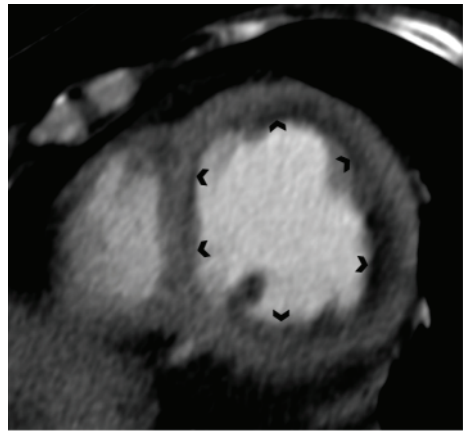

Figure 2: Contrast enhanced CT scan demonstrating diffuse subendocardial hypodensity of the left ventricle in a patient with histologically proven eosinophilic myocarditis.

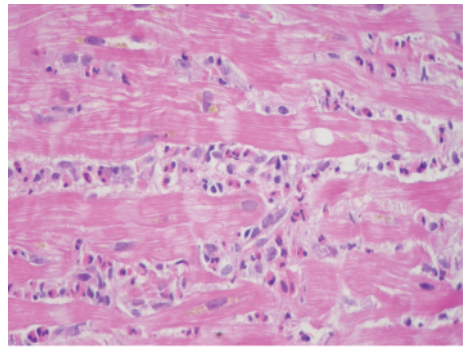

Figure 3: Endomyocardial biopsy demonstrating eosinophilic myocarditis (hematoxylin-eosin, magnification 600x).

subsequent exclusion of sporting activities in next 6 months [17]. Pharmacological and nonpharmacological treatment of patients with EM manifesting with heart failure or arrhythmias are managed according to current guidelines.

Specific treatment of EM differs significantly based on its underlying aetiology. In patients with suspicion for hypersensitivity or allergic aetiology of EM, it is of utmost importance to eliminate possible causative factors. If EM is related to infectious agents such as parasites, targeted antimicrobial treatment is obviously essential. In myeloproliferative disorders associated with fusion genes FIP1L1 (FIP 1 like 1), PDGFRA (platelet derived growth factor receptor alpha), and PDGFRB (platelet derived growth factor receptor beta), respectively, tyrosine kinase activity is constitutively present. The administration of the tyrosine kinase inhibitor imatinib is clearly indicated in this situation. Oral treatment with imatinib can effectively suppress but not eliminate the FIP1L1-PDGFRA clone in most patients, although some may experience remissions after imatinib discontinuation [3]. In patients with EM, prophylactic use of steroids during the first days of imatinib treatment is recommended [3]. The majority of individuals with EM are treated with immunosuppressive treatment, namely, corticosteroids. Nevertheless, the evidence supporting this widely

used therapy in non-CSS patients is modest and is based only on case reports, case series, and small nonrandomized studies [15]. Moreover, the initial dosage of corticosteroids and the treatment duration vary among the published studies and thus no clear evidence-based recommendations can be given at this time. It seems reasonable to adjust the dosage of corticosteroids and the treatment duration with respect to the severity of EM manifestation as well as the primary underlying disorder. In patients with CSS, corticosteroids are the mainstay of treatment. Patients with CSS are most frequently treated with 1 mg/kg per day of prednisone or its equivalent administered orally. When a clinical response is reached, usually in several weeks, steroids are tapered down slowly [13]. If a more advanced stage of the disease is present, combined immunosuppressive therapy comprising corticosteroids and cyclophosphamide or azathioprine is usually administered [13]. In a study conducted by Miszalski-Jamka et al., patients suffering from CCS in whom non-corticosteroid immunosuppressive treatment was initiated at the time of diagnosis less frequently had new onset or progression of heart failure in comparison with subjects in whom this therapy was started later on [23]. In patients with CSS or HES there is also evidence showing the usefulness of mepolizumab administration. Mepolizumab is a humanized monoclonal antibody that inhibits binding of IL-5 to its receptor expressed on eosinophils. Initial experience with mepolizumab demonstrates its safety and tolerability; a main advantage is its corticoid-sparing effect [24].

Interestingly, there is also evidence that certain patients with EM do not need to be treated with corticosteroids. In a retrospective study by Yanagisawa et al. [25], which included 22 patients with idiopathic eosinophilia and histologically proven EM and 7 subjects with lymphocytic myocarditis, a similar outcome in terms of LV ejection fraction improvement as well as mortality was observed at 1-year follow-up in both study groups with only conventional heart failure therapy.

Recently, new therapeutic strategies for eosinophil-associated disorders have been suggested. Among the plethora of eosinophils receptors described so far, only several receptors such as IL-5 receptor alpha, chemokine receptor CCR3, and sialic acid-binding immunoglobulin-like lectin 8 are considered to be relatively specific for eosinophil lineage and are thus potentially suitable for antibody targeting [26]. Among the drugs affecting these receptors benralizumab, representing humanized antibody to IL-5 receptor alpha, seems to be very promising and has evidence on blood and tissue eosinophilia reduction from several randomized studies [26]. Unfortunately, benralizumab similar to other new drugs targeting specifically eosinophil receptors lacks evidence from randomized trial for the treatment of EM.

14. Conclusions

Eosinophilic myocarditis is a rare myocardial disorder with heterogeneous aetiology. Peripheral blood eosinophilia associated with cardiac symptomatology should always raise suspicion for EM. Noninvasive imaging methods, namely, CMR, play an important role in the diagnostic process of EM. However, the definite diagnosis of EM usually needs to be confirmed by endomyocardial biopsy. Specific treatment of EM differs significantly based on its underlying aetiology. Immunosuppressive therapy represents the mainstay of treatment in the majority of patients with EM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Causes of HIV-Associated Cardiomyopathy: A Tale of Two Worlds

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Antiretroviral therapy (ART) has transformed the clinical profile of human immunodeficiency virus (HIV) from an acute infection with a high mortality into a treatable, chronic disease. As a result, the clinical sequelae of HIV infection are changing as patients live longer. HIV-associated cardiomyopathy (HIVAC) is a stage IV, HIV-defining illness and remains a significant cause of morbidity and mortality among HIV-infected individuals despite ART. Causes and clinical manifestations of HIVAC depend on the degree of host immunosuppression. Myocarditis from direct HIV toxicity, opportunistic infections, and nutritional deficiencies are implicated in causing HIVAC when HIV viral replication is unchecked, whereas cardiac autoimmunity, chronic inflammation, and ART cardiotoxicity contribute to HIVAC in individuals with suppressed viral loads. The initiation of ART has dramatically changed the clinical manifestation of HIVAC in high income countries from one of severe, left ventricular systolic dysfunction to a pattern of subclinical cardiac dysfunction characterized by abnormal diastolic function and strain. In low and middle income countries, however, HIVAC is the most common HIV-associated cardiovascular disease. Clear diagnostic and treatment guidelines for HIVAC are currently lacking but should be prioritized given the global burden of HIVAC.

1. Introduction

Dramatic gains have been made in the treatment of human immunodeficiency virus (HIV) over the last decade. By 2013, 35 million people globally were infected with HIV, and there were 2.1 million new HIV infections, nearly 40% lower than in 2001 [1]. The number of acquired immunodeficiency syndrome (AIDS) related deaths also declined by 35% over the same time period [1]. Much of the survival gains seen for people infected with HIV/AIDS are due to better availability of antiretroviral therapy (ART). The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that 13.6 million people were receiving ART as of June 2014 and that 15 million will receive ART by 2015 [1]. HIV-infected individuals on ART can expect to live longer and, as a result, they are at risk of developing chronic, noncommunicable diseases including many forms of cardiovascular disease [2].

HIV-associated cardiomyopathy (HIVAC) has evolved since its first description in the mid-1980s [3]. Throughout the 1980s and 1990s, before the widespread availability of ART, the presence of heart failure in HIV-infected individuals was mainly in the context of myocarditis, related to direct effects of HIV, opportunistic infections, autoimmunity, nutritional deficiencies, or severe immunosuppression [4]. HIVAC was characterized as symptomatic, systolic dysfunction associated with a dilated left ventricle and indicated a poor prognosis for HIV-infected patients. Median survival was 101 days for HIV-infected patients after diagnosis with dilated cardiomyopathy, compared to 472 days for patients with normal findings on echocardiogram at a similar stage of immunosuppression [5]. Today, systolic dysfunction is being replaced by subclinical diastolic dysfunction as the hallmark of HIVAC in individuals with well controlled HIV [6].

No consensus criteria currently exist to define "HIV-associated cardiomyopathy," but studies have outlined multiple subtypes of this evolving disease. Manifestations of HIVAC include symptomatic heart failure with left ventricular dysfunction with or without concurrent ventricular

Table 1: Etiologies and Characteristic Phenotypes of HIVAC.

	Etiology of HIVAC	Characteristic HIVAC Phenotype
Uncontrolled HIV Disease: (i) Immunosuppressed host (ii) High viral load (iii) Low CD4 count (<400 cells/mm^3)	(i) Myocarditis (a) Direct HIV toxicity (b) Opportunistic Infections (1) Viral: Coxsackie B, CMV, EBV (2) Non-viral: Toxoplasmosis, Cryptococcus, MAC (ii) Tuberculous Myopericarditis (iii) Micronutrient Deficiency (a) Selenium Deficiency	(i) More commonly seen in LMIC (ii) Symptomatic, systolic dysfunction +/− dilated ventricles (iii) Poor prognosis
Controlled HIV Disease: (i) Immunocompetent host (ii) Undetectable viral load	(i) Cardiac Autoimmunity (ii) Cardiac inflammation (iii) ART toxicity (a) AZT-induced cardiomyopathy	(i) More commonly seen in HIC (ii) Subclinical diastolic dysfunction with increased strain patterns

dilation, any systolic impairment or diastolic dysfunction in asymptomatic HIV patients, and new onset heart failure in stage IV HIV disease [7]. This broadened classification of HIVAC illustrates the increasingly complex relationship between HIV and cardiac dysfunction.

This transition in disease profile results from important disparities in the epidemiology and pathogenesis for HIVAC between high income countries (HICs) and low and middle income countries (LMICs), which, to the best of our knowledge, relate to differences in ART availability, HIV viral suppression, comorbidities, and opportunistic infections (Table 1) [6]. Thus, our understanding of the epidemiology and etiology of HIVAC in the pre-ART era remains relevant in many parts of the world where ART availability remains low. This review will explore the contributing etiologies of HIVAC while highlighting the current, disparate burden of HIVAC between HICs and LMICs.

2. Etiology of HIV-Associated Cardiomyopathy

Much of our understanding about the etiology of HIVAC is derived from studies performed in HICs before the availability of ART. As a result, the literature focused on direct and indirect cardiotoxicity of infections and HIV itself. More recent literature suggests an expanded role of autoimmunity and drug toxicity in the setting of ART. Studies from LMICs have also explored the role of nutrition in disease development. While large knowledge gaps remain, there are a number of prevailing hypotheses about the multifactorial etiology of HIVAC.

2.1. Myocarditis. Myocardial inflammation caused by HIV and related infections is implicated as a key inciting factor in the development of HIVAC. Various viral and opportunistic infections trigger myocarditis in the setting of uncontrolled HIV infection. Direct invasion of cardiac myocytes by cardiotropic viruses, including HIV, leads to a local cytokine release and subsequent infiltration of the myocardium with clonal expansion of B cells [8]. Myocarditis is particularly common in late stages of HIV infection. High rates of myocarditis are associated with CD4 counts of less than 400 cells/mm^3 and up to two-thirds of untreated AIDS patients having histological evidence of myocarditis on autopsy [8, 9].

Both viral and nonviral opportunistic infections have been linked to myocarditis and subsequent left ventricular dysfunction in untreated HIV patients. One of the largest clinical pathology studies done to date found that Italian patients with AIDS and myocarditis were often coinfected with cardiotropic viruses, most commonly Coxsackie B3 virus (32%), Epstein-Barr virus (8%), and *Cytomegalovirus* (4%) [10, 11]. Even higher rates of *Cytomegalovirus* (48%) have been seen in patients with left ventricular dysfunction using in situ hybridization [12]. *Toxoplasma gondii*, *Cryptococcus neoformans*, and *Mycobacterium avium-intracellulare* have also been isolated from the myocardium of end-stage AIDS patients with evidence of myocarditis and left ventricular dysfunction on autopsy [13]. Reduction in opportunistic infections in patients on ART may be responsible for the impressive drop in myocarditis rates and declining prevalence of HIVAC as seen in HICs [14, 15].

