

# Histopathological comparison of intramural coronary artery remodeling and myocardial fibrosis in obstructive versus end-stage hypertrophic cardiomyopathy

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## ABSTRACT

**Background:** Although imaging techniques have demonstrated the existence of microvascular abnormalities in hypertrophic cardiomyopathy (HCM), a detailed histopathological assessment is lacking as well as a comparison between different phases of the disease. We aimed to compare microvasculopathy and myocardial fibrosis in hypertrophic obstructive cardiomyopathy (HOCM) versus end-stage (ES) HCM.

**Methods:** 27 myectomy specimens of HOCM patients and 30 ES-HCM explanted hearts were analyzed. Myocardial fibrosis was quantitatively determined with dedicated software and qualitatively classified as scar-like or interstitial. Intramural coronary arteries were evaluated separately according to lumen diameter: 100–500  $\mu$  versus <100  $\mu$ . Microvasculopathy assessment included the description of medial and intimal abnormalities and stenosis grading. The two subgroups were compared considering only the anterobasal septum of ES explanted hearts.

**Results:** Median value of fibrosis in the anterobasal septum of explanted hearts was 34.6% as opposed to 10.3% of myectomy specimens ( $p < 0.001$ ). Scar-like fibrosis was widely found in ES hearts while interstitial fibrosis was distinctive of HOCM ( $p < 0.001$ ). All slides showed 100–500  $\mu$  microvasculopathy without any differences between subgroups in terms of lumen narrowing, extent of the disease and type of parietal involvement. Among ES hearts these lesions were associated with scar-like fibrosis ( $p = 0.034$ ). <100- $\mu$  microvasculopathy was also frequent with no differences between subgroups.

**Conclusions:** Microvasculopathy is an intrinsic feature of HCM with similar characteristics across the natural phases of the disease. Conversely, myocardial fibrosis changes over time with ES hearts showing a three-fold greater amount, mainly scar-like. ES showed a closer association between microvasculopathy and replacement fibrosis.

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## 1. Introduction

Hypertrophic cardiomyopathy (HCM) is the most frequent genetically determined cardiomyopathy, characterized by heterogeneous clinical, genetic and pathologic features [1,2].

It has progressively become clear that the histopathology of HCM is not limited to myocyte hypertrophy and disarray but also includes intramural “small vessel” coronary artery disease (IMCD) and myocardial fibrosis, both scar-like and interstitial [3,4].

In their seminal paper dated over 30 years ago, Maron et al. documented an elevated frequency of microvascular abnormalities and scar-like fibrosis in HCM patients suggesting a pathophysiological link between the two conditions mediated by ischemia [3].

The functional correlate of vascular remodeling can be found in the prevalence of coronary dysfunction, shown by PET and MRI studies, which carry a significant prognostic value [5–7].

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Major areas of uncertainty still remain, with regard to the characteristics and distribution of IMCD, the association between vascular abnormalities and the two types of myocardial fibrosis (replacement and interstitial) and its variations within the different phenotypic expressions of the disease: the hypercontractile-obstructive form, characterized by limited or no evidence of replacement fibrosis and the late hypocontractile end-stage phase, characterized by marked fibrotic remodeling. Because the two stages may occur at different times in the same individuals, clarifying the interplay of IMCD and fibrosis may help unravel the pathologic basis of disease progression.

We had the opportunity to study myocardial histopathology from patients in different HCM subgroups at two very different points in the evolution of the disease: post-myectomy samples from hypertrophic obstructive cardiomyopathy (HOCM) patients and explanted hearts of patients with end-stage HCM (ES-HCM).

The present study is an extension of our previous work [8] and introduces two new elements: a detailed characterization of IMCD with its topographic association with myocardial fibrosis and the comparison between HOCM and ES-HCM.

The aim of our work was to assess the degree and extent of intramural “small vessel” coronary artery disease, to define the contribution of medial and intimal layer abnormalities to vessel narrowing, and to explore the relationship of IMCD with the amount and type of myocardial fibrosis and chronic myocyte alterations, based on the stage of disease.

## 2. Methods

### 2.1. Study design

This observational study was performed in our cardiac pathology center (Sant’Orsola University Hospital in Bologna) evaluating cases from Bologna and “Careggi University Hospital” in Florence.

We analyzed whole hearts of ES-HCM patients who underwent heart transplantation in Bologna Centre due to heart failure with refractory symptoms, and myectomy specimens of HOCM patients who underwent septal myectomy in Florence. The ES specimens were analyzed in our previous histopathological study [8].

