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Structural analysis of arteriolar and myocardial remodelling in the subendocardial region of patients with hypertensive heart disease and hypertrophic cardiomyopathy

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Abstract Left ventricular hypertrophy is a risk factor for cardiovascular morbidity and mortality. In arterial hypertension and in hypertrophic cardiomyopathy it may be accompanied by clinical signs of myocardial ischaemia resulting from microcirculatory dysfunction in the absence of coronary macroangiopathy. Structural changes of the vascular and interstitial compartment of the heart are involved in the pathogenesis of impaired microcirculation. We investigated patients with hypertensive heart disease (HHD; n=12) and hypertrophic cardiomyopathy (HCM; n=19) without coronary macroangiopathy but with signs of myocardial ischaemia. Right septal endomyocardial biopsies were evaluated to quantify the structure of intramyocardial arterioles, collagen content and myocytic diameter by morphometric rules. Nine normotensive subjects served as controls. The groups differed significantly (P<0.05) in myocytic diameter and total collagen content. The myocytic diameter correlated with the thickness of the interventricular septum. Arterioles in HHD showed a significant increase in cross-sectional medial area and in HHD patients the periarteriolar collagen area increased both in absolute terms and when standardized to medial area. Arteriolar density was significantly reduced in HCM. In a multivariate discriminant analysis the positive predictive value for differentiation of the groups by non-myocytic variables was 72.5% (P=0.013). HHD and HCM differ in the structural alterations in the arteriolar bed. Medial hypertrophy and periarteriolar fibrosis prevail in HHD, and reduced arteriolar density is found in HCM. Different microvascular remodelling at the level of arterioles indicates distinct pathophysiologic processes that may contribute to the clinically observed disturbance of coronary microperfusion in these two diseases.

Key words Microangiopathy · Arteriolar density · Fibrosis · Remodelling · Cardiac hypertrophy

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Introduction

Left ventricular hypertrophy (LVH) is associated with an elevated incidence of adverse cardiovascular events. Patients with LVH have higher morbidity and mortality [35], and the prevalence of heart failure, myocardial ischaemia, malignant arrhythmias and sudden cardiac death is increased [30].

It is known that myocardial ischaemia can occur in the absence of coronary macroangiopathy [9, 39]. In patients with hypertensive heart disease (HHD) [2, 7, 48] and hypertrophic cardiomyopathy (HCM) [10, 11] myocardial ischaemia has been reported in the absence of significant coronary stenoses. This microvascular angina is possibly caused by structural and/or functional changes in intramyocardial resistance vessels beyond the resolution of coronary angiography. More than 50% of the coronary resistance resides in the small intramyocardial arterioles of less than 100 μm in diameter [13, 37], which represent about 80% of all intramyocardial arterial vessels [3, 31]. It is reasonable to assume that these vessels play a crucial role in the pathogenesis of myocardial ischaemia with decreased coronary reserve, in the absence of coronary macroangiopathy.

From a pathophysiological point of view a decrease of the total cross-sectional area of the resistance vessels leads to an increase of minimal coronary resistance and to a potential shortage of blood and oxygen supply. Different structural mechanisms may lead to a reduction in the total cross-sectional area of the microvasculature. Rarefaction, inadequate angiogenesis for the degree of myocytic hypertrophy, reduction of the luminal area by rearrangement of already existing material in the wall (remodelling) or the addition of material to the wall area (hypertrophy) may be involved in the process [25, 51]. Apart from structural vascular changes the process of hypertrophy alters myocardial structure qualitatively and quantitively for both the myocytic and interstitial compartments. The interstitium (especially the collagen matrix) is a major determinant of myocardial architecture, structural integrity and mechanical properties. The sum

of these changes, referred to as remodelling, is the hallmark of pathologic hypertrophy [57]. Animal studies suggest a close relationship between structural maladaptation and cardiac dysfunction or vulnerability [4, 58], and remodelling may cause myocardial ischaemia that determines the adverse clinical events in LVH [56].

However, the pathogenesis of LVH in HHD and HCM is distinct. HHD is a secondary cardiomyopathy with increased coronary perfusion pressure and increased wall stress imposed by pressure load on the ventricle. In contrast, HCM is a genetically linked disease of the sarcomeres while coronary perfusion pressure is in the normal range [50]. It is not known whether the remodelling of the coronary microvasculature is different in the two conditions.

