Diagnostic Evaluation of Hypertrophic Cardiomyopathy in its Clinical and Preclinical Phases

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Abstract
Hypertrophic cardiomyopathy is a familial, genetic disease caused by mutations in genes encoding sarcomeric proteins. It is characterized by various degrees of left ventricular hypertrophy, usually diffuse, predominantly involving the interventricular septum. The asymptomatic forms with mild or no segmental hypertrophy makes it difficult to establish the diagnosis and screening for familial forms. Its high penetrance is often incomplete and, as a result, 20% to 30% of adults who carry disease-causing gene mutations do not express the phenotype. The susceptibility to sudden death and likelihood of late expression makes establishing a preclinical diagnosis all the more important. The use of Doppler echocardiography and magnetic resonance imaging, in conjunction with a detailed ECG analysis, may be useful in this process. Molecular genetic studies can identify mutations in 60% to 80% of the cases. However, its complex, time-consuming and costly nature, coupled with an inadequate assessment of genotype-phenotype relationships, limits its routine application. Major advances in imaging methods and the introduction of more simplified molecular techniques may contribute to clinical and preclinical diagnosis of hypertrophic cardiomyopathy, in addition to allowing implementation of therapeutic strategies to prevent or delay the development of the disease.

Hypertrophic cardiomyopathy (HCM) is characterized by left ventricular hypertrophy (LVH) in the absence of chamber dilation and any other cardiovascular or systemic condition capable of producing similar changes. The presence of cellular disarray, fibrosis, and myocyte hypertrophy contributes to the development of diastolic dysfunction, myocardial ischemia, and arrhythmias, which are the substrate of the disease’s clinical manifestations. Since it was first described, more than four decades ago, HCM has been a subject of intense and fruitful investigation. It is the most prevalent genetic cardiovascular disease, affecting one in every 500 individuals. This is a familial disease with predominantly autosomal dominant pattern of inheritance.

Key Words
Cardiomyopathy; hypertrophic/diagnosis; cardiomyopathy, hypertrophic, familial; hypertrophy, left ventricular

More than 400 mutations in genes encoding sarcomeric proteins have already been identified (Table 1). Mutations in the β-myosin heavy chain (MYH7) and myosin-binding protein C (MYBPC3) genes seem to account for 60% to 80% of the cases.

The great molecular, pathological, and clinical heterogeneity of HCM complicates its diagnosis. Clinical diagnosis is based on predominantly asymmetric LVH associated with normal or reduced cavity on two-dimensional Doppler echocardiography or magnetic resonance imaging. Atypical forms with mild, localized or nondetectable LVH are usually challenging, making screening for affected individuals more difficult in families known to carry the disease.

Hypertrophic cardiomyopathy is the most common cause of sudden death in young people and athletes, including asymptomatic patients without prior diagnosis or signs of LVH. The susceptibility to devastating complications, such as sudden death, and progression to disabling conditions, such as heart failure, have prompted a search for indicators capable of identifying the disease at earlier stages. The advent of molecular genetic diagnosis has significantly contributed to the detection of gene mutation carriers without evidence of disease. Because of the incomplete phenotypic penetrance of HCM, Doppler echocardiography fails to detect LVH in 20% to 30% of the genetically affected adult patients. These individuals may show premature predisposition to sudden death or develop the phenotype later in life, as it is the case with mutations in troponin T and cardiac myosin-

Table 1 - Sarcomeric genes known to cause hypertrophic cardiomyopathy

<table>
<thead>
<tr>
<th>Sarcomeric protein</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac β-myosin heavy chain</td>
<td>MYH7</td>
</tr>
<tr>
<td>Myosin-binding protein C</td>
<td>MYBPC3</td>
</tr>
<tr>
<td>Troponin T</td>
<td>TNNT2</td>
</tr>
<tr>
<td>α-tropomyosin</td>
<td>TPM1</td>
</tr>
<tr>
<td>Essential myosin light chains</td>
<td>MYL3</td>
</tr>
<tr>
<td>Regulatory myosin light chains</td>
<td>MYL2</td>
</tr>
<tr>
<td>Troponin I</td>
<td>TNNI3</td>
</tr>
<tr>
<td>α-actin</td>
<td>ACTC</td>
</tr>
<tr>
<td>Titin</td>
<td>TTN</td>
</tr>
<tr>
<td>Troponin C</td>
<td>TNNC1</td>
</tr>
<tr>
<td>α-muscarinic heavy chain</td>
<td>MYH6</td>
</tr>
<tr>
<td>Muscle LIM protein</td>
<td>CRP3</td>
</tr>
</tbody>
</table>
Clinical features

Hypertrophic cardiomyopathy affects both genders, occurring in patients of different racial backgrounds and in multiple geographic areas. It usually develops during adolescence, although its clinical manifestations may appear earlier or later, after the fifth decade of life. Typically, HCM occurs between the ages 13 and 17 in carriers of HCM-causing gene mutations. Morphological features are usually complete at the age of 18, and there is no progression of left ventricular hypertrophy after this age. The elderly represent 25% of the cases, of which 40% to 50% have obstructive forms of hypertrophic cardiomyopathy.