Nonsarcomeric HCM phenocopies were excluded from the study.

Comparison between subgroups was performed using the whole myectomy samples of HOCM patients and the anterobasal septum of ES-HCM hearts (Supplemental Fig. 1). The myocardium surfaces assessed using morphometric analysis in the two subgroups were largely similar (myectomy mean value:  $232.3 \pm 118.13 \text{ mm}^2$ ; anterobasal septum mean value:  $250.5 \pm 114.15 \text{ mm}^2$ ).

Written consent for scientific analyses of specimens was obtained in all cases. The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the local ethics committee.

### 2.2. Clinical definitions

HCM was defined as wall thickness  $\geq 15 \text{ mm}$  in one or more left ventricle (LV) myocardial segments as measured by any imaging technique (echocardiography, MRI, or computed tomography) in the absence of any concomitant pressure or volume overload conditions capable of generating the observed degree of hypertrophic LV remodeling [2].

HOCM was defined in cases of LV outflow tract pressure gradient  $\geq 30 \text{ mmHg}$  at rest or during Valsalva manoeuvre [2]. Patients were referred to myectomy in presence of dynamic LV outflow tract obstruction associated with refractory symptoms, despite optimal medical therapy.

ES-HCM was defined by the presence of LV ejection fraction  $\leq 50\%$ , with or without LV dilatation.

Genetic testing for the most common sarcomeric genes associated with HCM was routinely offered.

### 2.3. Pathological analysis

Pathological analysis was performed by two expert cardiovascular pathologists using the most up-to-date pathology criteria, definitions and standards [9,10].

For each explanted heart a total of 16 myocardial specimens from LV, interventricular septum (IVS) and right ventricle (RV) were evaluated: 3 at the basal level (1 from LV, 1 from IVS, 1 from RV), 3 at the apical level (1 from LV, 1 from IVS, 1 from RV) and 10 from an entire midventricular section (5 from the anterior, anterolateral, lateral, inferolateral, and inferior LV walls; 3 from the anterior, medium, and posterior IVS and 2 from the anterolateral and inferolateral RV walls).

For each HOCM case, the entire myectomy specimen was examined.

Myocardial fibrosis extent was quantitatively assessed by histomorphometric analysis using dedicated software (Image-Pro Plus version 7.0, Media Cybernetics MD, USA) as

previously described [8], and expressed as a percentage value of the whole myocardial area examined.

Type of fibrosis was qualitatively recorded as replacement (scar-like) or interstitial myocardial fibrosis as previously described [8] and each specimen was considered to have mainly replacement fibrosis or mainly interstitial myocardial fibrosis when that type was present in  $\geq 60\%$ , and to have mixed fibrosis when the two types were near equal.

The presence of myocyte vacuolization, a sign of chronic myocardial injury, was also noted and classified in degree (mild when vacuoles were present in  $<30\%$  of myocardial fibers; moderate when present in  $30\text{--}60\%$  and severe when vacuoles filled  $>60\%$ ) and extent (focal when present in  $<30\%$  of the specimen; multifocal from  $30\%$  to  $60\%$ ; diffuse when present in  $>60\%$ ) (Supplemental Fig. 2).

Intramural coronary arteries were separately evaluated according to lumen diameter: approximately  $100$  to  $500 \mu$  (intermediate compartment) versus  $<100 \mu$  (distal compartment) [11].

For each specimen histopathology findings were assessed in all intramural coronary arteries viewed in cross section of parietal myocardium.

IMCD of  $100\text{--}500 \mu$  vessels was semi-quantitatively assessed in terms of: presence/absence; pattern of the disease (medial, intimal, mixed); type of alteration (medial layer hypertrophy/fibrosis/mixed changes; intimal layer cellular/fibrous/mixed thickening); distribution on vessel wall (eccentric, concentric, mixed). IMCD was classified as mild if causing lumen stenosis  $<30\%$ , moderate  $>30\%$  and  $<60\%$ , severe  $>60\%$  (Fig. 1).

In  $<100 \mu$  arterioles, IMCD was evaluated only in terms of presence/absence and of whole wall thickening; it was rated mild when causing lumen stenosis  $<50\%$  and moderate/severe with stenosis  $>50\%$  (Fig. 1).

Distribution of IMCD in each specimen was classed as focal (when present in  $<30\%$  of the specimen), multifocal (from  $30\%$  to  $60\%$ ), and diffuse (when present in  $>60\%$ ).