It is hypothesized that the HIV-1 virus causes myocarditis directly through myocyte toxicity, although debate about the exact pathogenesis exists. In vitro studies of human and rat cardiomyocytes have shown that HIV can enter myocytes directly through pathways independent of CCR5 and CXCR4 receptors. Invasion is thought to occur through macropinocytosis as HIV-1 virion particles with their nucleocapsid cores can be seen in vacuoles within myocytes on scanning electron microscopy [10, 16, 17]. HIV-1 nucleic acid sequences can be detected within the myocardial tissue of HIV-infected patients with myocarditis by in situ DNA hybridization [12, 18].

HIV also catalyzes a cascade of indirect pathways that induce myocardial inflammation and damage. Cardiomyocyte apoptosis and myocardial macrophage infiltration are more common in patients with HIVAC than in HIV-infected patients without cardiomyopathy [17]. Cardiomyocyte expression of HIV-1 associated protein, gp-120, and transactivator of transcription (Tat) protein signaling pathways have been implicated in mitochondrial dysfunction and cardiomyocyte apoptosis [16, 17, 19]. Additionally, myocardial

dendritic cells including macrophages and endothelial cells have been considered "reservoir cells" for HIV-1 invasion and contribute to localized myocardial cell death through activation of inflammatory cytokines [20]. Macrophages initiate proapoptotic signaling through mitochondrial injury, activation of caspases, and receptor-mediated signaling through tumor necrosis factor- (TNF-) alpha and Fas ligand expression [16, 17]. The release of TNF-alpha specifically has been shown to have a negative ionotropic effect on cardiomyocytes by altering intracellular calcium homeostasis and inducing nitric oxide synthesis [10]. Myocardial damage from these indirect pathways ultimately leads to left ventricular systolic dysfunction, increased left ventricular mass, and expression of natriuretic peptides that may lead to hemodynamic compromise as demonstrated in vivo in rats [21].

2.2. Cardiac Autoimmunity. Higher levels of serum autoantibody titers have been seen in HIV-infected adults and children with myocardial disease compared to HIV-uninfected individuals. Significantly higher concentrations of anti-alpha myosin antibodies are found in HIV-infected individuals compared to HIV-negative controls [22]. The level of cardiac autoantibodies is progressively higher comparing HIV-uninfected controls (3%), HIV-infected individuals without heart disease (19%), and HIV-infected patients with left ventricular systolic dysfunction (43%) [22]. Autoantibody concentrations correlate with mortality in HIV-infected patients.

Infection with common and opportunistic viruses may facilitate the onset of cardiac autoimmunity in HIV-infected individuals by modifying cardiomyocyte surface antigens and exposing otherwise hidden cell surface epitopes, resulting in abnormal autoimmune responses against endogenous cardiomyocytes [22]. Persistent, latent myocardial infection with cardiotropic viruses, like *Cytomegalovirus*, may trigger clonal expansion of autoreactive CD8 T cells that target normal myocytes and lead to myocarditis [12].

2.3. Micronutrient Deficiency. Micronutrient deficiency is common in HIV-infected individuals due to gut malabsorption, diarrhea, and wasting syndrome. The resulting free radical formation and myocardial injury have been linked to the development of HIVAC. Selenium is the most widely studied micronutrient deficiency, as it plays a significant role in other forms of dilated cardiomyopathy. Selenium is an essential element used in the generation of glutathione peroxidase, an enzyme which protects lipid membranes from oxygen radicals and plays a crucial role in the prevention of myocardial injury [23]. Abnormalities in immunologic defense, phagocyte function, and T cell response, as seen with selenium deficiency, predispose to further myocardial injury [23]. Animal models have shown that selenium-deficient mice are more susceptible to myocyte damage and myocarditis when exposed to stressors, such as Coxsackie B virus [24].

Selenium deficiency has been associated with cardiomyopathy in untreated HIV-infected individuals. A prospective study of 416 HIV-infected patients in Rwanda found that low serum selenium levels were associated with nearly twice the odds of developing cardiomyopathy in multivariate analysis (OR 1.92, 95%CI 1.73–2.04) [25]. Low levels of serum selenium correlate directly with other known HIVAC risk factors, including low socioeconomic status and CD4 count [25, 26].

While selenium deficiency may have role in risk of HIVAC, the role of selenium supplementation in preventing or treating HIVAC remains unknown. Case reports have shown improvement in cardiac function with supplementation in targeted, selenium-deficient patients but, despite numerous salutary effects of selenium supplementation in HIV-infected individuals, no prospective evidence exists to support selenium supplementation for treating or preventing HIVAC [23, 27–29].

2.4. Antiretroviral Toxicity. In general the initiation of ART has decreased the prevalence of HIVAC in HIC, although use of zidovudine (AZT) based regimens may be associated with greater risk of cardiomyopathy [15, 27]. Zidovudine, a reverse nucleoside transcriptase inhibitor, inhibits mitochondrial DNA polymerase, causes mitochondrial damage, and leads to focal myocardial necrosis [30]. Treatment with AZT is associated with reversible, dose-dependent damage to skeletal and cardiac myocytes [30, 31]. Case reports of HIV-infected adults in the USA in the early 1990s revealed high rates of cardiac dysfunction associated with AZT monotherapy that rapidly reversed with cessation of AZT [32]. Increased left ventricular mass and peak wall stress have also been noted in HIV-infected children after treatment with AZT [33]. More recently, AZT exposure has also been linked to diastolic dysfunction in HIV-infected subjects [34].

2.5. Tuberculous Myopericarditis. Pericardial disease is often the first manifestation of cardiac disease in HIV-infected individuals and carries high mortality in LMICs [35]. Pericarditis caused by *Mycobacterium tuberculosis* (TB) is the leading cause of pericardial disease in HIV-infected individuals in highly endemic areas, accounting for up to 70% of all pericardial effusions and 90% of pericardial effusions in HIV-infected individuals in parts of Sub-Saharan Africa (SSA) [35, 36]. HIV infection is the most important predisposing factor for TB infection, and HIV infection is thought to alter the clinical manifestation of pericardial disease [36]. Direct pericardial invasion in HIV coinfection occurs through hematogenous spread of TB, unlike indirect lymphatic invasive in HIV-uninfected hosts [37]. In HIV-infected individuals, pericardial TB infection often results in larger pericardial effusions, more myopericardial involvement, and less constrictive pericarditis compared to HIV-uninfected individuals [36]. The Investigation of the Management of Pericarditis in Africa (IMPI Africa), a registry of 185 patients with suspected TB pericarditis from Cameroon, Nigeria, and South Africa, showed that patients with HIV were more likely to present with dyspnea and electrocardiographic changes, indicating myopericardial disease, and less likely to present with ascites, suggestive of a lower incidence of constrictive pericardial disease [38].

HIV coinfection with TB myopericarditis is a leading cause of cardiac death among HIV-infected patients, with

a nearly sixfold increase in mortality compared to HIV-uninfected individuals [37]. HIV infection does not seem to alter the response to TB pericarditis treatment, although HIV-infected individuals have a higher rate of pericardial disease relapse [36]. The use of adjuvant corticosteroids to treat TB pericarditis in HIV-infected population remains controversial. A large, randomized controlled trial investigating the use of corticosteroids and/or *Mycobacterium indicus pranii* immunotherapy in TB pericarditis showed no difference between prednisolone and placebo or *M. indicus pranii* and placebo in the primary combined outcome of death, cardiac tamponade, and constrictive pericarditis, though patients receiving prednisolone as compared to placebo had significantly lower rates of the secondary outcomes of progression to constrictive pericarditis and fewer repeat hospitalizations [39]. There was also a significant increase in HIV-associated malignancy in HIV-infected patients receiving both prednisolone and *M. indicus pranii* versus placebo [39].

3. Effects of ART on Clinical Manifestations of HIVAC

The widespread use of ART has changed the phenotype of HIVAC as subclinical cardiac abnormalities, including diastolic dysfunction and impaired cardiac strain patterns, become increasingly common in HIV-infected individuals on effective HIV treatment [40]. A growing prevalence of asymptomatic ventricular dysfunction, abnormal strain patterns, and a higher incidence of diastolic dysfunction has been noted in HIV-infected populations on ART. The prevalence of systolic dysfunction has decreased in HICs whereas diastolic dysfunction is now seen in up to 64% of asymptomatic HIV-infected patients on ART [6, 40, 41]. Magnetic resonance imaging studies suggest that these subclinical changes may be due in part to myocardial fibrosis and steatosis seen in patients on ART [42]. While ART has dramatically reduced the burden of HIVAC in HICs, the incidence and mortality rates have risen in LMICs [14].

3.1. Burden of HIVAC in HICs. Most of our understanding of HIVAC emanates from studies performed in HICs, mostly from the United States and throughout Europe. Since the widespread initiation of ART the prevalence of HIVAC has dropped by 30% in these regions [14]. In the late 1980s, roughly one-third of all HIV-related cardiac deaths were due to dilated cardiomyopathy, and autopsy studies found evidence of myocarditis in up to 40% of noncardiac deaths in HIV-infected patients [35, 43]. A prospective study out of Johns Hopkins University in the early 1990s estimated the incidence of global left ventricular dysfunction to be 18% per year in HIV-infected patients [43]. However, with consistent access to antiretroviral medication and early initiation of treatment, myocarditis and dilated cardiomyopathy have virtually disappeared as manifestations of cardiac disease in HIV-infected patients in HICs today.

With the early advent of ART in HICs, the incidence of systolic dysfunction has decreased but diastolic abnormalities are increasing. A 2013 meta-analysis of 11 studies from HICs revealed that, among 2242 HIV-infected individuals on ART, only 8.3% had left ventricular systolic dysfunction whereas 43.4% had evidence of diastolic dysfunction [44]. Higher rates of subclinical cardiac abnormalities, such as abnormal left ventricular relaxation or pseudonormal filling patterns, higher pulmonary artery pressure, and decreased exercise tolerance are more frequently observed in patients on ART [40].

Additionally, the burden of cardiac disease in HIV infection in HICs are transitioning towards increasing atherosclerosis and ischemic heart disease. Patients on ART in HICs are living longer and exposed to more traditional cardiac risk factors such as tobacco use, hyperlipidemia, and diabetes. Antiretroviral therapies have been linked to an increased risk of coronary artery disease and myocardial infarction as well as acceleration of atherosclerotic formation and metabolic disturbances. Generally, immune reactivation with ART and chronic low-grade inflammation have been shown to promote subclinical atherosclerotic changes and arterial stiffness [45, 46]. The three major classes of ART, protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), and nonnucleoside reverse transcriptase inhibitors (NNRTIs), have all been associated with some degree of dyslipidemia; PIs and the NRTIs, stavudine and zidovudine, are indirectly implicated in the development of atherosclerosis via significant alterations in lipid metabolism and insulin resistance [45, 47, 48]. The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study, an international collaboration representing over 30,000 HIV-infected patients across Europe, the United States, and Australia, found an increased risk of myocardial infarction with use of PIs and certain NRTIs, namely, abacavir and didanosine [45, 49–51]. However, a 2013 systematic review of 27 studies addressing the risk of cardiovascular disease from ART did not find a consistent relationship between these drugs and myocardial risk [52]. Further prospective, randomized controlled trials are needed to better assess the relationship between ART and myocardial infarction risk. Meanwhile, the long term benefits of ART on controlling HIV infection and disease sequelae are thought to outweigh the increased relative risk of cardiovascular disease in the HIV-infected population.

3.2. Burden of HIVAC in LMICs. The impact of HIVAC may be most severe in LMICs, where HIVAC remains a relevant cause of morbidity and mortality despite the expanding use of ART [4]. HIVAC is associated with low socioeconomic status, a longer duration of HIV infection, low total lymphocyte count, low CD4 count, high HIV-1 viral load, and low plasma levels of selenium [37]. A CD4 count <100 cells/mm^3 appears to be an important threshold below which the risk of developing HIVAC increases significantly [5]. Cross-sectional studies in the pre-ART era from SSA indicated a prevalence of cardiomyopathy in up to 57% in hospitalized patients [35]. A prospective study of 157 HIV- infected patients in Kinshasa, Democratic Republic of Congo, showed that about half of the patients developed a cardiac abnormality over 7 years [13].

More recently, the Heart of Soweto study found that, of the 5328 newly diagnosed cases of cardiac disease at a major hospital in South Africa between 2006 and 2008, 518 cases

were in HIV-infected patients and only half of them (54%) were taking ART [7]. The most common cardiac diagnosis among all HIV-infected patients was HIVAC (38%) with an average left ventricular ejection fraction of 46%. The viral loads were significantly higher (110,000 versus 90,000 RNA copies/mL) and CD4 counts significantly lower (180 versus 211 cells/mm3) in cases of HIVAC compared to those HIV-infected patients without cardiomyopathy [7]. Results from the Sub-Saharan Africa Survey of Heart Failure study, a multinational registry of patients across Africa presenting to hospitals with acute, decompensated heart failure, showed that HIV was the direct cause of heart failure in 2.6% of all cases [53].