Since endomyocardial biopsies are useful in the morphometric investigation of vascular changes of the human myocardium [54], quantitative data on the structural changes in the myocardium in these diseases were obtained from our biopsy material.

Materials and methods

All patients presented with angina pectoris and signs of myocardial ischaemia on the exercise ECG. After completion of diagnostic procedures, which included non-invasive and invasive testing, the final diagnosis was established. Coronary angiogram excluded coronary artery disease in all patients. HHD was diagnosed if the blood pressure was in excess of 140 mmHg systolic and/or 90 mmHg diastolic on different occasions. No secondary cause of hypertension could be found, and the patients were therefore regarded as essential hypertensives. HCM was diagnosed if the ratio of the interventricular septum (IVS) to the left ventricular posterior wall (LVPW) thickness exceeded 1.5 in the absence of any other cause for LVH and the thickness of IVS was greater than 15 mm in the basal portion. We investigated 12 patients with HHD (5 male/7 female; age 53±9 years), 19 patients with HCM (15 male/4 female; age 52±11 years) and 9 normotensive control (C) subjects (7 male/2 female; age 43±11 years; Table 1). In control patients no cardiac cause for the reported chest pain was found. None of the patients was diabetic. Each patient was grouped according to his or her physical capacity by an experienced cardiologist (New York Heart Association classification).

Two-dimensional transthoracic cardiac ultrasound was performed using a Toshiba SSH 140 model. The thickness of the IVS, the LVPW and the end-diastolic left ventricular diameter (LVEDD) was measured according to the American Society of Echocardiography convention. The LV mass index was then calculated using the Devereux equation [15]. LVH was defined by a LV mass index greater than 100 g/m^2 for women and greater than 131 g/m^2 for men [34].

A coronary macroangiopathy with over 50% narrowing of the epicardial arteries was ruled out by coronary angiography (via the femoral approach). The ejection fraction was greater than 60% on biplane ventriculography. Outflow gradients were determined in HCM either by echocardiography or invasively. A gradient greater than 30 mmHg at rest or after adequate provocation (Valsalva manoeuvre) defined the obstructive form of HCM. In addition, right septal endomyocardial biopsies (REMCB) were taken under fluoroscopic control to rule out specific causes of LVH including myocarditis or storage diseases of the heart. The femoral vein approach was routinely chosen. A modified bioptome manufactured at the University of Düsseldorf was routinely used. In former studies [45]; (Schwartzkopff et al., unpublished data) the arteriolar density in the right subendocardial septal region 2.5 mm beneath the endocardial layer of autopsied hearts was over 1.5 arterioles/mm^2. Therefore, the bioptome was equipped with jaws of 2.4 mm × 1.2 mm (length × width) area, which was thought to be a compromise between the jaw size needed for the determination of arteriolar density and the risk of complications that might occur with a larger device. Each patient gave informed consent before the procedure. No major complications (defined as events that required interventions resulting in hospitalization or prolongation of the hospital stay) were seen. The study was approved by the Ethics Committee of the Heinrich-Heine University, Düsseldorf, Germany, and conformed with the principle of the Declaration of Helsinki.

Right endomyocardial biopsies were taken and were formalin fixed and paraffin embedded. A defined sampling volume was given by the jaw size of the bioptome. The myocardial structure is partially anisotropic; mainly this is due to the alignment of the myocytes and the direction of the intramyocardial arterioles running from epicardial to endocardial layers. Since stereological rules are established exclusively for isotropic conditions, precautions have to be observed in partially anisotropic tissue. It has been shown that sections cut under isotropic conditions allow the application of common rules of stereology [22]. Therefore right endomyocardial biopsies were embedded in a sphere and rolled in random directions, thus generating an isotropic uniform random section, which allowed us to measure myocardial structure without bias (Fig. 1). Morphometric measurement was done on Elastica van Gieson (EvG)-stained sections for arterioles and in hematoxylin eosin (HE) staining for myocytes (Fig. 2). Morphometric analysis was performed exclusively on transversely cut arterioles resulting in a circular appearance of the vessel under investigation. Serial sections were cut (usually five per patient) to follow arterioles through the tissue block and afterwards the transversely cut arterioles within the sections were chosen for measure-