We also assessed the topographic association between abnormal small arteries and myocardial fibrosis, i.e. whether the abnormal small arteries lay in the areas of fibrosis, specifying the type (scar-like or interstitial).

### 2.4. Statistical analysis

Categorical variables were expressed as number and percentage; continuous variables as mean  $\pm$  standard deviation (SD) or median (min-max). Categorical variables were compared with Chi-square test or Fisher exact test in cases of small numbers. Shapiro-Wilk W test was performed to assess normality distribution of continuous variables; then comparisons were performed with Student’s *t*-test or Mann-Whitney test accordingly. For all comparisons, a *p* value  $< 0.05$  was considered significant. The Stata software v.14.2 package (StataCorp; College Station, TX) was used for all analyses.

## 3. Results

A total of 57 cases were analyzed: 27 post-myectomy samples from the HOCM subgroup and 30 whole hearts of ES-HCM patients. Baseline clinical characteristics are listed in Supplemental Table 1. Mean age was 45.4 years at myectomy for HOCM patients and 46.8 years at transplantation for the ES-HCM subgroup. Both groups showed a prevalence of male gender.

No large epicardial coronary artery showed any significant lumen stenosis at angiography, nor was there any myocardial bridging.

All specimens showed myocyte hypertrophy and multiple myocardial disarray.