The mortality due to HIVAC is significant and reaches as high as 15–20% in parts of SSA [54]. HIV status is an independent predictor of death at 180 days for patients with acute decompensated heart failure and is associated with increased in-hospital, 60-day, and 180-day mortality rates [55].

3.3. Reconciling HIVAC Disparities in HICs and LMICs. The causes contributing to HIVAC seem to depend on the degree of viral suppression which are strongly related to region of the world [35]. Opportunistic and viral infections, nutritional deficiencies, and direct HIV toxicity are leading causes in uncontrolled disease, especially with high viral loads or CD4 counts <100 [4]. When viral suppression is adequate and immune function is restored, ART, chronic inflammation, and autoimmunity may be more pronounced contributors to HIVAC [4]. Thus, HIVAC may truly represent yet another syndrome of heart failure with numerous individual causes, each of which may warrant specific therapy in addition to generally accepted therapy for heart failure. As life expectancy for HIV-infected individuals continues to increase worldwide, we are likely to see more subclinical manifestations of HIVAC which warrant more attention to screening in the presymptomatic individual.

4. Current Diagnostic and Screening Tools

Identifying early markers of myocardial dysfunction in HIV-infected individuals at high risk of cardiac disease may provide early intervention of life-saving therapy. To date, however, there have been no diagnostic criteria or screening guidelines defined for HIVAC. Echocardiography remains the standard for detection of ventricular dysfunction [56]. Diastolic dysfunction and abnormal myocardial strain are often the only echocardiographic abnormalities in asymptomatic HIV-infected patients on ART [4]. Early detection of subclinical myocardial dysfunction can be assessed by 2-dimensional strain and strain rate using speckle tracking echocardiography [37]. Further, cardiac magnetic resonance can now detect signs of subclinical cardiac steatosis and myocardial fibrosis [42]. However, the clinical significance of some of these structural and metabolic cardiac changes remains unknown.

The role of screening echocardiography in HIV-infected populations is unclear. Timing and frequency of echocardiography testing is undetermined. Starc et al. have recommended that pediatric patients have an echocardiogram done at the time of HIV-diagnosis, followed by repeat testing every couple of years for asymptomatic patients or annual testing in patients with symptoms of heart failure, unexplained respiratory illness, or symptomatic HIV infection [57]. Given the increasing prevalence of subclinical disease and poor outcomes in late detection of systolic dysfunction in HIV-infected patients, developing clear screening guidelines should be a high priority. However, even with optimized screening practices and diagnostic criteria, targeted treatment options remain limited once HIVAC develops.

5. Current Treatment Options

Best practices for treatment of HIVAC have not been rigorously tested. Early initiation of beta-blockers and ACE-inhibitor therapy may be beneficial in subclinical disease to prevent progression to severe systolic dysfunction through common mechanisms, such as afterload reduction and sympathoadrenal modulation [56, 58]. In the absence of specific guidelines to the contrary, patients with HIV and heart failure should be treated with standard therapy for heart failure according to current consensus guidelines [59].

ART has been shown to positively impact outcomes in retrospective studies, but there is no prospective evidence that ART has a beneficial effect on cardiac outcomes in HIVAC [20]. Such evidence is unlikely to be forthcoming, however, as the latest WHO guidelines recommend initiating ART regardless of CD4 count. Adjunctive therapies for HIVAC such as supplementation with carnitine, selenium, and multivitamins have been proposed in an attempt to preserve left ventricular function in micronutrient deficient populations but warrant further evidence before wide scale adoption [60]. Immunomodulatory therapy has been shown to improve left ventricular structure and function in some patient populations. Patients with biopsy-proven autoimmune myocarditis, for example, improve left ventricular function and dimensions after therapy with corticosteroids [61]. Monthly intravenous immunoglobulin (IVIG) infusions have also been shown to improve cardiac function in HIV-infected children with subclinical cardiac abnormalities [20, 62]. However, there are no controlled trials investigating efficacy of corticosteroids or IVIG in treating HIVAC in adult populations. Further investigation is needed to identify best treatment practices for HIVAC.

Mechanical support devices and cardiac transplantation are definitive treatment options for end-stage HIVAC, although their use is still limited in HIV-infected populations. HIV infection was previously considered a contraindication to mechanical support and transplant, but since advanced ART has improved outcomes and mortality rates from end-stage heart failure continue to rise, the United Network for Organ Sharing (UNOS) declared that asymptomatic HIV-infected individuals should not be excluded from heart transplant consideration solely based on their HIV status [63]. Data from case series and small cohort studies in the USA and Canada suggest that good outcomes with survival rates for HIV-infected patients are similar to those of HIV-uninfected

patients up to 3 years after cardiac transplantation [64, 65]. Despite this evidence, a recent survey of cardiac transplantation centers found that 57% of programmes still considered HIV infection to be a contraindication to transplantation due to scarcity of organ supply, concerns for posttransplant immunosuppression enhancing progression to AIDS, and possible postoperative drug interactions between ART and immunosuppressive therapies [66]. Left ventricular assist devices (LVADs) are also scarcely used in HIV-infected individuals, with most centers citing risks of device-related infection [66]. A case study of two HIV-infected individuals who underwent implantation with HeartMate XVE pulsatile-flow LVAD found no HIV-related infectious complications, and a recent analysis of all 22 HIV-infected LVAD cases in the USA revealed outcomes similar to the general LVAD population with comparable mortality rates at 3, 6, 12, and 24 months [66, 67].

6. Biomarkers for HIVAC Screening

The use of novel biomarker testing to screen for cardiac dysfunction in HIV-infected persons is a growing area of investigation. B-natriuretic peptide (BNP) screening combined with collaborative care has been shown to reduce the rates of systolic and diastolic dysfunction in patients at risk of heart failure [68]. An inverse correlation between BNP levels and left ventricular function in HIV-infected patients has been seen in small case studies [69, 70], but the specificity of BNP for cardiac disease in HIV-infected individuals is unclear [69–71]. More research is needed to assess whether this cost-effective and simple test may be a useful screening tool for identifying HIVAC.

Soluble ST2, a novel biomarker of cardiac stress, and GDF-15, a growth differentiation factor expressed in cardiac injury, are associated with cardiac dysfunction and all-cause mortality in a controlled study of HIV-infected individuals [72]. ST2 was also associated with diastolic dysfunction, suggesting its role as a possible profibrotic mediator in HIVAC. Other novel markers requiring further investigation include serum autoantibody titers for cardiac-specific autoantibodies, like anti-alpha myosin, that have been identified in left ventricular dysfunction in HIV-infected individuals and may serve as a target for immunomodulatory treatment [10].

7. Conclusion

HIV-associated cardiomyopathy remains a significant cause of morbidity and mortality in both HICs and LMICs despite the widespread use of ART. Overall, the clinical presentation of HIVAC is changing as life expectancy increases in HIV-infected individuals. Severe, symptomatic dilated cardiomyopathy, as previously seen in end-stage AIDS, is declining as the predominant clinical manifestation of HIVAC. Subclinical, diastolic dysfunction and abnormal ventricular strain patterns are being seen more frequently in HIV-infected individuals with adequate HIV viral control. The etiology for this variable phenotype likely depends on the degree of viral replication and immunosuppression. Myocarditis, opportunistic infections, micronutrient deficiencies, and HIV itself play a large role in individuals with inadequate viral suppression and poor immune function, whereas ART toxicity and cardiac autoimmunity are seen more when disease is controlled.

The prevalence of HIVAC has declined in HICs with successful ART and decreased opportunistic infections, whereas HIVAC remains a significant contributor to disease burden in LMICs [7, 14, 27]. These diverging epidemics result from a combination of factors. Poor soil composition across SSA has predisposed a quarter of the population to selenium and other micronutrient deficiencies that have been seen to worsen cardiomyopathy [4]. Limited access to effective ART is a critical challenge faced in many LMICs. Frequent use of AZT in first-line therapy persists in many LMICs due to its low cost despite international recommendations for other, less cardiotoxic regimens [73].

Emphasis needs to be placed on designing clear guidelines for screening protocols and diagnostic criteria for HIVAC. Appropriate timing and tools for cardiac screening in HIV-infected individuals beg clarification. Using advanced echocardiographic imaging to evaluate for contractile reserve, diastolic dysfunction, and abnormalities in myocardial deformation can identify higher-risk patients [4], but it remains unknown whether this alters clinical decision making for HIV-infected patients. Establishing diagnostic criteria that account for stage of HIV and degree of immunosuppression should be a high priority. Recommendations regarding the timing and frequency of routine cardiac evaluation for HIV-infected individuals are needed, as well as partnership between infectious disease specialists and cardiologists in identifying and managing patients at high risk of HIVAC. HIV-associated cardiomyopathy will continue to be a significant contributor to the global cardiac disease burden as the HIV population ages, and more research is needed to understand best practices in diagnosis and treating the disease worldwide.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Vascular Endothelial Growth Factor Is Associated with the Morphologic and Functional Parameters in Patients with Hypertrophic Cardiomyopathy

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Background. Hypertrophic cardiomyopathy (HCM) is mostly autosomal dominant disease of the myocardium, which is characterized by myocardial hypertrophy. Vascular endothelial growth factor (VEGF) is involved in myocyte function, growth, and survival. The aim of study was to analyze the clinical significance of VEGF in structural and functional changes in patient with HCM. *Methods*. In a group of 21 patients with nonobstructive HCM, we assessed serum VEGF and analyzed its association with morphological and functional parameters. Compared to healthy controls, serum VEGF was increased: 199 (IQR: 120.4–260.8) ng/L versus 20 (IQR: 14.8–37.7) ng/L, $P < 0.001$. VEGF levels were associated with left atrium diameter ($r = 0.51$, $P = 0.01$), left ventricle ejection fraction ($r = -0.56$, $P = 0.01$), fractional shortening ($r = -0.54$, $P = 0.02$), left ventricular mass ($r = 0.61$, $P = 0.03$), LV mass index ($r = 0.46$, $P = 0.04$), vena cava inferior diameter ($r = 0.65$, $P = 0.01$), and peak gradient of tricuspid regurgitation ($r = 0.46$, $P = 0.03$). *Conclusions*. Increased VEGF level is associated with structural and functional parameters in patients with HCM and serves as a potential tool for diagnostic process of these patients.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a heterogeneous myocardial disorder characterized by myocardial hypertrophy with structural and functional abnormalities. The incidence of HCM is approximately 1 in 500 (0.2%) of the general population [1]. The clinical outcome of HCM is diverse, ranging from asymptomatic patients to cardiac arrhythmias, congestive heart failure, and sudden cardiac death. According to the guidelines, patients with HCM require lifelong follow-up to detect changes in symptoms, risk of adverse events, left ventricle outflow tract obstruction (LVOTO), LV function, and cardiac rhythm [1, 2]. The recommended follow-up includes clinical evaluation, 12-lead ECG, and transthoracic echocardiography. The use of laboratory markers seems to be promising for diagnosis and risk stratification in HCM. There is a broad spectrum of biomarkers in peripheral blood, which are potentially useful for diagnosis and risk stratification in patients with HCM. But only cardiac troponins and natriuretic peptides have the most robust data [2]. The previous studies showed that high levels of natriuretic peptides and cardiac troponins are associated with cardiovascular events, heart failure, and death [3]. Systemic evaluation of these parameters revealed an association of these markers with the morphological and functional parameters [4–6]. The association of the myocardial hypertrophy and cardiac troponin and natriuretic peptide levels is not specific for the degree of left ventricle remodeling in HCM. The increase of these

parameters is driven probably more by myocardial damage or pressure overload in patients with obstructive type of LV hypertrophy and not by hypertrophy *per se*.

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen *in vitro* and angiogenic inducer in *in vivo* models. VEGF is secreted as a glycosylated homodimeric protein of 46 kDa that is made up of two 24 kDa subunits linked by disulphide bonds.

The tissue distribution of these VEGF receptors includes vascular smooth muscle cells, osteoblasts, cardiomyocytes, myofibroblasts, neurons, and various tumor cells [7]. It has been shown that VEGF is highly expressed in cardiomyocytes and myofibroblasts, indicating that VEGF family plays an autocrine/paracrine role in the regulation of myocyte and myofibroblast function and growth/survival [8–10]. Vascular endothelial growth factor plays an important role in the process of myocardial hypertrophy. However, the data about the association of VEGF and morphological and functional parameters in patients with hypertrophic cardiomyopathy are missing. The aim of our study was to assess VEGF level in peripheral blood in patients with HCM and to analyze its association with clinical markers of the disease.