Table 1 Demographic data (mean ± SD: HCM hypertrophic cardiomyopathy, HHD hypertensive heart disease, *C* control, *NYHA* New York Heart Association, *LVEDP* left ventricular enddiastolic pressure, *LVEDD* left ventricular enddiastolic diameter, *IVS* interventricular septum, *LVPW* left ventricular posterior wall)

	HCM (*n*=19)	HHD (*n*=12)	C (*n*=9)
Age [range] in years	52 ± 11 [25–67]	53 ± 9 [36–69]	43 ± 11 [29–59]
Sex (male/female) [%]	15/4 [79%/21%]	5/7 [42%/58%]	7/2 [78%/22%]
Obstruction (yes/no)	15/4	–	–
NYHA classification	I=2/II=7 II=9/IV=1	I=0/II=7 III=5/IV=0	–
Cardiac index [l/min per m^2]	3.07 ± 0.73 ^{2*}	4.21 ± 1.39 ^{2*}	3.23 ± 0.54
Systolic aortic pressure [mmHg]	129 ± 15 ^{2*}	153 ± 20 $^{2*/3*}$	125 ± 12
Diastolic aortic pressure [mmHg]	70 ± 8 ^{2**}	84 ± 11	72 ± 9
LVEDP [mm Hg]	18 ± 9 $^{1**/2***}$	7.6 ± 2.3^{2***}	9.7 ± 3.2
LVEDD [mm]	42.7 ± 4.9 ^{1**}	46.8 ± 5.4	48.8 ± 3.3
IVS [mm]	23.6 ± 5.2 $^{1**/2**}$	13.0 ± 2.6 ^{2**}	11.0 ± 1.1
LVPW [mm]	11.5 ± 1.3	11.3 ± 0.9	10.6 ± 1.3
LV hypertrophy [%]	100%	33%	0%
LV mass [g]	323 ± 55 $^{1**/2**}$	222 ± 63^{2**}	188 ± 28
LV mass index [g/m^2]	172 ± 31 $^{1**/2**}$	115 ± 22 ^{2**}	95 ±14

Statistical analysis is done by multiple comparison and alpha adjustment according to the Bonferoni rule; 1 HCM/C; 2 HCM/HHD; 3 HHD/C;
* $P \leq 0.05$; ** $P \leq 0.01$;
*** $P \leq 0.001$

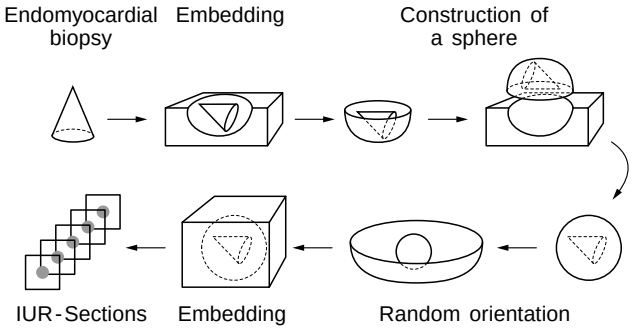

Fig. 1 Generation of isotropic uniform random (IUR) sections in partially anisotropic tissue

ment. Differentiation of arterioles and veins were done according to Weiß et al. [59]. Collagen was determined on Picro Sirius Red-stained sections. This staining has been found to specifically stain collagen and to correlate well with biochemical methods with regard to collagen content [29]. The measurements were done on the video-based image analyser Quantimet 570 (Leica, Cambridge). The following variables were measured (stain and magnification in brackets):

1. Outer arteriolar diameter (μm) excluding the elastic external membrane (EvG; ×800).
2. Medial area (μm²) of all circular cut arterioles. Arterioles were clearly detectable (EvG ; ×800) by their internal elastic lamina (black stained) and yellow-stained tunica media containing at least one layer of vascular smooth muscle cells, and by the reddish stained tunica adventitia, which consists mainly of collagen [59].
3. Periarteriolar collagen area (μm²) was defined as the collagen area neighbouring the tunica adventitia and being directly connected with it (Picro Sirius Red; ×800). Taking into account the differences in arteriolar diameter, this parameter was standardized to the medial area.
4. Myocytic diameter (HE; ×800) as the average of a minimum of 20 myocytes longitudinally cut and measured at the site of the nuclei (μm).
5. Collagen content (%) as the whole of reddish stained interstitial space (Picro Sirius Red; ×400).
6. Numerical arteriolar density (n/mm²) by counting all arteriolar profiles in the whole section (EvG; ×800).
7. Biopsy area (EvG; ×40) as defined by the area covered by myocardial tissue avoiding large artificial spaces (mm²).