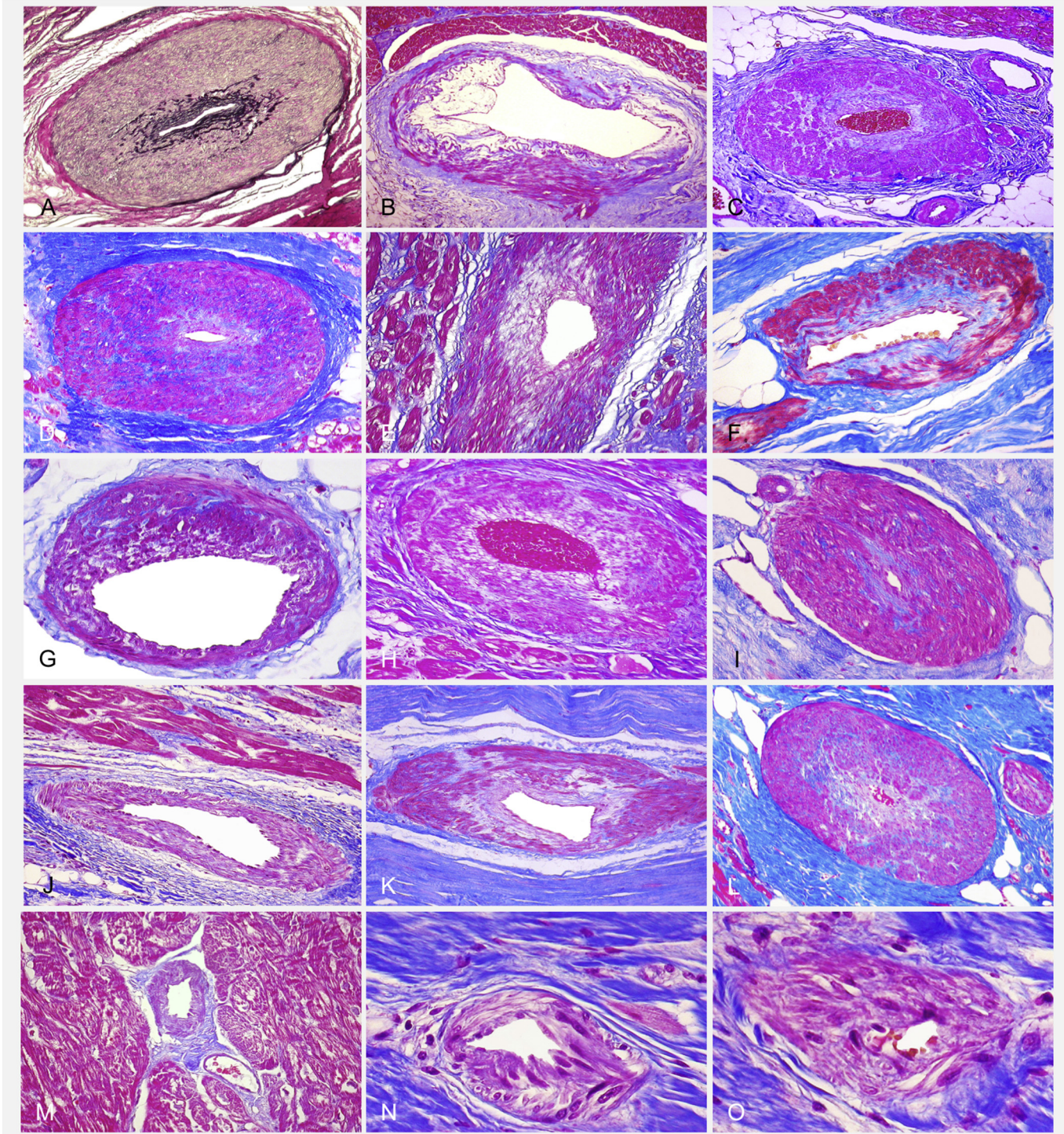
### 3.1. Post-myectomy samples – HOCM

Median value of fibrosis in myectomy specimens was 10.3% (1.6%–39.4%); qualitative analysis revealed replacement fibrosis in 4 patients (14.8%), interstitial in 22 (81.5%) and mixed in 1 case (3.7%). IMCD affecting  $100\text{--}500 \mu$  vessels was present in all myectomy specimens; parietal distribution was eccentric in 37%, concentric in 18.5% and mixed in 44.4%, resulting in severe stenosis in one quarter. Both tunica media and intimal layer were involved in over  $\frac{3}{4}$  of slides. Among medial layer alterations, fibrosis coexisted with hypertrophy in over half specimens. IMCD  $<100 \mu$  was frequently detected with a predominance of mild parietal thickening. Myocyte vacuolization was present in almost 90% of specimens, mostly mild to moderate and focal/multifocal.

### 3.2. Anterobasal septum of ES-HCM

Median value of myocardial fibrosis in the anterobasal septum of ES-HCM hearts was 34.6% (1.3%–87.7%); over half slides (53.3%) showed scar-like fibrosis while 8 cases (26.7%) revealed interstitial fibrosis; mixed type fibrosis was found in the remaining specimens.





**Fig. 1.** “Spectrum of histopathologic findings”. A–L: 100–500  $\mu$  vessel intermediate compartment: medial (A), intimal (B) and mixed (C) pattern; medial mixed hypertrophy/fibrosis (D), cellular intimal thickening (E), fibrous intimal thickening (F); eccentric (G) and concentric (H–I) lesions; mild (J), moderate (K), severe (L) lumen stenosis (Azan Mallory trichrome stain, original magnification 200 $\times$ ). M–O: <100  $\mu$  vessel distal compartment: mild lumen stenosis <50% (M); moderate-severe lumen stenosis >50% (N–O) (Azan Mallory trichrome stain, original magnification 400 $\times$ ).

Microvasculopathy affecting the intermediate compartment (100–500  $\mu$ ) was found in 93.3% of cases; a concentric distribution of parietal abnormalities was often evident, in over half cases along with eccentric lesions. These wall alterations generated severe lumen stenosis in 30% of slides. Medial layer abnormalities included hypertrophy in all cases, 56.7% accompanied by fibrosis. Among intimal

lesions, cellular thickening was predominant while fibrosis coexisted in nearly a quarter.

Distal vascular compartment (<100  $\mu$ ) was altered in 73.3% slides with moderate/severe lesion in a third. Myocyte vacuolization was widely encountered (93.3%) showing mainly moderate/severe and multifocal/diffuse alterations.