2. Material and Methods

2.1. Study Population. Study population consisted of patients with nonobstructive hypertrophic cardiomyopathy. The diagnosis of hypertrophic cardiomyopathy was based on a history of illness, physical examination, echocardiography, and cardiac catheterization in accordance with European Society of Cardiology recommendations [2]. Based on the results of previous cardiac catheterization, patients with resting or provoked LVOTO were not included in the study. Also, patients with significant concomitant disease, such as pulmonary disease, arterial hypertension, malignancy, autoimmune disorders, neurodegenerative disorders, thyroid disease, or concurrent viral disease, were excluded. VEGF levels were compared to control group of healthy 20 blood donors (40.4 ± 8.5 years) with no evidence of cardiovascular disease according to ECG stress test and echocardiography. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committee of our institution. Informed consent was obtained from each patient. Baseline demography and clinical characteristics of the study population are shown in Table 1.

2.2. Echocardiography. Echocardiography was performed in agreement with the American Society of Echocardiography and European Association of Cardiovascular Imaging standards evaluating the following parameters: left ventricular end-diastolic (LVEDD) and end-systolic (LVESD) diameters, left atrium diameter (LA), right ventricle diameter (RV), end-diastolic interventricular septum (IVST) and posterior wall thickness (PWT), inferior vena cava diameter (IVC), LV ejection fraction (LV EF), left ventricle fractional shortening (FS), peak gradient of tricuspid regurgitation (Pgrad TR), left ventricle mass (LVM), and left ventricle mass index (LVMI) [11]. Left ventricle outflow tract gradient was evaluated in accordance with European Society of Cardiology guidelines at rest and after provocation by Valsalva maneuver [2].

Table 1: Patient demographic and clinical characteristics.

	HCM (n = 21)
Age (years)	58.4 ± 13.2
Female gender, n (%)	6 (28)
Atrial fibrillation, n (%)	7 (33)
Creatine level (μmol·L^{-1})	92 ± 14
Diabetes mellitus, n (%)	9 (43)
Hyperlipidemia, n (%)	14 (66)
Smoking, n (%)	7 (33)
NYHA functional class, n (%):	
I	8 (38)
II	10 (47)
III-IV	3 (14)
Therapy	
Medication, n (%)	
Calcium channel blocker	10 (47)
β-blockers	12 (57)
Diuretics	8 (38)
ACE inhibitors/sartans	9 (42)
Implantable devices, n (%)	
DDD pacing	5 (24)
ICD/BiV	1 (4)

NYHA, New York Heart Association; ACE, angiotensin converting enzyme; DDD, dual chamber pacing; ICD, implantable cardioverter/defibrillator; and BiV, biventricular pacing.

2.3. Assessment of VEGF. Blood samples were obtained from venous catheters, introduced into tube collectors containing no preservatives. Within 1 h, the blood samples were centrifuged for 10 min at 2500 g and the supernatant was removed and kept at −70°C until the assay was performed.

VEGF concentrations were measured using cytokine array for the evidence investigator protein biochip system (Randox Laboratories, UK). Simultaneous quantitative detection of multiple analytes based on sandwich chemiluminescent immunoassay was carried out from a single patient sample. The core technology consists of a solid plate containing discrete test regions with immobilized antibodies specific to different markers. Increased levels of marker in a specimen lead to increased binding of antibody labeled with horseradish peroxidase and thus to an increase in the luminescent signal emitted. The light signals generated from each of the test regions on the biochip are simultaneously detected using a charge coupled device camera. The analytical range of the VEGF assay was 3.24–1000 ng/L. The interassay coefficient of variability (n = 20) was, for 146.4 ng/L, 10.8% and, for 456.3 ng/L, 7.4%. Internal quality control measurements were carried out using samples provided by the kit manufacturer.

2.4. Statistics. Statistical analysis was performed by MedCalc Software, version 14 (MedCalc Software bvba, Ostend, Belgium). Normally distributed variables are expressed as means ± standard deviation, while nonnormally distributed

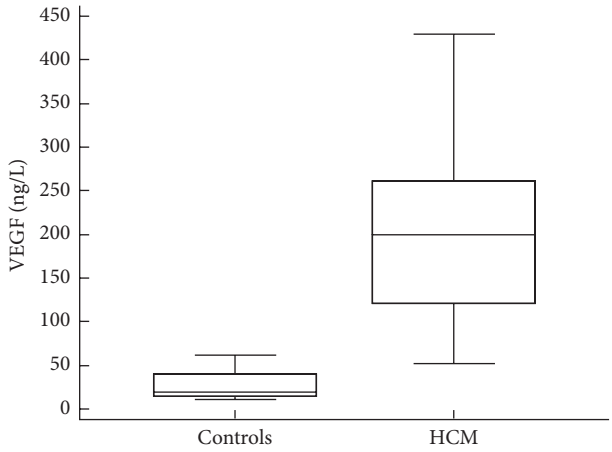

Figure 1: Serum VEGF in patients with hypertrophic cardiomyopathy and controls. HCM, hypertrophic cardiomyopathy.

variables are expressed as median (interquartile range). Categorical variables are presented as percentages. Continuous variables were compared using Student's t-test, Mann-Whitney, or Wilcoxon's tests, where appropriate. Linear regression was applied to evaluate the relationship between continuous variables. The degree of association between continuous variables was calculated using Pearson's correlation coefficient. A P value <0.05 was considered statistically significant.

3. Results

3.1. Serum VEGF. Serum VEGF was increased in all 21 patients. Compared with controls, HCM patients had significantly increased serum VEGF level 199 (IQR: 120.4–260.8) ng/L versus 20 (IQR: 14.8–37.7) ng/L, $P < 0.001$ (Figure 1). In patients with New York Heart Association (NYHA) functional classes I and II, serum VEGF values were lower compared to patients with NYHA classes III and IV: 146.4 (IQR: 113.6–235.2) ng/L versus 328.1 (IQR: 286.7–406.3) ng/L, $P < 0.01$. In patients with atrial fibrillation, VEGF values were not significantly increased compared to patients with sinus rhythm: 234.1 (IQR: 132.2–307.5) ng/L versus 158.5 (IQR: 121.8–250.1) ng/L, P 0.43.

3.2. Echocardiography. All patients underwent two-dimensional and Doppler echocardiography. Table 2 shows the summary information of echocardiographic parameters of patients with hypertrophic cardiomyopathy. The mean of the anteroposterior left atrium dimension was 45.2 ± 5.6 mm, and it exceeded reference values (female: 38 mm, male: 40 mm) in 17 (81%) patients. The mean internal diameter of right ventricle was 26.3±3.2 mm, and it exceeded reference value (31 mm) in 1 (5%) patient. The mean of the internal end-systolic left ventricle dimension was 31.8 ± 8.8 mm, and it exceeded reference values (female: 34.8 mm, male: 39.8 mm) in 2 (10%) patients. The mean of the internal end-diastolic left ventricle dimension was 47 ± 6.3 mm, and it exceeded reference values (female: 52.2 mm, male: 58.4 mm) in 1 (5%) patient. The mean of the interventricular septum thickness was 18.7 ± 2.2 mm, and it exceeded recommended thickness for diagnosis of hypertrophic cardiomyopathy (≥15 mm) in all patients. The mean left ventricle mass was 408.4 ± 11.8 g, and it exceeded reference values for two-dimensional method (female: 150 g, male: 200 g) in all patients. The mean left ventricle mass index was 202.4 ± 53.9 g·m^{-2}, and it exceeded reference values for two-dimensional method (female: 88 g·m^{-2}, male: 102 g·m^{-2}) in all patients. The mean of the left ventricle ejection fraction was 65.4 ± 12.2%, and, only in 1 (5%) patient, the LV ejection fraction was below the reference values (female: 54%, male: 52%). The mean of the left ventricle fractional shortening was 0.32 ± 0.09, and it exceeded the reference values (female: 27–45, male: 25–43) in 3 (15%) patients. The mean peak tricuspid regurgitation gradient was 22.6 ± 11.8 mmHg. The inferior vena cava diameter was 18 ± 3.7 mm.

Table 2: Echocardiographic parameters of the study population.

Echocardiography	
LA (mm)	45.2 ± 5.6
RV (mm)	26.3 ± 3.2
IVST (mm)	18.7 ± 2.2
LV ESD (mm)	31.8 ± 8.8
LV EDD (mm)	47.0 ± 6.3
PWT (mm)	13.7 ± 2.0
LV EF (%)	65.4 ± 12.2
LV FS	0.32 ± 0.09
IVC (mm)	18.0 ± 3.7
Pgrad TR (mmHg)	22.6 ± 11.8
LVM (g)	408.4 ± 11.8
LVMI (g·m^{-2})	202.4 ± 53.9

LA, left atrium; RA, right ventricle diameter; IVST, interventricular septal thickness; LV ESD, left ventricular end-systolic diameter; LV EDD, left ventricular end-diastolic diameter; PWT, posterior wall thickness; LV EF, left ventricular ejection fraction; LV FS, left ventricular fractional shortening; IVC, inferior vena cava diameter; Pgrad TR, peak gradient of tricuspid regurgitation; LVM, left ventricle mass; and LVMI, left ventricle mass index.

None of the patients had left ventricle outflow tract obstruction. The peak left ventricle outflow tract gradients were 6 (IQR: 1–7.5) mmHg during at rest measurement and 15 (IQR: 2.2–29) mmHg during Valsalva maneuver. The median values for the peak LVOT pressure gradient were 3 (IQR: 2.2–5) mmHg for at rest measurements and 14 (IQR: 2–22) mmHg during Valsalva maneuver.

3.3. Association of VEGF and Morphological and Functional Echocardiographic Parameters. The analysis of echocardiographic parameters showed association of the morphological and functional parameters with serum VEGF level. Serum levels of increased vascular endothelial growth factor left were significantly associated with left atrium diameter ($r = 0.51$, 95% CI: 0.09–0.77, and $P = 0.01$), left ventricle ejection fraction ($r = -0.56$, 95% CI: −0.80–−0.17, and $P = 0.01$), LV fractional shortening ($r = -0.54$, 95% CI: −0.79–−0.14, and $P = 0.02$), LV mass ($r = 0.61$, 95% CI: 0.24–0.82, and $P = 0.03$), LV mass index ($r = 0.46$, 95% CI: 0.02–0.75, and $P = 0.04$), vena cava inferior diameter ($r = 0.65$, 95% CI: 0.30–0.84,

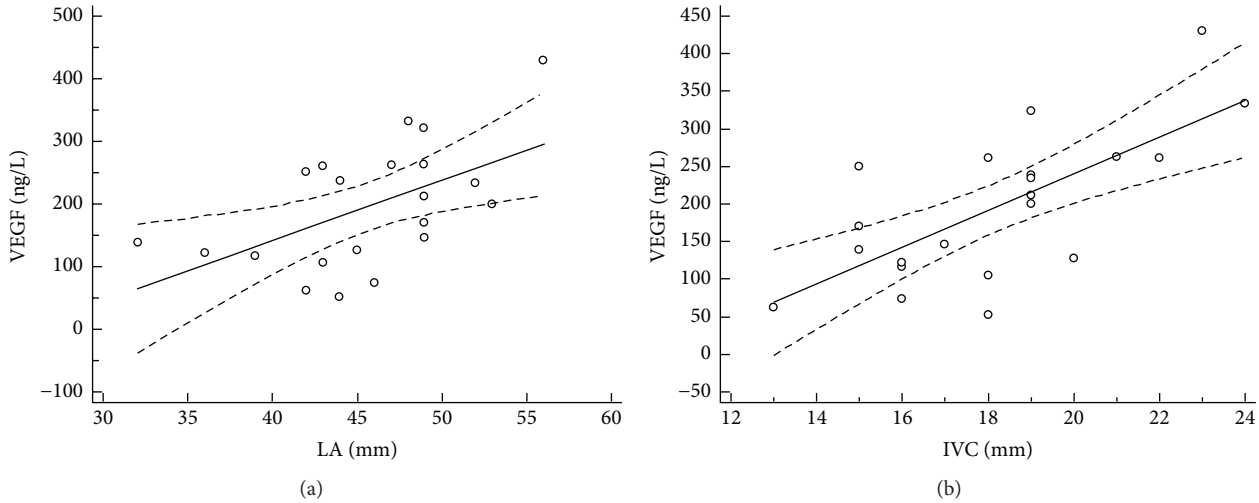

Figure 2: Association of VEGF and left atrium diameter (a) and inferior vena cava diameter (b) in patients with hypertrophic cardiomyopathy. Left atrium (a): $n = 21$, coefficient of determination R^2: 0.31, regression equation: $y = -276.4 + 10.4x$, and significance level: $P = 0.0082$. Inferior vena cava diameter (b): $n = 21$, coefficient of determination R^2: 0.49, regression equation: $y = -273.9 + 25.9x$, and significance level: $P = 0.0004$. LA, left atrium diameter; IVC, inferior vena cava diameter.