The mean number of arteriolar profiles morphometrically evaluated was 14 per patient. A subset of the hypertensive patients (7 of 12) had been previously investigated by means of a point-counting technique [46]. All the measured variables – apart from the arteriolar diameter – are independent of the relaxation/constriction state of the vasculature and myocardium [14]. Therefore the parameters investigated were suitable for morphometric studies in non-perfusion-fixed tissue.

Descriptive data are expressed as mean±standard deviation [$\bar{X}\pm SD$]. The coefficient of variance (CV) is calculated as $SD/\bar{X}\times 100\%$. The coefficient of error (CE) is defined by $CV/\sqrt{n}$ (number of measurements) and defines the precision of the estimate. The Wilcoxon test, Mann-Whitney U test with Bonferoni adjustment and linear regression analysis were used as indicated. Furthermore a multivariate discriminant analysis using Wilks' Lambda [5] was done on morphometric data to determine the factors that discriminated best between the different groups. Sensitivity, specificity and the positive predictive value were defined as follows: sensitivity, (number of correctly classified patients with HCM or HHD compared with the total number of patients with HCM or HHD)×100; specificity, (number of correctly classified controls to total number of controls) ×100; positive predictive value, (number of correctly classified patients to total number of patients) ×100. Statistical significance was inferred at a two-tailed *P* value of less than 0.05. The analysis was done on the SPSS®-PC+ package (version 4.0).