**Table 1**  
Histopathological findings in HOCM myectomy specimens and in anterobasal septum of ES-HCM.

	Overall	HOCM	ES-HCM Anterobasal septum	p value
	(n = 57)	(n = 27)	(n = 30)	
Fibrosis	100% (57)	100% (27)	100% (30)	/
Median value (min–max)	19.5% (1.3%–87.7%)	10.3% (1.6%–39.4%)	34.6% (1.3%–87.7%)	<0.001
Type				
Replacement	35.1% (20)	14.8% (4)	53.3% (16)	<0.001
Interstitial	52.6% (30)	81.5% (22)	26.7% (8)	
Mixed	12.3% (7)	3.7% (1)	20% (6)	
Vacuolization	91.2% (52)	88.9% (24)	93.3% (28)	0.660
Degree				
Mild	24.6% (14)	40.7% (11)	10% (3)	0.022
Moderate	45.6% (26)	33.3% (9)	56.7% (17)	
Severe	21% (12)	14.8% (4)	26.7% (8)	
Extent				
Focal	21% (12)	40.7% (11)	3.3% (1)	<0.001
Multifocal	22.8% (13)	48.1% (13)	66.7% (20)	
Diffuse	12.3% (7)	0	23.3% (7)	
100–500 μ IMCD	96.5% (55)	100% (27)	93.3% (28)	0.492
Vessel wall distribution				
Eccentric	22.8% (13)	37% (10)	10% (3)	0.092
Concentric	22.8% (13)	18.5% (5)	26.7% (8)	
Mixed	50.9% (29)	44.4% (12)	56.7% (17)	
Degree				
Mild	26.3% (15)	29.6% (8)	23.3% (7)	0.887
Moderate	42.1% (24)	44.4% (12)	40% (12)	
Severe	28.1% (16)	25.9% (7)	30% (9)	
Extent				
Focal	14% (8)	14.8% (4)	13.3% (4)	0.179
Multifocal	63.2% (36)	55.6% (15)	70% (21)	
Diffuse	19.3% (11)	29.6% (8)	10% (3)	
Disease pattern				
Medial	12.3% (7)	14.8% (4)	10% (3)	0.956
Intimal	5.3% (3)	7.4% (2)	3.3% (1)	
Medial + intimal	78.9% (45)	77.8% (21)	80% (24)	
Medial abnormalities				
Hypertrophy	36.8% (21)	40.7% (11)	33.3% (10)	0.877
Fibrosis	0	0% (0)	0% (0)	
Hypertrophy + fibrosis	54.4% (31)	51.8% (14)	56.7% (17)	
Intimal abnormalities				
Cellular thickening	61.4% (35)	66.7% (18)	56.7% (17)	0.444
Fibrous thickening	7% (4)	11.1% (3)	3.3% (1)	
Mixed thickening	15.8% (9)	7.4% (2)	23.3% (7)	
<100 μ IMCD	75.4% (43)	77.8% (21)	73.3% (22)	0.765
Degree				
Mild	47.4% (27)	55.6% (15)	40% (12)	0.347
Moderate/severe	28.1% (16)	22.2% (6)	33.3% (10)	
Extent				
Focal	17.5% (10)	18.5% (5)	16.7% (5)	1
Multifocal	54.4% (31)	55.6% (15)	53.3% (16)	
Diffuse	3.5% (2)	3.7% (1)	3.3% (1)	

HOCM: hypertrophic obstructive cardiomyopathy; ES-HCM: end-stage hypertrophic cardiomyopathy; IMCD: intramural coronary artery disease; numbers in brackets aside percentages are the absolute values.

### 3.3. Comparison of HOCM and ES-HCM

Table 1 compares the histopathological findings in myectomy samples and in the anterobasal septum of explanted hearts.

The average amount of fibrosis in the anterobasal septum of explanted hearts was more than three times greater than that of post-myectomy surgical samples (34.6% vs 10.3%,  $p < 0.001$ ). The type of fibrosis also differed between subgroups: interstitial fibrosis was widely present in myectomies (over 80%) while scar-like fibrosis prevailed in ES hearts (53.3% vs 14.8%,  $p < 0.001$ ). Almost all slides showed 100–500 μ IMCD with similar features between subgroups; the majority of specimens

showed involvement of both tunica media and intimal layer and approximately one quarter of cases presented severe lumen narrowing.

The distal compartment (<100 μ IMCD) was also frequently involved, without significant differences between subgroups.

Topographic association between IMCD and different types of myocardial fibrosis (scar-like and interstitial) is shown in Supplemental Table 2. Intermediate compartment vasculopathy was diffusely associated with interstitial fibrosis (87.7% overall), with no differences between subgroups. ES specimens showed however a closer association between 100 and 500 μ IMCD and scar-like fibrosis (70% vs 40.7%,  $p = 0.034$ ) (Supplemental Table 2, Fig. 2).

Myocyte vacuolization was equally present in both groups, although ES-HCM specimens had more severe and diffuse alterations.

No differences were found in vessel abnormalities according to the sarcomeric mutations most commonly encountered (Supplemental Table 3).

### 3.4. Additional findings from whole heart examination in ES-HCM

The characteristics of ES-HCM subgroup myocardial fibrosis were previously described in detail [8].