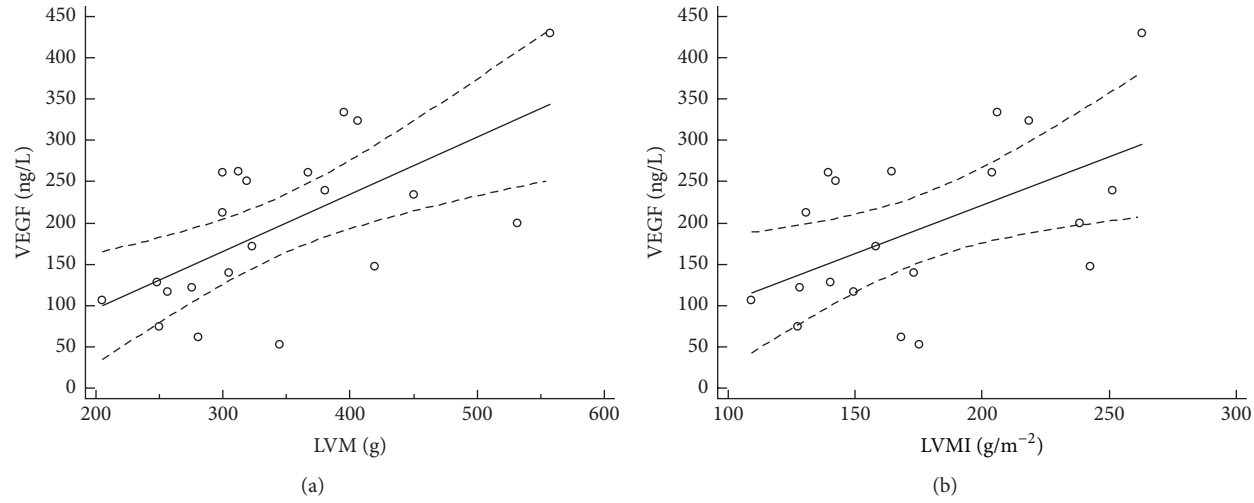

Figure 3: Association of VEGF and left ventricular mass (a) and left ventricular mass index (b) in patients with hypertrophic cardiomyopathy. Left ventricular mass (a): $n = 21$, coefficient of determination R^2: 0.43, regression equation: $y = -61.3 + 0.76x$, and significance level: $P = 0.0011$. Left ventricular mass index (b): $n = 21$, coefficient of determination R^2: 0.31, regression equation: $y = -27.5 + 1.3x$, and significance level: $P = 0.0115$. LVM, left ventricular mass; LVMI, left ventricular mass index.

and $P = 0.01$), and peak gradient of tricuspid regurgitation ($r = 0.46$, 95% CI: 0.04–0.74, and $P = 0.03$). Figures 2, 3, 4, and 5 illustrate regression analysis of the morphological and functional echocardiographic parameters.

4. Discussion

Hypertrophic cardiomyopathy is an autosomal dominant inherited myocardial disease defined by the presence of increased left ventricular wall thickness that is not solely explained by abnormal loading conditions [2]. The pathophysiology of HCM is complex and consists of multiple interrelated abnormalities, including left ventricle hypertrophy, left ventricle outflow tract obstruction, diastolic dysfunction, mitral regurgitation, myocardial ischemia, and arrhythmias. The natural history of HCM varies from an asymptomatic and benign clinical course to sudden premature death. Therefore, the new markers are searched with the aim for detecting risk patients and improving their prognosis. Until now, there have been published many studies focused on a broad spectrum of biomarkers covering most of the pathophysiological processes described in HCM, for example, myocardial necrosis and wall stress markers, inflammatory markers, markers of endothelial dysfunction, markers of

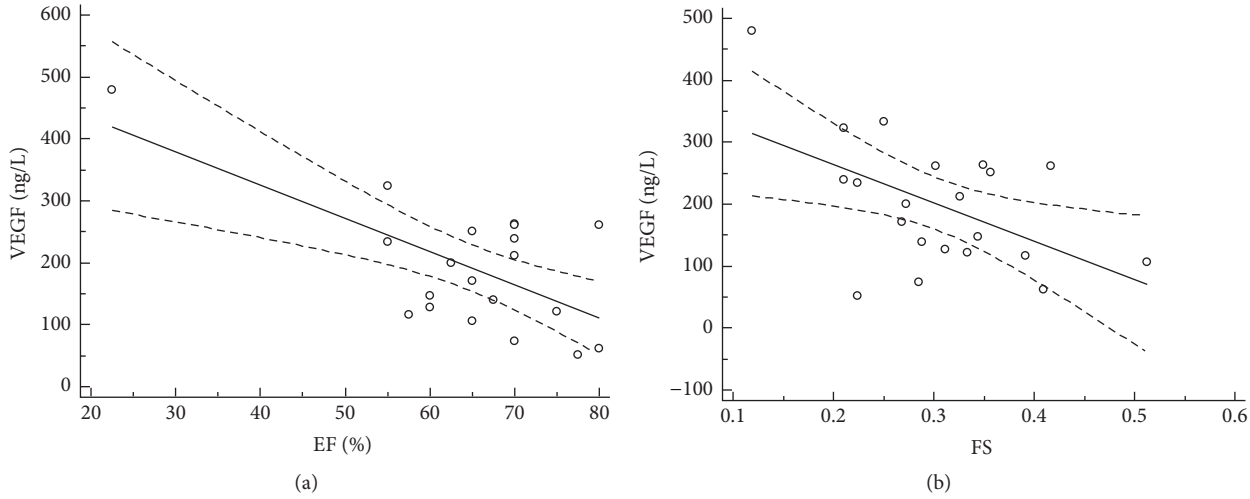

Figure 4: Association of VEGF and left ventricle ejection fraction (a) and left ventricle fractional shortening (b) in patients with hypertrophic cardiomyopathy. Left ventricle ejection fraction (a): $n = 21$, coefficient of determination R^2: 0.42, regression equation: $y = 541.3 - 5.4x$, and significance level: $P = 0.018$. Left ventricle fractional shortening (b): $n = 21$, coefficient of determination R^2: 0.27, regression equation: $y = 387.4 - 618.9x$, and significance level: $P = 0.0165$. LV EF, left ventricle ejection fraction; FS, left ventricular fractional shortening.

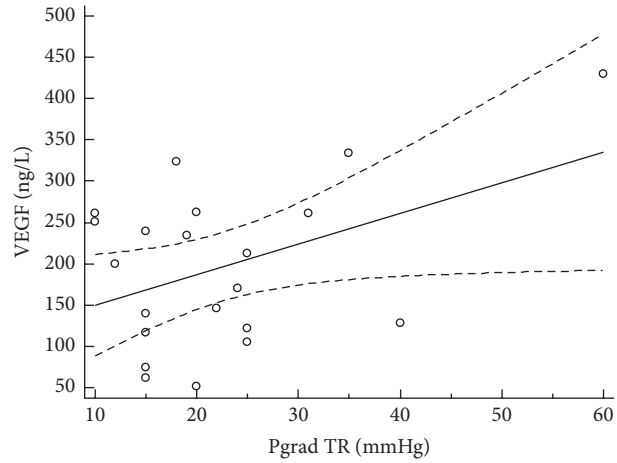

Figure 5: Association of VEGF and peak gradient of tricuspid regurgitation in patients with hypertrophic cardiomyopathy. Peak gradient tricuspid regurgitation: $n = 21$, coefficient of determination R^2: 0.47, regression equation: $y = 58.4 + 6.2x$, and significance level: $P = 0.005$. TR, tricuspid regurgitation.

apoptosis, matrix metalloproteinases, platelet function markers, prothrombotic markers, hormones [12–14]. However, no disease specific marker was approved for a routine clinical practice.

In our study, we focused on VEGF and its clinical significance in patients with HCM.

It has been shown that VEGF is an endothelial cell-specific mitogen *in vitro* and an angiogenic inducer in *in vivo* models [15] and plays an important role in myocardial hypertrophy [16]. In our study, we confirmed increased serum VEGF level in patients with nonobstructive HCM when compared to normal population. Furthermore, VEGF levels were significantly associated with the degree of left ventricle hypertrophy (left ventricle mass and its index) in the absence of left ventricle outflow tract obstruction.

The progression of left ventricle hypertrophy leads to development of heart failure and is associated with a poor prognosis [17]. In various studies, plasma natriuretic peptide levels were significantly increased in HCM compared with normal subjects. In HCM patients, both BNP and ANP are significantly higher in the subgroup that shows evidence of obstruction and both correlate positively with left intraventricular pressure gradient. As prognostic factors, plasma levels of NT-pro-BNP and ANP are independent predictors of cardiovascular events in patients with HCM [17–20]. Therefore, we analyzed VEGF level and its relation to clinical and hemodynamic parameters. We observed that the New York Heart functional classes III and IV were characterized by higher VEGF level when compared to NYHA classes I and II. Hemodynamic parameters were evaluated by the use of echocardiography. We found significant negative association of left ventricular functional parameters (ejection fraction and fractional shortening). Also, we have noticed that VEGF is associated with increased pressure in pulmonary artery circulation. The increase of this pressure is passive and it is driven by volume and pressure left ventricle overload. This observation supports left atrium enlargement in the presence of LV hypertrophy. Our results are supported by the previous observations of the pathogenetic role of the VEGF in left ventricle remodeling process in patients with HCM [21–23]. As compared to other markers, VEGF could have a potential to reflect morphological and functional parameters in patients with hypertrophic cardiomyopathy.

5. Conclusions

The results of our present study indicate that serum VEGF level in patients with HCM was significantly increased in comparison to control group and was associated with NYHA

functional class. The VEGF levels were positively associated with the morphological parameters of HCM (left ventricle mass and its index); functional parameters of the left ventricle showed negative association. Serum VEGF correlated also with peak tricuspid regurgitation gradient (marker of pulmonary circulation pressure), inferior vena cava diameter, and left atrium diameter (markers of volume and pressure overload).

Despite the study limitations (relatively small number of the patients and morphological and hemodynamic parameters were evaluated by echocardiography), serum VEGF has emerged as an interesting and promising parameter useful for risk stratification of patients with hypertrophic cardiomyopathy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

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Research Article

Effect of Left Ventricular Outflow Tract Obstruction on Left Atrial Mechanics in Hypertrophic Cardiomyopathy

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Left atrial (LA) volumes are known to be increased in hypertrophic cardiomyopathy (HCM) and are a predictor of adverse outcome. In addition, LA function is impaired and is presumed to be due to left ventricular (LV) diastolic dysfunction as a result of hypertrophy and myocardial fibrosis. In the current study, we assess the incremental effect of outflow tract obstruction (and concomitant mitral regurgitation) on LA function as assessed by LA strain. Patients with HCM (50 obstructive, 50 nonobstructive) were compared to 50 normal controls. A subset of obstructive patients who had undergone septal myectomy was also studied. Utilising feature-tracking software applied to cardiovascular magnetic resonance images, LA volumes and functional parameters were calculated. LA volumes were significantly elevated and LA ejection fraction and strain were significantly reduced in patients with HCM compared with controls and were significantly more affected in patients with obstruction. LA volumes and function were significantly improved after septal myectomy. LVOT obstruction and mitral regurgitation appear to further impair LA mechanics. Septal myectomy results in a significant reduction in LA volumes, paralleled by an improvement in function.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder characterized by pathological left ventricular (LV) hypertrophy, complex pathophysiology, and diverse clinical outcomes. Increased LV mass and diastolic dysfunction are associated with progressive left atrial (LA) dilatation and dysfunction, often compounded by the presence of LV outflow tract (LVOT) obstruction and concomitant mitral regurgitation. LA size and volume have been shown to be determinants of both exercise capacity [1] and major adverse cardiac and cerebrovascular events in patients with HCM [2–4]. In addition, LA dysfunction and in particular LA booster pump function have been shown to correlate with heart failure symptoms in HCM [5] as well as being a strong predictor for the development of atrial fibrillation (AF) requiring hospitalization [6]. A recent study of a large cohort of HCM patients undergoing CMR has demonstrated LA ejection fraction (LAEF) and minimum LA volumes as predictors of the development of AF [7].