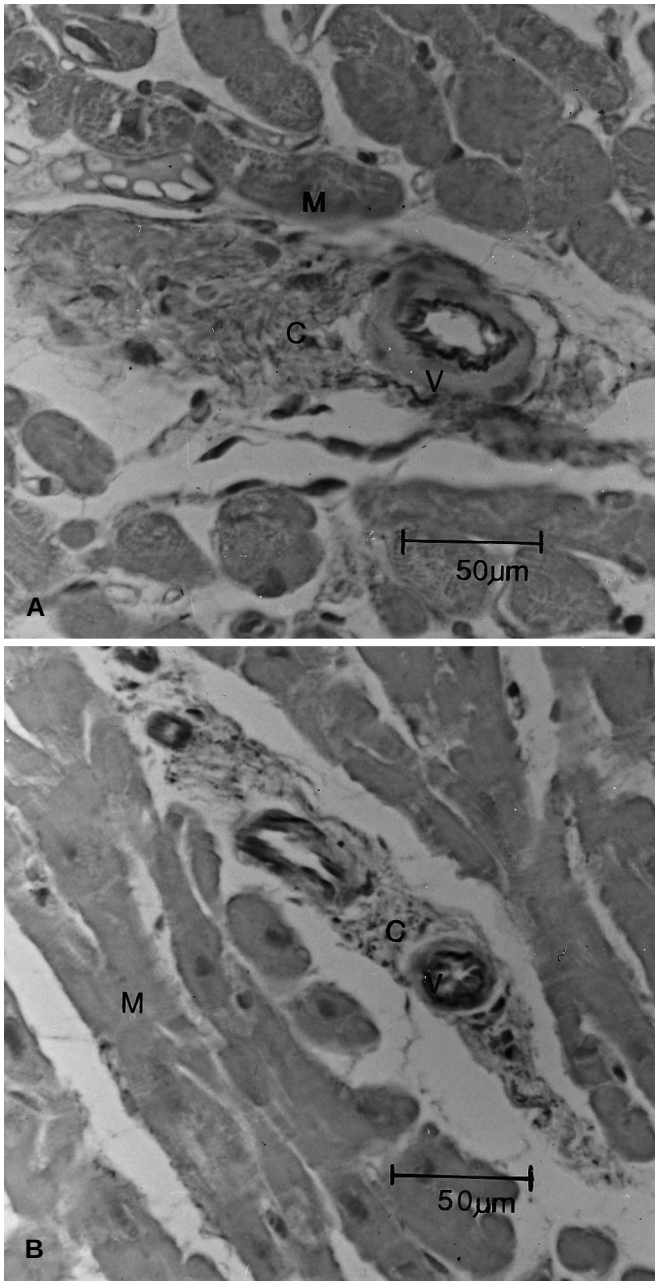

Fig. 2A, B Tissue sections of myocardial biopsies. **A** Hypertensive heart disease with arteriolar media thickening. **B** Normal subject. Elastica van Gieson, ×400. (*V* Arteriolar vessel, *C* collagen, *M* myocyte)

Results

There were no significant differences in age, sex and NYHA classification between HCM and HHD (Table 1). Patients with HCM showed a significantly higher LV mass index than those with HHD and C. 4 pts. with HHD

had LV-hypertrophy. In 4 patients with HCM no outflow gradient was detectable. During catherization the cardiac index (measured by thermodilution) was significantly higher in HHD than HCM patients. In those with HCM the left ventricular end-diastolic pressure (LVEDP) was significantly increased.

According to a hierachical variance model [21, 44] we evaluated the biopsy area, number of biopsies and number of sections necessary to achieve a coefficient of error under 5% for the individual patient in regard to the numerical density of the arterioles. The biological variance can be estimated according to Eq. 1 [21]:

$$Os_g^2=(s_a^2/n_a)+(s_b^2/n_a\times n_b)+(s_f^2/n_a\times n_b\times n_f) \quad \text{(Eq. 1)}$$

(Os_g^2 = total biological variance; s_a^2 = variation among individuals; s_b^2 = variation among different specimen from the same individual; s_f^2 = variation between sections from the same specimen; n_x = number of units under investigation on each level).

From the observed standard deviation of multiple biopsies from one heart, we estimated the number of biopsies necessary for the numerical arteriolar density to reach a coefficient of error of 5%, which is regarded as an acceptable degree of precision for the estimation of biological parameters [44]. In biopsy samples greater than 1.22 mm^2, one or more arterioles were found in 80% of all biopsies (Fig. 3). In biopsies of that size, 60% of the total biological variance of the arteriolar density is dependent on the interindividual variance (Table 2). A coefficient of error of less than 5% for the individual can be achieved with a total number of more than two sections and a minimum of five biopsy specimens per patient. Hence, in our study a subgroup of 12 patients with HCM, 7 patients with HHD and 5 C were eligible for quantification of numerical arteriolar density, with five or more biopsies being available with a biopsy area greater 1.22 mm^2.