Histopathological features of IMCD in explanted hearts are shown in Supplemental Table 4. IMCD in 100–500 μ vessels was widely encountered (92.3% of all slides) with a predominant involvement of IVS and LV over RV ( $p < 0.001$ ); nevertheless, over 4/5 of right ventricles were involved in some degree (Supplemental Fig. 3). Severe and multifocal/diffuse alterations were more frequent in the IVS, followed by the LV free wall (44.7% and 30% respectively); RV was relatively spared (Supplemental Fig. 4). As for parietal distribution, vascular lesions were generally concentric. Both tunica media and intimal layer contributed to vessel narrowing, especially in the IVS and in the LV free wall. Among medial layer abnormalities, fibrosis coexisted with hypertrophy in 40% of all slides, especially in IVS. Cellular thickening was the most frequent intimal layer lesion, with fibrous intimal thickening coexisting mostly in the IVS. In the right ventricle, intermediate compartment (100–500 μ) IMCD tended to involve only the medial layer. Distal compartment (<100 μ) IMCD was often found (73% of all slides); IVS, followed by LV, were mainly involved also in terms of narrowing severity while RV was relatively spared ( $p < 0.001$ ). Myocyte vacuolization was present in almost 90% slides, especially in LV and IVS while RV was less involved, especially in terms of severity and extent ( $p < 0.001$ ).

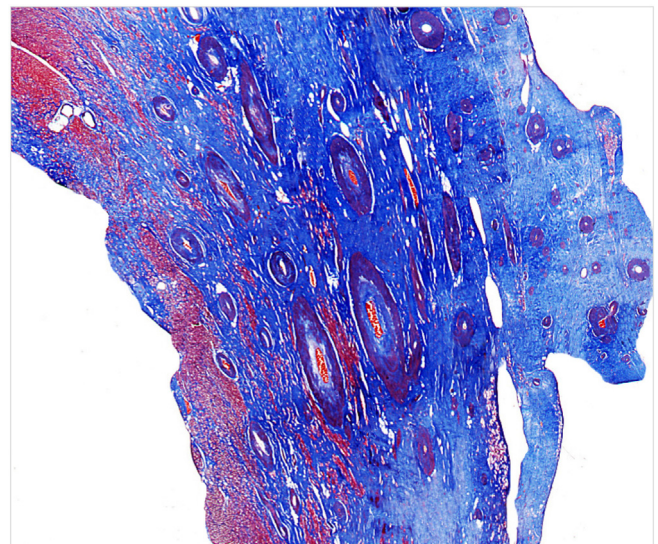
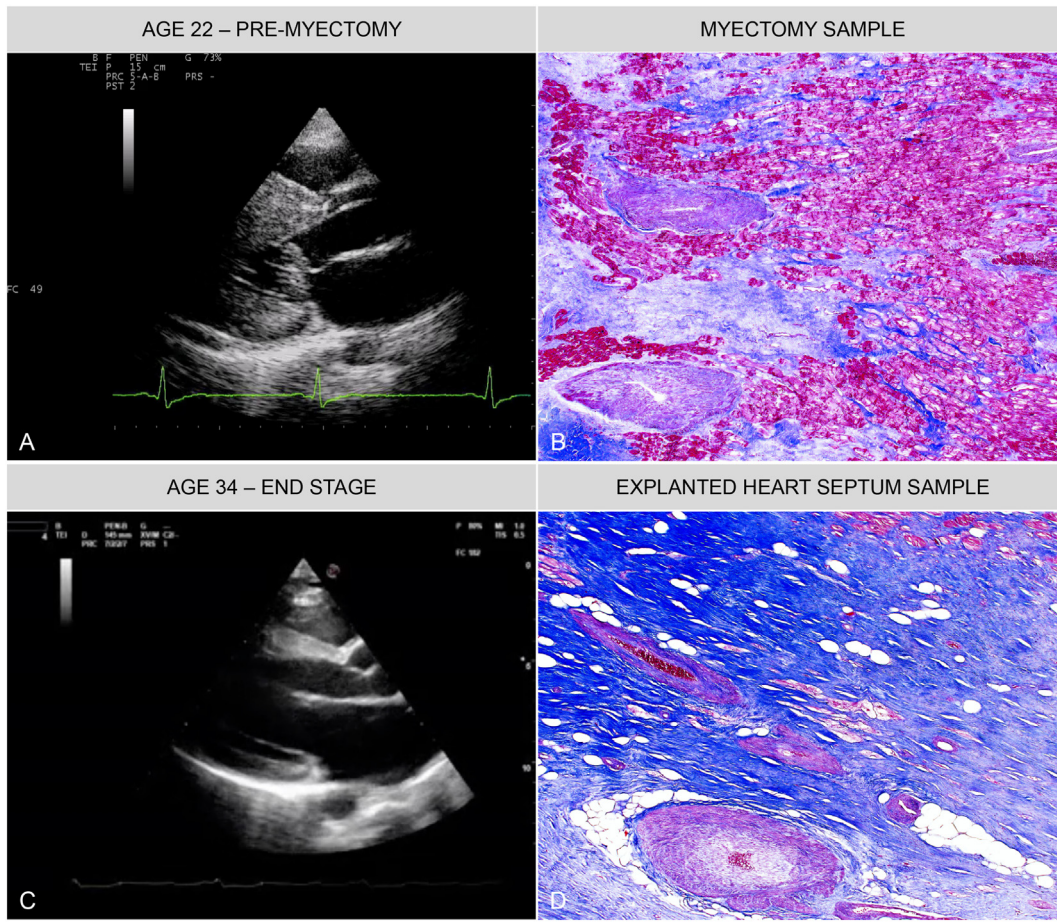