Occasionally, LVH can be present in neonates and children. In infants, it is associated with heart failure and high mortality rates. Differential diagnosis should include neuromuscular binding protein-C genes, respectively. Improvements in imaging methods for assessing left ventricular function and larger scale use of molecular genetic diagnosis may contribute substantially to the identification of the disease in its clinical and preclinical phases.

Electrocardiogram

The electrocardiogram is abnormal in 75% to 95% of the patients. These changes are seen early, even before adolescence, when Doppler echocardiogram is usually normal. In adult-onset hypertrophic cardiomyopathy, ECG abnormalities may precede the appearance of LV hypertrophy. The following are considered major electrocardiographic diagnostic criteria: left ventricular overload, deep Q-waves, > 40 ms intraventricular conduction disturbances, T-wave inversion, minor VR changes, deep S-waves in V2.

Doppler echocardiogram

LV wall thickness = 12 mm in the anterior septum or posterior wall and/or 14 mm in the posterior septum or free wall associated to moderate SAM or redundant leaflets. LV outflow tract obstruction is not frequent among these patients, of which 40% to 50% have obstructive forms of hypertrophic cardiomyopathy.

LV hypertrophy 15. The following are considered major electrocardiographic criteria: left ventricular overload, deep Q-waves, > 40 ms intraventricular conduction disturbances, T-wave inversion, minor VR changes, deep S-waves in V2.

Magnetic resonance

Structural segmental abnormalities of the myocardium and LV, focal fibrosis in areas with segmental hypertrophy.

Molecular genetic diagnosis

Disease-causing gene mutations

Table 2 - Preclinical diagnosis of hypertrophic cardiomyopathy: changes in adults with familial forms

| Electrocardiogram | LV hypertrophy, deep Q-waves > 40 ms intraventricular conduction disturbances, T-wave inversion, minor VR changes, deep S-waves in V2 |
| Doppler echocardiogram | LV wall thickness = 12 mm in the anterior septum or posterior wall and/or 14 mm in the posterior septum or free wall associated to moderate SAM or redundant leaflets |
| Tissue Doppler Strain/strain rate | Decreased LV systolic and early diastolic velocities; Decreased LV strain |
| Magnetic resonance | Structural segmental abnormalities of the myocardium and LV, focal fibrosis in areas with segmental hypertrophy |
| Molecular genetic diagnosis | Disease-causing gene mutations |

SVE - left ventricular overload, VR - ventricular repolarization, LV - left ventricle, SAM - systolic anterior motion of the mitral valve.

Electrocardiogram is a valuable tool for screening asymptomatic carriers of the disease without echocardiographic abnormalities in affected families. Electrocardiographic changes are seen in 20% to 50% of these cases and should be appropriately evaluated, particularly during preadolescence. Assessment of carriers of HCM-causing mutant genes without LVH on conventional Doppler echocardiogram shows that the presence of a single major electrocardiographic criterion should be interpreted as diagnostic, considering its low prevalence in the general population. On the other hand, it must be kept in mind that minor electrocardiographic criteria,
such as interventricular conduction disturbances, minor changes in ventricular repolarization, and deep S-waves in lead V2, may occasionally occur in the absence of heart disease\(^{8}\).

Holter ECG monitoring shows rhythm disturbances in 90% of the adults affected by the disease\(^{2}\). Ventricular extrasystoles and nonsustained ventricular tachycardia are found in 20% to 30% of the patients\(^{23,24}\). Bradyarrhythmias, supraventricular tachycardias, and atrial fibrillation may precede the development of ventricular tachycardia\(^{2}\).

Repetitive, prolonged episodes of nonsustained ventricular tachycardia predispose to ventricular fibrillation, particularly in patients younger than 30\(^{25}\). Ventricular arrhythmias are rarely seen in children, adolescents and young adults, but when present they have a higher positive predictive value for sudden death\(^{26}\). Sustained ventricular tachycardia may indicate association with LV apical aneurysms or ischemic heart disease\(^{1}\).