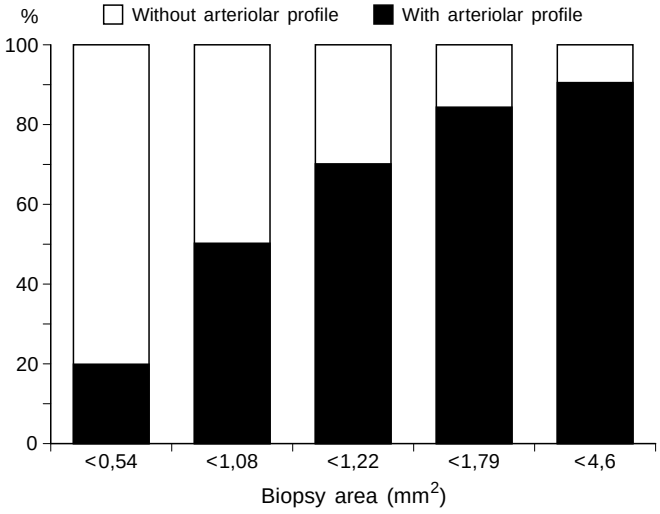

Fig. 3 Probability of finding an arteriole in dependance of biopsy area

Repeated measurements were done to study the intra- and interobserver variability of the morphological data for the estimated group mean of 10 patients, who were randomly chosen from the study subgroup population. To define the variability of repeated studies we calculated the coefficient of variance between the initial and second measurement by using the standard deviation of the differences from measurements of morphometric parameters in these patients [44]. The standard deviation was calculated separately for two measurements of the same observer (M.M.) and for the first measurement of both observers (M.M., B.S.). In the intraobserver study the coefficient of variance was 28% for cross-sectional medial area, 15% for periarteriolar collagen area, 19% for the total collagen content and 9% for the arteriolar density. Hence, the precision of the estimate of the mean for the variables measured in the control group of 9 patients reached a coefficient of error below 10% in the intraobserver study. With regard to the estimate of the mean of the morphometric parameters in the HCM group (n=19) and the HHD group (*n*=12 in brackets), the coefficient of error was calculated as 6.4% (8.1%) for the cross-sectional medial area, 3.4% (4.3%) for the periarteriolar collagen area, 4.4% (5.5%) for the collagen area and 2.6% (3.4%) for the arteriolar density (subgroup: [HCM; *n*=12] and [HHD; *n*=7]). Therefore it is unlikely that statistically significant differences between the groups were hampered by a lack of precision in the estimate. The interobserver variability had a coefficient of error of 11% for cross-sectional medial area, 13% for the periarteriolar collagen area, 15% for the total collagen content and 7% for the arteriolar density (subgroup [HCM; *n*=12] and [HHD; *n*=7]). Statistically the estimates between the two observers were not different.

Biopsy size was not correlated with any of the other morphological findings. The myocytic diameter and the collagen content were significantly increased in HCM, but while in HCM the total amount of collagen was increased, the total amount of collagen in HHD was non-significantly altered. Nevertheless, patients with HHD were found to have significantly more periarteriolar collagen than HCM and C patients. The distribution of the outer arteriolar diameter in the groups investigated is

Table 2 Hierachical variance model for the numerical density of arterioles in REMCB (from randomly chosen pts. with HCM, HHD and C)

[a] (calculated by equation 1): 1.42=0.84+1.77/4 + 2.51/20 expressed in percents as: 100%=60% + 31% + 9%

	Number of sections	Number of biopsies	Patients (*n*=10)	Biological variance
For each patient	5	4		
Mean of numerical density [n/mm^2]	1.7	1.7	1.7	1.7
Variance [n/mm^2]	2.51	1.77	0.84	1.42
Coefficient of variance (CV) [SD/X]	0.94	0.81	0.54	0.70
Share of the biological variance [a](%)	9	31	60	100

shown in Fig. 4. An increase in the medial area was present in patients with HHD. In the subgroup of patients eligible for evaluation of the numerical density of arterioles, this variable was significantly reduced in HCM patient. A reduction of arteriolar density was inversely correlated with an increase in myocytic diameter for all groups (Fig. 5). Between myocytic diameter and the IVS we found a highly significant correlation (r=0.77; P<0.01). The morphological data and statistics are summarized in Table 3.

The medial area was the variable with the best discriminatory ability for non-myocytic parameters, followed by collagen content and the standardized periarteriolar collagen area. Table 4 summarizes the discriminant analysis according to the classification results. The analysis was highly significant with a positive predictive value reaching 72.5% (P=0.0134). Although there was overlap in the diagnosis of HHD and HCM, only 5 patients (16%) were incorrectly classified. Patients with HHD showed the widest range in medial area (Fig. 6). There were 3 HCM patients who showed marked arteriolar hypertrophy in the range of the values of patients with HHD. Moreover, 5 patients had a normal collagen content and non-hypertrophied arterioles and were thus classified in the control group by analysis.

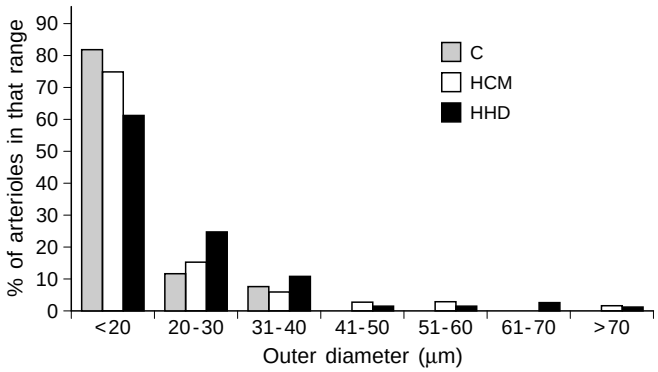

Fig. 4 Distribution of the outer arteriolar diameter in right septal endomyocardial biopsy specimens (*HCM* hypertrophic cardiomyopathy, *HHD* hypertensive heart disease, *C* control)