The relationship between the LV and LA is highly dynamic and interdependent. All phasic aspects of LA function are to some degree affected not only by LV contractility,

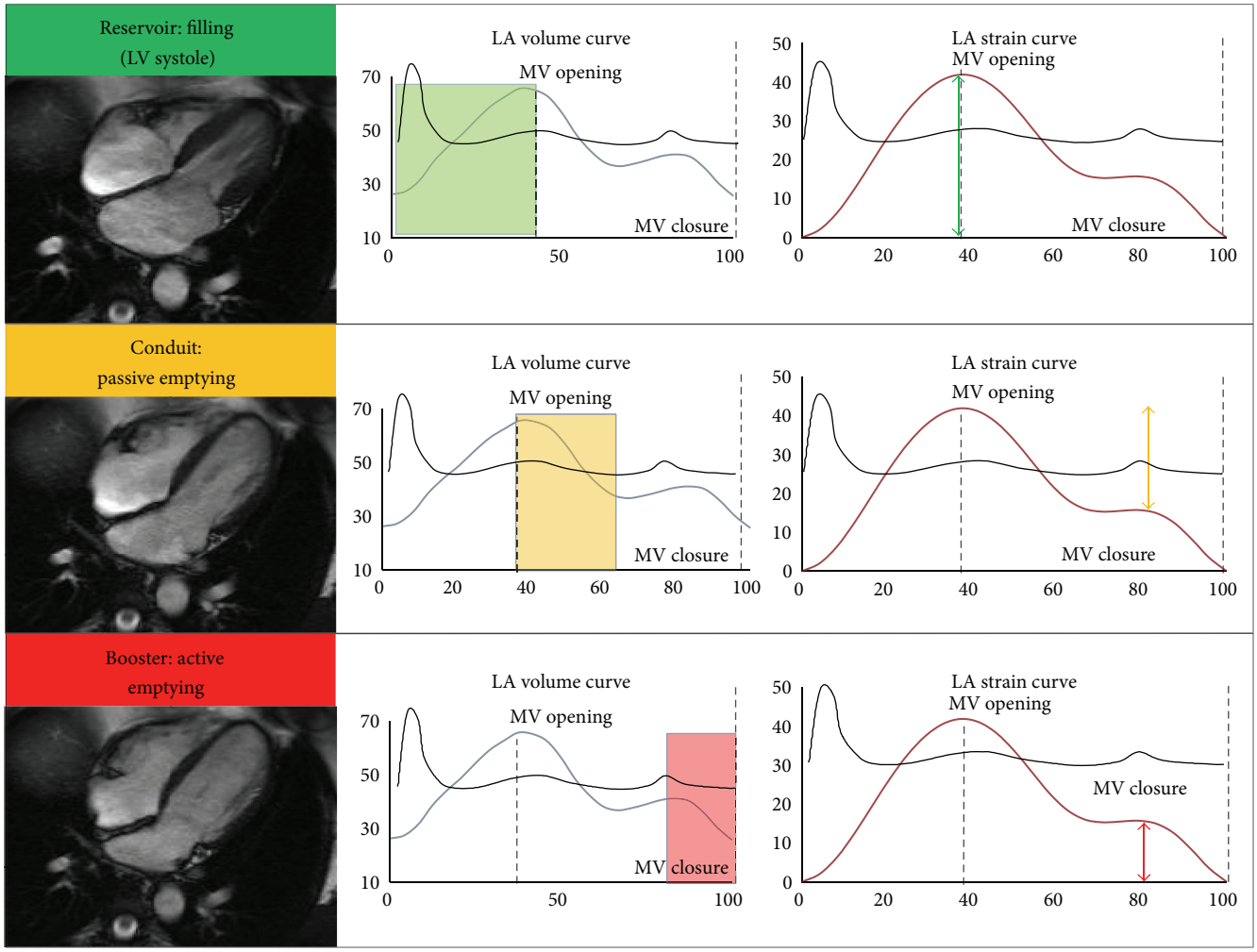

Figure 1: Diagrammatic representation of phasic LA function, LA volume, and strain curves. An ECG curve is superimposed to highlight timing of LV systole and diastole.

relaxation, compliance, and filling pressures, but in addition by intrinsic LA contractility, relaxation, and compliance [8, 9].

LA strain and strain rate analysis by means of feature tracking provides a feasible noninvasive method of assessment of LA function [10–12]. Three distinct phases of atrial function assessed are (1) reservoir function, which represents the storage of pulmonary venous return during ventricular systole and isovolumic relaxation, (2) conduit function, which represents the period of passive emptying of the LA down a pressure gradient during early diastole, and (3) booster or active contractile function, which represents intrinsic atrial contractility during which the atria empty before the end of ventricular diastole (see Figure 1). A recent study has demonstrated the feasibility and reliability of quantification of LA strain and strain rate using CMR myocardial feature tracking in both normal controls and patients with HCM [13].

The aim of this study was to evaluate alterations in LA volumes and function in patients with HCM, particularly between patients with and without LVOT obstruction, and to identify the determinants of LA myopathy in this disease state. In addition, we aimed to study the effects of relief of LVOT obstruction on LA function in a subset of patients undergoing septal myectomy.

2. Methods and Materials

2.1. Study Population. A retrospective cohort of one hundred adult patients from the Hypertrophic Cardiomyopathy Clinic at the Toronto General Hospital with HCM (maximal septal thickness of ≥15 mm and a septal-to-posterior wall thickness ratio of ≥1.3, in the absence of another cardiac or systemic disease that could cause LV hypertrophy) was included in the study. All patients had preserved LV systolic function (defined as a CMR-derived LVEF ≥55%). Patients were in sinus rhythm at the time of both the CMR study and echocardiogram. Studies were performed on active cardiac medications. Patients were subdivided into two groups as follows: (1) a nonobstructive HCM subgroup with LVOT gradient of <30 mmHg both at rest and with provocation (with Valsalva and amyl nitrate) and (2) an obstructive HCM subgroup with

resting LVOT gradient of ≥30 mmHg. Patients with latent obstruction only were not included in the current study. A cohort of fifty normal controls who had previously undergone CMR at the Tufts Medical Center and the Minneapolis Heart Institute were included for comparison. In addition, a subset of twenty patients with obstructive HCM who had undergone CMR studies before and after septal myectomy was studied.

2.2. Clinical and 2D Echocardiographic Data. A retrospective chart review was performed in order to obtain demographic data and symptomatic status. Standard 2D echocardiographic data was obtained from the study performed closest to the CMR, with all echocardiographic measurements acquired as per ASE guidelines [14, 15]. Mitral regurgitation was qualitatively assessed by a single observer and graded as none, trivial, mild, moderate, or severe. The study was approved by the Research Ethics Board of the Toronto General Hospital and the Investigational Review Board of the participating centers in the United States of America.

2.3. CMR Protocol. At the Toronto General Hospital, CMR imaging was performed on 1.5T or 3T whole body magnets (Magnetom Avanto, Magnetom Verio, Siemens Healthcare, Erlangen, Germany) using a 32-element phased-array coil. At Tufts Medical Center, CMR imaging was performed on a Philips Gyroscan ACS-NT 1.5T scanner (Best, Netherlands) and at the Minneapolis Heart Institute on a Siemens Avanto 1.5T scanner (Erlangen, Germany). Cine steady state free precession (SSFP) images were acquired in short axis (sequential 10 mm slices from the atrioventricular ring to the apex) and 2-, 3-, and 4-chamber long axes. LV ejection fraction, ventricular volumes, ventricular mass, and maximal wall thickness were measured by standard offline analysis using customized software (QMassMR, Medis, Leiden, Netherlands).

2.4. MR Velocity Vector Imaging. VVI is a feature-tracking method which incorporates feature and endocardial contour tracking. VVI quantifies myocardial motion by automatically tracking user-defined endocardial and epicardial contours to define the inward and outward myocardial motion. Based on motion of the tracked points between the frames and knowledge of the time interval between frames, 2D tissue velocity is computed. Strain and strain rate are computed by the range in the relative distance between localized tracked trace points, combined with the difference in the relative displacement of the tissue motion between tracked points. Strain was defined as the instantaneous local trace lengthening/shortening and strain rate as the rate of lengthening/shortening.

The feature-tracking program, VVI Version 3.0.0 (Siemens Healthcare, Mountain View, CA), was applied to the cine SSFP images from archived studies, allowing for strain parameter assessment. Cine SSFP data derived from CMR images were converted from Digital Imaging and Communications in Medicine (DICOM) to Audio Video Interleave (AVI) format creating 30 cardiac phases. Subsequently, LA motion was quantified by automatic tracking of user-defined points in both the subendocardial and subepicardial regions.

Table 1: Phasic LA function parameters.

Reservoir function	
Expansion index (%)	$(V_{max} - V_{min})/V_{min} \times 100$
Left atrial ejection/emptying fraction (%)	$(V_{max} - V_{min})/V_{max} \times 100$
Conduit function	
Passive emptying index/fraction (%)	$(V_{max} - V\text{pre}_A)/V_{max} \times 100$
Conduit volume (mL/m^2)	LV stroke volume – $(V_{max} - V_{min})$
Booster/pump function	
Active emptying index/fraction (%)	$(V\text{pre}_A - V_{min})/V\text{pre}_A \times 100$

V_{max}: maximal left atrial volume; V_{min}: minimum left atrial volume; $V\text{pre}_A$: left atrial volume immediately prior to atrial contraction.

2.5. Left Atrial Volumes. LA volumes were determined by VVI software from the four-chamber view using Simpson's method of disks. Pulmonary veins and the LA appendage were excluded in the calculation of volumes. The following were measured (indexed to body surface area): (1) maximum volume at end-systole (V_{max}), (2) pre-*A* volume prior to the onset of atrial contraction ($V\text{pre}_A$), and (3) minimum volume at end-diastole (V_{min}). From these volumes, LA phasic parameters were derived as shown in Table 1.

2.6. Left Atrial Mechanics. In the long-axis four-chamber views, endocardial and epicardial borders were manually traced in the end-systolic frame. The software subsequently traced the borders in the other frames of the cardiac cycle automatically. Strain parameters were recorded after visual confirmation of the best endocardial and epicardial motion tracking (by operator subjective visual assessment). The strain curves were gated in systole (*R* wave), and longitudinal strain/strain rate parameters were calculated. A diagrammatic representation of the phases of LA function is shown in Figure 1.

2.7. Intra- and Interobserver Variability. Offline analysis of all cine SSFP data sets was performed by a single observer (LW). Ten randomly selected studies were reanalyzed by the same observer (LW) and a second observer (JM).

2.8. Statistical Analyses. Continuous and categorical data are expressed as mean (±SD) or *n* (%), respectively. Comparison between the HCM subgroups and normal controls was performed using an ANOVA. Intra- and interobserver variability were assessed using Bland-Altman analysis. Correlations between variables were assessed using Pearson's correlation or Spearman's rank correlation coefficient test where appropriate. The independent effects of LVOT obstruction on LA strain parameters were tested using multivariable linear regression models. All statistical analyses were performed using SAS 9.3 (Cary, North Carolina) and MedCalc version 11.6.0.0 (MedCalc Software, Belgium). Statistical significance was defined as a 2-sided p value <0.05.