Fig. 2. “Example of severe and extensive microvasculopathy topographically associated with marked scar-like fibrosis in an end-stage HCM specimen.”



**Fig. 3.** “Evolution from the hypercontractile-obstructive to the end-stage phase of HCM.” Pre-myectomy echocardiography at age 22 (A); at this stage myectomy sample histology (B) may show severe IMCD in presence of limited replacement fibrosis associated with interstitial myocardial fibrosis. Echocardiography at age 34 (C). In ES anterobasal septum histology (D) often shows the coexistence of severe IMCD and extensive replacement fibrosis (Azan Mallory trichrome stain, original magnification 50 $\times$ ).

Longitudinal base-apex microvasculopathy distribution showed that while the prevalence of 100–500  $\mu$  IMCD was ubiquitous, LV and IVS apical segments showed more severe stenosis ( $p = 0.013$ ). Moreover, in LV and IVS apical thirds a trend of more distal vascular compartment thickening was found although without a statistical significance (Supplemental Table 5).

#### 4. Discussion

Over the last 30 years the histopathological features of HCM have been explored [3,4,9,10] and the role of IMCD has been recognized as a possible determinant of replacement myocardial fibrosis, LV dysfunction and outcome, while its functional and structural correlates have been elucidated in PET and MRI studies [5–7]. In a previous study, we described the topographic distribution of interstitial and replacement fibrosis within the ventricles in explanted end-stage hearts and suggested a relationship between IMCD severity and extent of scar [8]. In the present study, we provide novel evidence in support of this hypothesis, by comparing the extent and distribution of IMCD and different types of fibrosis in HOCM undergoing myectomy and end-stage HCM. Our main results are: 1. Intramural coronary artery disease can be found in both phenotypic expressions of HCM (HOCM and ES), with similar frequency and histopathological characteristics; 2. Both intramural intermediate and distal vascular compartments are altered; 3. Interstitial fibrosis prevails in myectomy specimens while replacement fibrosis is distinctive of ES hearts, where a basal–apical gradient of IMCD severity (paralleling the scar-like gradient) is evident; 4. Although microvasculopathy is very frequently associated with interstitial

fibrosis, ES hearts show a more stringent topographic association between 100 and 500  $\mu$  IMCD and myocardial scarring, consistent with the role of microvascular dysfunction demonstrated *in vivo* by PET, a technique primarily aimed at the intermediate vascular compartment.

Our data demonstrate the presence of extensive comparable vascular abnormalities in two radically different phases of HCM: 100–500  $\mu$  IMCD was encountered in over 90% of all slides examined, in both HOCM and ES-HCM with similar characteristics in terms of degree of lumen narrowing, extent of the disease and type of parietal involvement (medial/intimal pattern, type of parietal alterations and distribution of lesions on the vessel wall). The distal vascular compartment – <100  $\mu$  – was also frequently altered and again without any differences between the two subgroups.

These results lead to the conclusion that intramural coronary abnormalities represent an intrinsic feature of HCM equally present in the various natural phases of the disease. In fact, small coronary artery disease appears to be the common denominator shared by patients at opposite stages and phenotypes of HCM.

Although the genesis of coronary microvascular remodeling remains an enigma, several clues point to embryological factors acting in response to a hypercontractile myogenic tube in the early phases of development, with possible impact on the morphogenesis of intramural coronary circulation [12]. Of note, recent insights from transgenic HCM models support a developmental hypothesis for extra-myocardial HCM abnormalities [13].