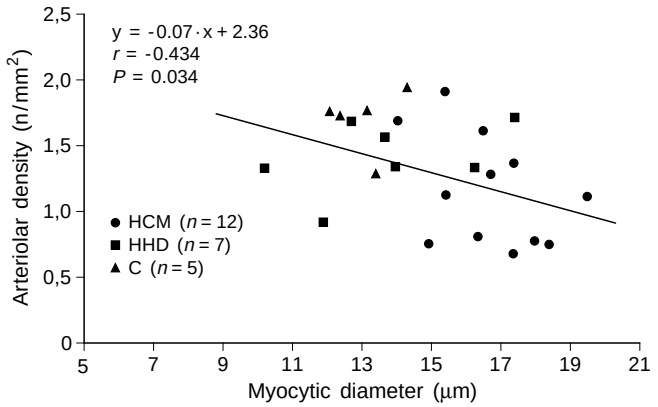

Fig. 5 Plot of myocytic diameter against arteriolar density

Table 4 Classification results. Percent of "grouped" cases correctly classified: 72.50%

Actual	Group	No. Of cases	Predicted group membership		
			HCM	HHD	C
Group	HCM	19	11 57.9%	3 15.8%	5 26.3%
Group	HHD	12	2 16.7%	9 75.0%	1 8.3%
Group	C	9	0 0.0%	0 0.0%	9 100.0%

Discussion

This study reveals vascular wall thickening of arterioles by an increase in cross-sectional medial area and periarteriolar fibrosis in HHD, and a reduced arteriolar density in HCM.

We found that in both HHD and HCM remodelling of the myocardial structure is present. However, the changes are not uniform, and it seems that the remodelling process involves distinctly different compartments. In our study myocytic hypertrophy correlated with an increase in the thickness of the septum, thus indicating that myocytic hypertrophy was an important factor in left ventricular hypertrophy. However, in HCM there might also be hyperplasia of myocytes [19]. Although the volume of myocytes is the main determinant of myocardial mass, their number accounts for only about one third of all myocardial cells; the greater proportion is contributed by interstitial and vascular cells [57]. With regard to the interstitium we found a significant increase of total collagen content in HCM and a 50% – but not significant – increase in HHD versus controls. Animal studies point to a reduced left ventricular compliance in myocardial diseases associated with fibrosis [16]. This may lead to an increase in LVEDP and to diastolic dysfunction with the clinical signs of dyspnoea and decreased exercise toler-

Table 3 Morphometric results

	HCM (n=19)	HHD (*n*=12)	C (*n*=9)
Arteriolar diameter [µm]	17.94 ± 4.49	21.07 ± 4.70	17.01 ± 2.04
Medial area [µm²]	200.93 ± 120.94[2*]	412.58 ± 243.97[2*/3*]	161.67 ± 43.90
Periarteriolar collagen area [µm²]	121.2 ± 80.34[2***]	361.2 ± 195.2[2***/3**]	104.67 ± 63.40
Periarteriolar collagen area/medial area	0.69 ± 0.55[2*]	1.00 ± 0.46[2*/3*]	0.60 ± 0.23
Myocytic diameter [µm]	16.08 ± 1.4[1***/2*]	13.30 ± 1.84[2*]	12.67 ± 0.95
Collagen content [%]	3.73 ± 1.50[1**]	2.87 ± 1.52	1.86 ± 0.49
Numerical arteriolar density [n/mm²][a)]	1.06 ± 0.34[1*]	1.40 ± 0.25	1.65 ± 0.23

Statistical significances are shown as follows: 1 HCM/C; 2 HCM/HHD; 3 HHD/C; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[a] For the numerical arteriolar density a subgroup of patients was examined (see Methods)