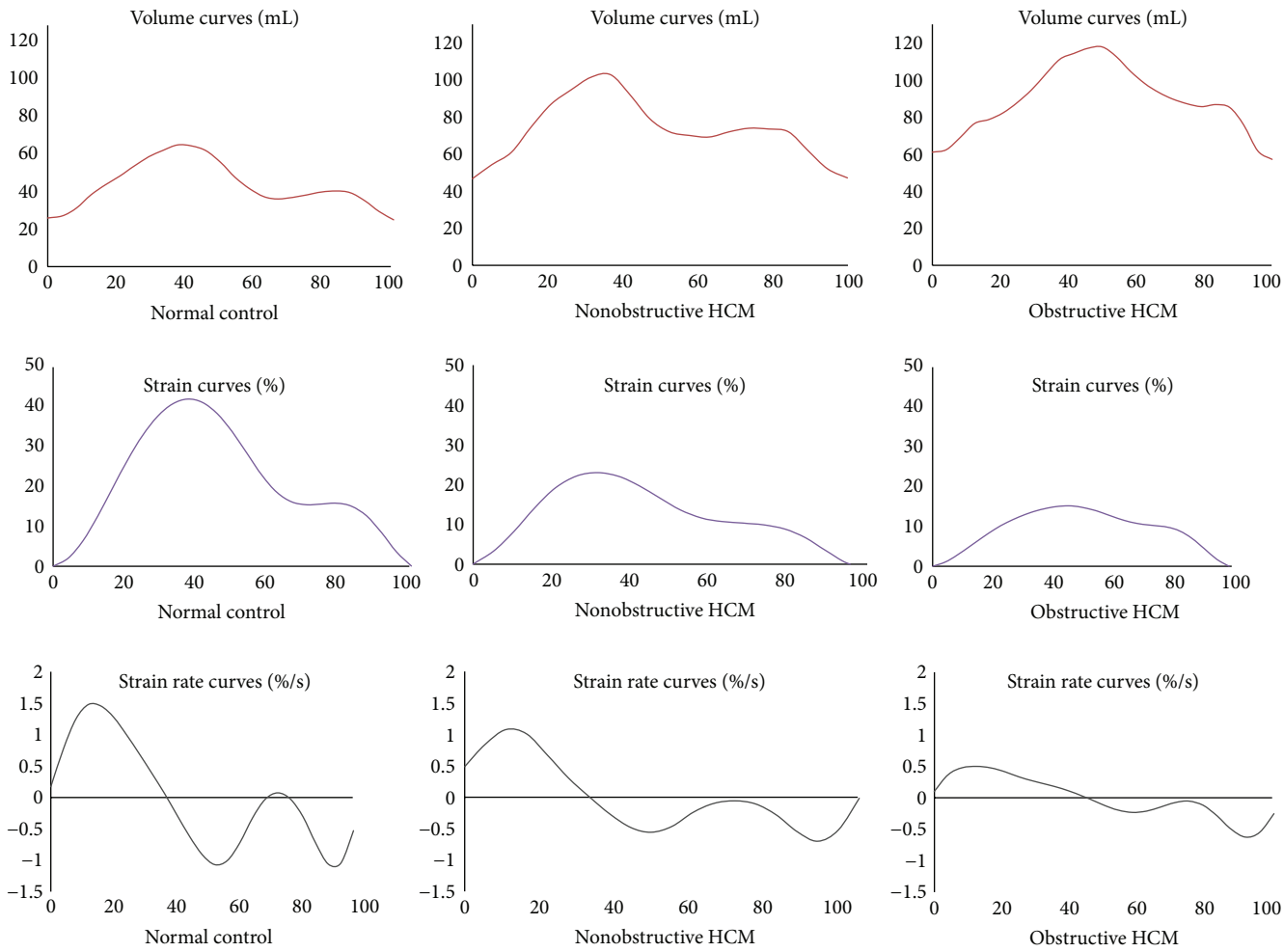

Figure 2: Representative volume, strain, and strain rate curves from a normal control and patients with nonobstructive and obstructive HCM.

3. Results

3.1. Patient Demographics and Clinical Data

3.1.1. Clinical, Demographic, and 2D Echocardiographic Parameters. Baseline demographic and echocardiographic parameters for the normal control group and the HCM group as a whole are shown in Table 2, with the data for obstructive and nonobstructive HCM subgroups shown in Table 3.

3.1.2. Cardiovascular Magnetic Resonance Imaging. Conventional CMR parameters are shown in Tables 2 and 3. While LVEF and LVEDVi were not significantly different between HCM patients and normal controls, LVMI was significantly elevated in patients with HCM (80.6±26 versus 52.5±11 g/m^2; $p < 0.0001$). There was no significant difference in LVEDVi between HCM patients with and without obstruction. However, patients with obstructive HCM had a higher LVMI (87.7 ± 24 versus 73.0 ± 26 g/m^2; $p = 0.006$).

3.2. LA Volumes and Myocardial Mechanics. All LA volumes were significantly elevated in patients with HCM, and LAEF significantly reduced (44.8 ± 9 versus 65 ± 11%; $p < 0.0001$), with all phases of LA function (reservoir, conduit, and booster) affected. These findings remained even after adjustment for age, LVMI, and LVEDVi. Representative LA volume, strain, and strain rate curves are shown in Figure 2.

Table 2: Baseline clinical and CMR parameters.

	Normal controls ($n = 50$)	HCM ($n = 100$)	p value
Clinical characteristics			
Age at CMR (years)	42.6 ± 16	49.7 ± 15	*0.007*
Sex (male, *n*, %)	28 (56%)	70 (70%)	0.09
BSA	1.94 ± 0.3	1.94 ± 0.2	0.96
NYHA I/II/III/IV (%)	100/0/0/0	56/19/25/0	*<0.0001*
CMR parameters			
LVEF (%)	62.5 ± 6	63.1 ± 7	0.58
LVEDVi (mL/m^2)	87.7 ± 16	86.8 ± 13	0.72
LVMi (g/m^2)	52.5 ± 11	80.6 ± 26	*<0.0001*

CMR: cardiac magnetic resonance; BSA: body surface area; NYHA: New York Heart Association functional class; LVEF: left ventricular ejection fraction; LVEDVi: indexed left ventricular end-diastolic volume; LVMi: indexed left ventricular mass.

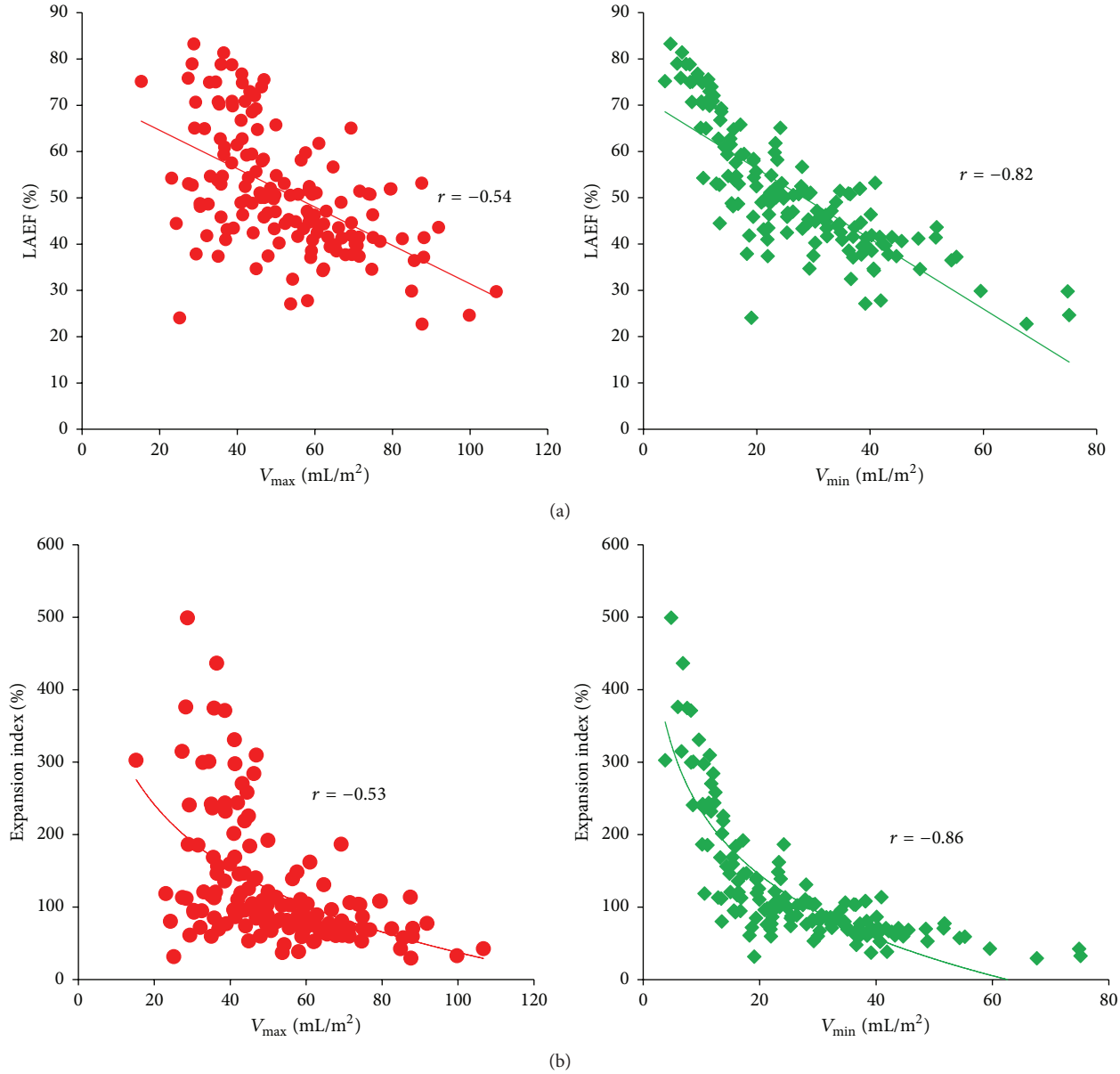

Figure 3: (a) Correlation between LA ejection fraction (LAEF %) and maximum/minimum LA volumes. (b) Correlation between LA expansion index and maximum/minimum LA volumes.

3.3. Relationship between LVOT Obstruction and LA Mechanics. Patients with obstructive HCM had a significantly greater impairment in LA function and larger LA volumes (Table 4), and LAEF was significantly lower (42.3 ± 8 versus 47.2 ± 9%; $p = 0.004$). Although conduit function was not different between the two HCM subgroups, patients with obstruction had significantly more impairment of LA reservoir function, with lower reservoir strain (14.5 ± 4 versus 17.7 ± 5%; $p = 0.002$) and strain rate (0.59 ± 0.2 versus 0.73 ± 0.2%/s; $p = 0.001$). In addition, there was a significant reduction in both booster strain (6.1±2 versus 7.5±3%; $p = 0.01$) and strain rate (−0.44±0.1 versus −0.58±0.25/s; $p = 0.004$). The reduction in LA function and increase in LA volumes remained significant even after adjusting for age, LVMI, LVEDVi, and degree of mitral regurgitation.

3.4. Correlation of LA Mechanics and Echocardiographic and CMR Parameters. There were no significant correlations between strain or strain rate parameters and 2D echocardiographic parameters of diastolic function or between resting/provocable LVOT gradients and either strain or strain rate parameters. LVMI correlated inversely with reservoir strain ($r = -0.61$; $p < 0.0001$), booster strain ($r = -0.52$; $p < 0.0001$), and LAEF (−0.56; $p < 0.0001$). Correlations between LA volumes and LA mechanics are shown in Figure 3 and Table 5.

3.5. Effect of Septal Myectomy on Left Atrial Volumes and Mechanics. The mean time from surgery to repeat CMR was 8.6 months (range: 5 to 18 months). No patients underwent a concomitant surgical MAZE procedure. Significant

Table 3: Baseline clinical, CMR, and 2D echocardiographic parameters in the nonobstructive and obstructive HCM subgroups.

	Nonobstructive HCM ($n = 50$)	Obstructive HCM ($n = 50$)	p value
Clinical characteristics			
Age at CMR (years)	44.7 ± 15	54.6 ± 12	*0.001*
Sex (male, *n*, %)	36 (72)	34 (68)	0.83
BSA	1.92 ± 0.2	1.96 ± 0.2	0.35
NYHA I/II/III/IV (%)	82/14/4/0	32/22/46/0	*<0.0001*
Atrial fibrillation (*n*, %)	3 (6)	4 (8)	0.69
Beta-blockers (*n*, %)	22 (44)	42 (84)	*0.0001*
Calcium channel blockers (*n*, %)	5 (10)	4 (8)	0.73
Disopyramide (*n*, %)	0 (0)	38 (76)	*<0.0001*
ACE-i/ARB (*n*, %)	7 (14)	6 (12)	0.77
Amiodarone (*n*, %)	2 (4)	1 (2)	0.56
Coumadin (*n*, %)	4 (8)	2 (4)	0.40
CMR parameters			
LVEF (%)	61.7 ± 6	64.6 ± 7	*0.03*
LVEDVi (mL/m^2)	86.1 ± 15	87.5 ± 12	0.62
LVMi (g/m^2)	73.0 ± 26	87.7 ± 24	*0.006*
2D echocardiographic parameters			
LVOT resting (mmHg)	6.6 ± 2	56.2 ± 28	*<0.0001*
LVOT provocable (mmHg)	12.1 ± 7	82.7 ± 30	*<0.0001*
Maximal wall thickness (mm)	19.1 ± 5	20.7 ± 4	*0.002*
MR: trivial/mild/moderate/severe (%)	74/26/0/0	18/46/28/8	*<0.0001*
E wave (m/sec)	0.68 ± 0.2	0.86 ± 0.2	*<0.001*
A wave (m/sec)	0.49 ± 0.2	0.73 ± 0.2	*<0.001*
E/A ratio	1.54 ± 0.6	1.32 ± 0.6	0.07
Deceleration time (ms)	217 ± 48	264 ± 62	*<0.001*
Isovolumic relaxation time (ms)	89 ± 17	94 ± 23	0.32
E/E' ratio	7.2 ± 3	11.2 ± 5	*<0.0001*

CMR: cardiac magnetic resonance; BSA: body surface area; NYHA: New York Heart Association functional class; LVEF: left ventricular ejection fraction; LVEDVi: indexed left ventricular end-diastolic volume; LVMi: indexed left ventricular mass; LVOT: left ventricular outflow tract gradient; MR: mitral regurgitation.

reductions in NYHA class, LVOT gradient, and degree of mitral regurgitation were seen after myectomy (Table 6). LA volumes decreased and LAEF increased significantly (41.6 ± 13 versus 48.4 ± 10%; $p = 0.006$). Although conduit function remained unchanged, an improvement in both reservoir (14.1±6 versus 17.3±7%; $p = 0.01$) and booster strain (6.8±4 versus 9.8 ± 5%; 0.0001) was seen.