**Doppler echocardiogram**

Doppler echocardiogram plays a decisive role in the diagnosis of HCM, since it identifies major structural and functional changes typical of the disease, as well as a wide phenotypic diversity. Left ventricular hypertrophy ranges from mild to severe, and from localized to diffuse. No morphological pattern of LVH is regarded as truly typical, although asymmetric forms with predominant involvement of the interventricular septum and diffuse hypertrophy are the most frequent\(^{14}\). Concentric forms represent 1% to 5% of the cases\(^{27,28}\). Less typically, hypertrophy can be confined to a single ventricular segment, such as the posterior portion of the septum or the anterolateral and posterior free walls, or even LV apical regions\(^{27}\).

The extent and pattern of LVH are inversely correlated with age, and are not associated with gender and functional class\(^{14}\). Adolescents and young adults often have extreme hypertrophy, with LV wall thickness \(\geq 30\) mm, which predisposes to sudden death\(^{27}\). Measurements ranging from 15 to 30 mm are common, revealing different degrees of myocardial involvement\(^{14}\). Borderline thicknesses \(\leq 15\) mm denote an incipient process and should be differentiated from physiological states, such as athlete’s heart\(^{27,14}\).

Any value for LV wall thickness may be identified in the presence of a mutant gene, even those regarded as normal\(^{18,12}\). Consequently, screening of families affected by HCM based on LV maximal wall thickness is obviously limited, particularly during childhood and preadolescence. In a recent study, an echocardiographic score calculated as the sum of wall thicknesses obtained in four different LV segments has been shown to be more accurate, especially among younger people\(^{10}\). Left ventricular wall thicknesses of 12 mm in the anterior septum or posterior wall or 14 mm in the posterior septum or free wall are regarded as criterion for the preclinical diagnosis of adult familial forms, when associated with moderate mitral valve systolic anterior motion (SAM) or redundant valve leaflets\(^{8}\).

There is a potential relationship between the degree of LVH and the responsible gene. Mutations in the gene encoding the \(\beta\)-myosin heavy chain are associated with diffuse, severe disease\(^{11}\). In troponin T mutations, hypertrophy is usually mild or absent\(^{11}\). Hypertrophic cardiomyopathy caused by myosin-binding protein C gene mutations is associated with normal LV wall thickness at a younger age\(^{11}\).

Doppler echocardiogram allows a distinction to be made between obstructive and non-obstructive forms of HCM. Obstruction affects more often LV outflow tract, due to the anterior or posterior mitral valve leaflet making contact with the basal portions of the interventricular septum\(^{13,14}\). Mitral valve deformities may contribute to a subaortic gradient. In 45% of the cases of obstructive HCM, the anterior mitral leaflet is elongated or has an anomalous insertion directly into the papillary muscle\(^{27}\). The systolic anterior motion (SAM) of the mitral valve, partly attributed to the Venturi effect, may lead to valvular regurgitation, with the regurgitant jet directed posteriorly\(^{14}\). Less frequently, there is mid-ventricular obstruction due to excessive papillary muscle hypertrophy and malalignment\(^{27}\).

The degree of obstruction, assessed by continuous Doppler, is dynamic, changing in response to several stimuli and in serial measurements. The gradient changes spontaneously in a same individual, being influenced by intravascular volume, contractility, and afterload\(^{2,12}\). Provocative maneuvers lack standardization and include Valsalva, amyl nitrite inhalation, postextrasystole potentiation, dobutamine infusion, and exercise\(^{2,26}\). A recent study, in which patients without subaortic obstruction under rest conditions were assessed by exercise Doppler echocardiography, has shown predominance of obstructive HCM, corresponding to 70% of the patients\(^{13}\). The presence of LV outflow tract obstruction is regarded as an independent predictor of progression to heart failure. The likelihood of death from HCM, heart failure, or stroke is higher in these cases\(^{22}\). While LV outflow tract obstruction was associated with elevated risk of sudden death\(^{14}\), its role as a predisposing factor is not well established yet\(^{12}\).

Conventional assessment of global LV systolic function, based on estimated ejection fraction, shows normal or elevated values\(^{1,24}\). It does not exclude contractile dysfunction, which is better documented by tissue Doppler and strain/strain rate imaging. Left ventricular enlargement with decreased ejection fraction and wall thinning occurs in 5% to 10% of patients who reach maturity\(^{13,35}\). Assessment of diastolic filling using transmitral Doppler shows abnormal LV relaxation, even though restrictive or pseudonormal filling patterns are also found\(^{3}\).