A central research question – due to its prognostic implications – is the relationship between vascular abnormalities and myocardial fibrosis. Previous histopathological studies, both qualitative and quantitative, have found vascular abnormalities in the proximity of replacement fibrosis



[3,4]. A causative link was hypothesized due to reduced coronary reserve causing recurrent mismatch between oxygen demand and supply in the course of daily activities, generating myocardial ischemia and ultimately resulting in scar formation [3]. Functional studies have reinforced this concept as impaired myocardial blood flow measured with PET has been found in areas with delayed gadolinium enhancement at MRI, generally associated with myocardial scarring [5]. Interestingly, early microvascular abnormalities and dysfunction in HCM have been found to precede myocardial alterations such as myocyte hypertrophy [14]. Long-term, intramural coronary dysfunction has been identified as a predictor of LV remodeling (including the end-stage phase), characterized by extensive progression of replacement fibrosis, as well as heart failure-related outcome [6,7].

In the present work the topographic association between microvasculopathy and myocardial fibrosis was thoroughly analyzed. Both intermediate (vessel diameter 100–500  $\mu$ ) and distal (vessel diameter < 100  $\mu$ ) vascular compartments were widely associated with interstitial fibrosis, with no differences between ES hearts and myectomy specimens. However, as far as the intermediate vascular compartment is concerned, ES samples showed a significantly closer topographic association between IMCD and scar-like fibrosis. This finding, together with the coexistence in ES apical segments of a greater amount of scar-like fibrosis and severe vessel narrowing, supports the hypothesis of a causative link between vascular abnormalities and myocardial scarring.

Indeed our study population includes patients at very different stages of HCM exhibiting opposite phenotypic expressions. According to our results, small coronary artery disease appears to be a common denominator equally shared by the two forms whereas extensive replacement fibrosis is distinctive of ES patients. Due to the non-prospective nature of our work we can only speculate that microvascular dysfunction may be the missing link between the two forms of HCM with chronic small vessel-mediated ischemia ultimately resulting in myocardial scarring. Supposedly, as ES-HCM is an advanced stage of the disease, the more frequent association between replacement fibrosis and severe vessel narrowing could be the expression of a vicious circle that increases the severity of both vasculopathy and fibrosis.

This chain of events is exemplified in Fig. 3: a patient with typical HOCM exhibiting hypercontractile state undergoes surgical septal myectomy, with optimal result. At this stage, IMCD is already fully represented and has begun to exert its negative effects, although limited or no replacement fibrosis is present. Twelve years later, the same patient has progressed to the end-stage phase, with extensive fibrotic remodeling and thinning of the septum, and marked reduction in systolic function. At this stage, the degree of IMCD is unchanged, but scar is now evident and diffuse. The extended time-frame from the first to the last stage of this process accounts for the recent finding of the ShaRe registry [15], demonstrating an average of almost two decades between the initial diagnosis of HCM and the onset of heart failure-related complications.

In terms of future management opportunities, this concept supports the rationale for agents that might limit the long-term ischemic burden of IMCD, such as ranolazine [16]. Nevertheless, further prospective studies are warranted to confirm our findings and possibly definitively unravel the pathophysiological mechanisms that promote ES evolution among HCM patients.

## 5. Study limitations

Although our study provides a histopathological characterization of different phenotypic expressions of HCM suggesting a pathophysiological mechanism responsible for the natural history of the disease, our cohort was not followed longitudinally and the two subgroups – HOCM and ES – are constituted by different patients at different phases of the disease. Furthermore, no case of nonobstructive, non-end-stage HCM was included in our cohort (no systematic indication to endomyocardial biopsy in this subgroup). Unfortunately patients were not systematically studied with MRI or PET, so that no correlation between histology and

noninvasive imaging of myocardial substrate and microvascular circulation was possible.

## 6. Conclusions

IMCD is an early and intrinsic feature of the HCM disease spectrum, and can be observed in myectomy samples from HOCM patients in similar degree to ES-HCM patients. Only in the latter, however, does IMCD appear to be associated with replacement fibrosis, suggesting progression of ischemic damage directly due to microvascular dysfunction. We speculate that small coronary arteries abnormalities induce prolonged intramyocardial ischemia resulting over time in extensive scar formation and consequent end-stage evolution. Further prospective studies are warranted to confirm the causative link between small coronary artery disease and myocardial scarring and possibly identify new ways of interrupting the vicious pathway from isolated IMCD to myocardial damage.

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## Declarations of interest

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2019.03.060>.

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