3.6. Intra- and Interobserver Variability. Intra- and interobserver variability demonstrated good agreement for both volumetric and strain parameters and are shown in Table 7.

4. Discussion

In the present study, we demonstrate not only a significant increase in LA volumes but also a marked impairment in all components of LA function in patients with HCM compared with normal controls. While previous studies have demonstrated abnormalities in LA function in patients with HCM, for the first time we have demonstrated significantly worse LA function in those with resting LVOT obstruction compared to patients with nonobstructive HCM. Septal myectomy, via relief of LVOT obstruction and reduction in degree of mitral regurgitation, results in both a significant reduction in LA volumes and improvement in LA function.

The pathological hypertrophy characteristic of HCM, myocardial ischemia secondary to abnormalities of the microvasculature, and the presence of myocardial disarray and fibrosis all serve to result in a reduction in LV compliance, abnormal ventricular relaxation, and diastolic dysfunction. The resultant elevation in LV filling pressures is transmitted back to the LA, necessitating an increase in LA pressures in order to maintain adequate diastolic filling. Subsequent increases in LA wall tension serve to drive LA enlargement, reflected in the significantly greater LA volumes seen in patients with HCM compared with normal controls.

In the present study, patients with LVOT obstruction were older and more symptomatic and had significantly greater LA volumes. In addition, they had evidence of a higher burden of hypertrophy, a greater degree of mitral regurgitation, and more severe diastolic dysfunction. LVMI was shown to correlate inversely with measures of LA strain, but no correlations were noted between absolute LVOT gradient and parameters of strain and strain rate. However, even after

Table 4: LA volumes and myocardial mechanics.

	Normal controls ($n = 50$)	Nonobstructive HCM ($n = 50$)	Obstructive HCM ($n = 50$)
LA volume indices			
V_{max} (mL/m^2)	38.3 ± 10	54.3 ± 15*	63.4 ± 17$^{*\#}$
Vpre$_A$ (mL/m^2)	22.3 ± 8	40.1 ± 13*	49.3 ± 14$^{*\#}$
V_{min} (mL/m^2)	13.3 ± 5	28.9 ± 10*	36.9 ± 13$^{*\#}$
Left atrial ejection/emptying fraction (%)	65.0 ± 11	47.2 ± 9*	42.3 ± 8$^{*\#}$
Left ventricular stroke volume (mL/m^2)	54.5 ± 9	52.6 ± 11	55.5 ± 9
Conduit volume	58.4 ± 26	52.3 ± 22	56.4 ± 23
Reservoir function			
Expansion index (%)	219.0 ± 113	95.2 ± 36*	76.3 ± 23$^{*\#}$
Longitudinal reservoir strain (%)	39.5 ± 13	17.7 ± 5*	14.5 ± 4$^{*\#}$
SRs (%/sec)	1.37 ± 0.4	0.73 ± 0.2*	0.59 ± 0.2*
Conduit function			
Passive emptying index/fraction (%)	41.6 ± 13	26.4 ± 9*	22.3 ± 7*
SRe (%/sec)	−0.99 ± 0.5	−0.43 ± 0.2*	−0.35 ± 0.2*
Booster function			
Active emptying index/fraction (%)	39.9 ± 14	28.0 ± 10*	25.7 ± 8*
Longitudinal booster strain (%)	15.3 ± 7	7.5 ± 3*	6.1 ± 2$^{*\#}$
SRa (%/sec)	−1.05 ± 0.5	−0.58 ± 0.2*	−0.44 ± 0.1$^{*\#}$

SRs: strain rate in systole; SRe: strain rate during passive emptying; SRa: strain rate during active emptying; $^*p < 0.05$ compared with normal controls; $^\#p < 0.05$ between obstructive and nonobstructive HCM.

Table 5: Correlations between LA volumes and myocardial mechanics.

	V_{max} (mL/m^2)		V_{min} (mL/m^2)	
	Correlation coefficient	p value	Correlation coefficient	p value
Left atrial ejection/emptying fraction (%)	−0.54	<0.0001	−0.82	<0.0001
Expansion index (%)	−0.53	<0.0001	−0.86	<0.0001
Reservoir strain (ST$_S$, %)	−0.49	<0.0001	−0.73	<0.0001
Booster strain (ST$_A$, %)	−0.32	0.0001	−0.52	<0.0001
Active emptying index/fraction (%)	−0.35	<0.0001	−0.57	<0.0001

multivariate analysis (adjusting for age, gender, LVMI, LVEDVi, and degree of mitral regurgitation), there remained a significant difference in both LA volumes and function between patients with and without LVOT obstruction.

LA reservoir function reflects both active left ventricular contraction (and systolic descent of the mitral annulus) and passive LA stretch and is largely determined both by LV systolic function and by LA compliance. Given that LV longitudinal strain has been shown to be reduced in patients with HCM despite a preserved LV ejection fraction, with an even greater degree of impairment in patients with LVOT obstruction [16], this may in part explain the differences in reservoir function seen in patients with obstructive versus nonobstructive HCM. In addition, the presence of a preexisting atrial myopathy or even fibrosis in the atrial wall may lead to an increase in LA stiffness and a concomitant reduction in compliance. LA chamber stiffness (assessed by invasively measured LA pressure-volume relations) has been demonstrated to be increased in patients with HCM compared with controls and to correlate with the degree of LV hypertrophy [17].

Conduit function appears to be governed mainly by LV relaxation, which is affected by increased LV mass, myocardial ischemia, and the presence of myocardial fibrosis. No significant difference was noted between patients with obstructive and nonobstructive HCM in conduit volume, passive empting index, or SRe, despite a higher LVMI and higher grade of diastolic dysfunction in patients with obstruction.

LA booster function is dependent on preload (via the Frank-Starling mechanism), afterload, and intrinsic LA contractility. While there is initially a compensatory increase in LA contractility in response to an increase in LA volumes, eventually further increases in LA volumes will result in a decline in atrial function, as evidenced by the inverse correlation we have demonstrated between V_{max} and LA ejection fraction, reservoir, and booster strain. Sanada et al. have previously demonstrated the effect of LA afterload mismatch on LA booster pump function in HCM [18]. The increase in LA afterload seen in patients with obstruction is likely to contribute to the greater reduction in LA booster strain seen in these patients and may explain the improvement in function seen after septal myectomy.

Table 6: Effects of septal myectomy on LA volumes and mechanics.

	Premyectomy ($n = 20$)	Postmyectomy ($n = 20$)	p value
NYHA I/II/III/IV (%)	0/0/100/0	80/20/0/0	*<0.0001**
LVOT resting (mmHg)	68.1 ± 40	11.0 ± 6	*<0.0001**
LVOT provocable (mmHg)	98.3 ± 27	21.2 ± 14	*<0.0001**
MR: trivial/mild/moderate/severe (%)	10/40/40/10	40/60/0/0	*0.0004**
LVEF (%)	65.9 ± 8	61.4 ± 9	0.08
LA volume indices			
V_{max} (mL/m^2)	66.2 ± 23	49.8 ± 20	*<0.0001**
$V\text{pre}_A$ (mL/m^2)	53.2 ± 24	39.3 ± 18	*<0.0001**
V_{min} (mL/m^2)	40.5 ± 23	26.6 ± 16	*<0.0001**
Left atrial ejection/emptying fraction (%)	41.6 ± 13	48.4 ± 10	*0.006**
Left ventricular stroke volume (mL/m^2)	55.5 ± 11	48.0 ± 10	*0.01**
Conduit volume	57.2 ± 21	47.3 ± 20	0.066
Reservoir function			
Expansion index (%)	80.5 ± 44	100.1 ± 36	*0.01**
Longitudinal reservoir strain (%)	14.1 ± 6	17.3 ± 7	*0.01**
SRs (%/sec)	0.51 ± 0.2	0.57 ± 0.2	0.15
Conduit function			
Passive emptying index/fraction (%)	21.4 ± 9	22.2 ± 9	0.66
SRe (%/sec)	−0.27 ± 0.2	−0.27 ± 0.2	0.98
Booster function			
Active emptying index/fraction (%)	26.4 ± 11	33.7 ± 8	*0.002**
Longitudinal booster strain (%)	6.8 ± 4	9.8 ± 5	*0.0001**
SRa (%/sec)	−0.48 ± 0.2	−0.68 ± 0.2	*0.001**

∗ denotes $p < 0.05$ between pre- and postmyectomy values.

Table 7: Inter- and intraobserver variability.

	Diff. means (±1.96 SD)	
	Interobserver	Intraobserver
V_{max} (mL/m^2)	1.5 (±7.7)	1.5 (±2.6)
$V\text{pre}_A$ (mL/m^2)	0.8 (±8.4)	1.5 (±3.0)
V_{min} (mL/m^2)	2.6 (±9.8)	1.5 (±5.8)
Longitudinal reservoir strain (%)	1.3 (±5.2)	0.2 (±6.6)
Longitudinal booster strain (%)	0.5 (±3.1)	0.1 (±3.7)
SRs (%/sec)	0.07 (±0.16)	0.01 (±0.18)
SRe (%/sec)	0.02 (±0.18)	0.00 (±0.37)
SRa (%/sec)	0.09 (±0.26)	0.02 (±0.24)

Difference of means ±1.96 SD: bias and limits of agreement derived from Bland-Altman analysis.

An intrinsic atrial myopathy may in addition affect active *LA* contraction and booster strain. Indirect evidence to suggest the presence of an intrinsic myopathy in HCM is the increase in the number of calcium antagonist receptors demonstrated in the atrial myocardium of patients with HCM, suggesting an abnormality in calcium fluxes through voltage-sensitive calcium channels that may play a role in atrial dysfunction [19].

In the present study, no correlations were noted between 2D echocardiographic parameters of diastolic function and strain or strain rate parameters. Importantly, no correlation was seen between *A* wave velocity on transmitral Doppler filling profiles and any parameters of LA booster function, suggesting that peak *A* wave may not accurately reflect LA contractile function but rather reflects the atrioventricular pressure gradient between the atrium and ventricle. While LA volumes and transmitral *A* wave are readily obtained from standard transthoracic echocardiography, our present findings highlight the need for more sophisticated techniques for the assessment of LA function if we are to use this information to predict risk of adverse events.

While strain analysis is not yet part of routine clinical practice, the simple addition of measurement of V_{min} to routinely measured V_{max} appears to provide valuable information regarding function. V_{min} demonstrates a stronger correlation with not only measures of global LA function and compliance, but also markers of LA contractility (active and booster function). The present data suggest that measurement of minimum volume provides a valuable surrogate marker of LA function.

5. Study Limitations

Although measurements using VVI applied to CMR images have not been previously validated against VVI applied to echocardiographic images in the same patient group for the analysis of LA function, a recent study utilizing the feature-tracking software has demonstrated both the feasibility and reliability of this technique for assessing LA strain and strain rate [13]. Although differences in signal characteristics

between different scanners and scans performed at different field strengths might also impact feature tracking, Kowallick et al. have demonstrated good reproducibility of measurements of LA function irrespective of scanner type [13]. The thin-walled LA may result in technical difficulties with the application of feature tracking using VVI. In addition, the LA appendage and origin of the pulmonary veins pose additional challenges to tracking. LA function has previously been demonstrated to be abnormal in patients with primary severe mitral regurgitation without HCM [20], and in the current study patients with obstructive HCM had a significantly greater degree of mitral regurgitation than the nonobstructive group. Some of the effects on left atrial volume and function after septal myectomy may be explained by the relief of mitral regurgitation in addition to relief of outflow tract obstruction. Left atrial fibrosis, which may represent an important intrinsic determinant of left atrial function, was not assessed on CMR in the current study but may account for some of the demonstrated changes in function noted.

6. Conclusions

Left atrial volumes and functional parameters are abnormal in patients with HCM and are significantly worse in those with obstruction. The associated effects of left ventricular outflow tract obstruction and mitral regurgitation appear to further impair left atrial mechanics. Surgical myectomy, via relief of obstruction, appears to have a positive impact on both left atrial volume and function, and further studies are needed to assess whether aggressive management of LVOT obstruction will result in a reduction in the occurrence of adverse events, particularly atrial fibrillation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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