**Tissue Doppler**

Tissue Doppler echocardiography is more sensitive than standard Doppler echocardiography for detecting minor changes in left ventricular function\(^{29,30}\). In patients with overt hypertrophic cardiomyopathy, it can detect left ventricular functional impairment with systolic velocities (S) lower than that obtained in normal controls\(^{37}\). Long-axis diastolic dysfunction is observed by delayed and reduced early (E') and late (A') velocities, as well as prolonged regional deceleration and isovolumic relaxation times\(^{38}\).

The E'/E ratio, which can reflect increased LV filling pressure, predicts the degree of exercise tolerance\(^{39}\). There is a correlation between septal E', functional class, and plasma levels of B-type natriuretic peptide (BNP)\(^{40}\).
This imaging modality is clearly superior to Doppler echocardiography for diagnosing LV apical hypertrophy[^52], as magnetic resonance imaging (MRI) provides information on LV morphology and performance, including ventricular mass and volumes, in addition to global and regional diastolic and systolic function. Flow dynamics in the LV outflow tract and the degree of mitral regurgitation can also be determined[^28]. It also reveals abnormalities in myocardial metabolism, is 30% sensitive and 71% specific for the detection of LV hypertrophy[^39]. Areas of late enhancement have prognostic value in HCM[^32]. Its extent is correlated with a greater degree of fibrosis and increased ejection fraction[^39]. Areas of late enhancement were not detected in mutation carriers without the HCM phenotype[^51], but warrants additional studies.

The histopathological substrate of the disease can be analyzed by gadolinium-enhanced MRI. Late enhancement is found in 80% of the patients, involving 0 to 48% of myocardial mass with different patterns of distribution[^56,57]. It is directly related to areas of reparative fibrosis, in which collagen is the predominant component[^58]. Two patterns of distribution are described: diffuse and confluent[^56]. Late enhancement has prognostic value in HCM. Its extent is associated with greater predisposition to sudden death and progressive left ventricular dilation. The diffuse pattern, more than the confluent, is associated with the presence of at least two risk factors for sudden death[^56]. When multifocal, it is correlated with a greater degree of fibrosis and decreased ejection fraction[^59]. Areas of late enhancement were not detected in mutation carriers without the HCM phenotype, suggesting that fibrosis only develops after appearance of LV hypertrophy[^57]. In 81% of the phenotype-negative subjects, MRI revealed the presence of triangular, deep, bright structural abnormalities in the basal and mid segments of the LV interseptal wall[^60]. The depth of these images decreased with increased wall thickness, which may explain the absence of such a description in histopathological studies, usually restricted to forms with complete phenotypic expression. Gadolinium-enhanced MRI may facilitate the differential diagnosis with Fabry disease[^61], which accounts for 4% of patients diagnosed with HCM[^61].

**Magnetic Resonance Imaging**

Magnetic resonance imaging (MRI) provides information on LV morphology and performance, including ventricular mass and volumes, in addition to global and regional diastolic and systolic function. Flow dynamics in the LV outflow tract and the degree of mitral regurgitation can also be determined[^28]. It also reveals abnormalities in myocardial metabolism, is 30% sensitive and 71% specific for the detection of LV hypertrophy[^39]. Areas of late enhancement have prognostic value in HCM[^32]. Its extent is correlated with a greater degree of fibrosis and increased ejection fraction[^39]. Areas of late enhancement were not detected in mutation carriers without the HCM phenotype[^51], but warrants additional studies.

**Endomyocardial biopsy**

Endomyocardial biopsy is performed to identify the histopathological substrate of the disease, the less specific feature and focal distribution of which limit its use in routine screening for HCM. More recently, studies based on necropsy or in explanted hearts have analyzed the interrelation between several histopathological components and their respective association with clinical outcomes[^10,21,63,64].
Endomyocardial biopsy, using light microscopy, shows various degrees of cellular hypertrophy and fibrosis. On electron microscopy, morphological changes are usually nonspecific. Cellular disarray, lying deep within the interventricular septum, is often beyond the reach of the biopotope, occupying approximately 30% of the left ventricular wall. It is not pathognomonic, affecting, in a localized form, subjects with normal hearts or with congenital heart diseases. Its extension is inversely related to age. Cellular disarray is not associated with specific LVH patterns, but is more diffuse in the presence of maximal wall thickness ≤20 mm, preserved systolic function, and young patients with sudden premature death.

Interstitial or reparative fibrosis may be focal or occupy extensive areas of the myocardium, and it is directly related to age, maximal LV wall thickness, and the presence of a dilated chamber. Fibrosis is more severe in patients who progress to sudden death at a more advanced age.

Microcirculation impairment is characterized by wall thickening due to myointimal hyperplasia and the ensuing luminal narrowing of small intramural arteries, being more pronounced in the interventricular septum. It is implicated in the development of reparative fibrosis and progression to dilated hypertrophic cardiomyopathy. This is an early finding that affects even the very young.

Endomyocardial biopsy may be used for the differential diagnosis with LVH of other etiologies, interventricular septal tumors, and infiltrative diseases, such as cardiac amyloidosis. Myocardial infiltrative processes may be clinically indistinguishable from HCM, such as Pompe disease, which affects children. In Fabry disease, an X-linked recessive lysosomal disorder, potentially treatable, deficiency of α-galactosidase A is associated with glycosphingolipid deposition in the myocardium in older men. Mutations in genes related to cell metabolism, described recently, mimic familial hypertrophic cardiomyopathy. Mutations in the gene encoding the β regulatory subunit of the AMP-activated protein kinase (PRKAG2) cause storage disease, in which Wolff-Parkinson-White syndrome and premature conduction disease are associated with varying degrees of pseudo ventricular hypertrophy. Mutations in the lysosome-associated membrane protein-2 (LAMP2) result in Danon disease, with massive myocardial hypertrophy accompanied by Wolff-Parkinson-White syndrome. In both diseases, histopathological examination reveals lack of cellular disarray and presence of glycosgen-containing vacuoles.

Electrophysiological studies

The role of electrophysiological studies (EPS) using programmed ventricular stimulation to assess the arrhythmogenic substrate of HCM has not yet been established. Although some relationship has been demonstrated between inducibility and prognosis, its predictive accuracy is debatable. It may be helpful in patients with unexplained syncope. High resolution ECG has low predictive accuracy as well. T-wave alternans is regarded as predictive of ventricular arrhythmias and sudden death. Its contribution to risk stratification in HCM may be limited and needs further evaluation.

Molecular genetic diagnosis

DNA analysis is the most definitive method for identifying HCM in its clinical and preclinical phases. The heterogeneous molecular substrate, represented by hundreds of mutations in multiple genes, adds complexity to the genetic diagnosis and limits its use in routine clinical practice. Because of the marked allelic heterogeneity, together with low individual prevalence of mutations, it is difficult to assess the genotype-phenotype relationship. The significant inter- and intrafamilial phenotypic variability is attributed to the action of modifying agents, either environmental or genetic, and to the likelihood of occurring more than one mutation in one or more genes.

Molecular genetic diagnosis is a valuable tool for assessing familial forms of the disease, particularly those associated with sudden death or late clinical expression. It enables the early release of normal family members and follow-up of those who carry mutations but have no evidence of the disease. Prospective studies are needed to determine whether these individuals will necessarily express the phenotype. Preclinical diagnosis may produce adverse psychological effects, particularly in children and adolescents. In this regard, multidisciplinary genetic counseling is considered mandatory.

Molecular genetic diagnosis helps differentiate other forms of LVH and phenocopies, including metabolic storage diseases, which are clinically indistinguishable from HCM.

The use of genetic analysis in risk stratification for sudden death is supported by descriptions of malignant mutations. Early studies based on large pedigrees relate certain phenotypes to the mutant genes and discriminate between mutations with good and poor prognosis. More recent studies provided new data on HCM clinical and genetic profile, revealing less specific phenotypes and lower prevalence of malignant mutations. For the purpose of prognostic evaluation, it is necessary to expand the analysis of genotype-phenotype relationships, including larger, unrelated families with a higher number of affected members.

As molecular genetic diagnosis is expensive and time-consuming, it has been limited to research centers. Single-strand conformation polymorphisms (SSCP) analysis can map 60% to 80% of the cases. The advent of automated, direct DNA sequencing contributes to its use on a clinical scale, since it allows rapid screening for up to eight sarcomeric genes, but is still expensive. The likelihood of false-negatives due to mutations in genes that were not assayed does exist.

Molecular genetic diagnosis should prompt implementation of measures designed to prevent or delay disease progression, such as gene therapy. The multitude of structural and functional disorders involving contractile proteins has been a drawback to the development of effective therapeutic strategies.

Conclusion

Hypertrophic cardiomyopathy is a disease with a heterogeneous molecular genetic substrate and significant phenotypic variability, which makes clinical and preclinical diagnosis very complex. Higher resolution imaging modalities and affordable molecular techniques will allow early diagnosis...
and implementation of measures that may prevent the development of LVH and progression to sudden death.

Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

References