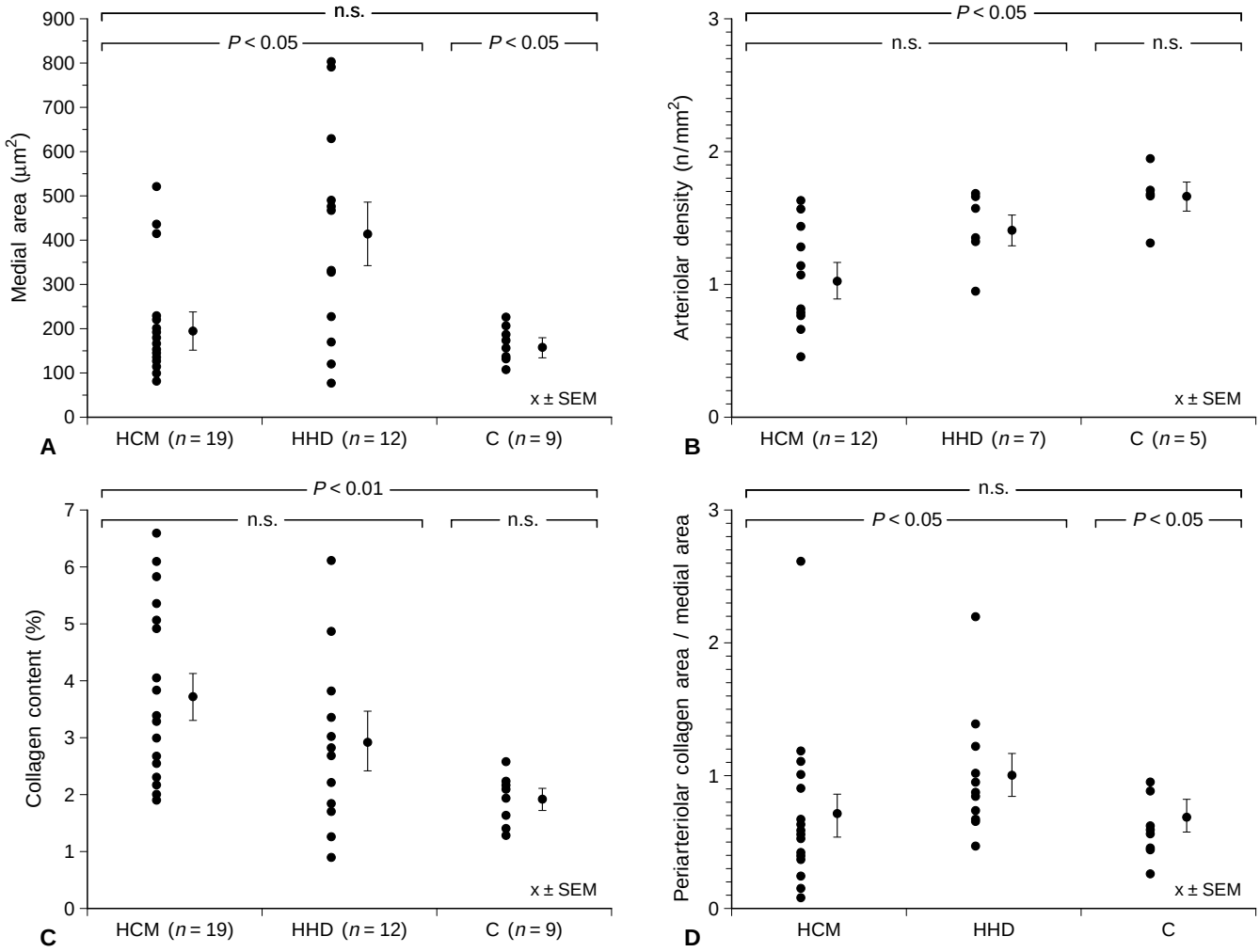

Fig. 6 **A** Medial area, **B** arteriolar density, **C** collagen content, **D** periarteriolar collagen area/medial area. In each column the dots represent the actual value for a patient. On the right of each column the dot plus bars expresses mean value ($\bar{X}$) ± S.E. for each group (Table 3; *n.s.* not significant)

ance [8]; features that are well recognized in HCM and HHD even when systolic function is still preserved [23]. This may in part explain the symptoms in our patients.

In patients with myocardial disease structural changes of the intramyocardial arterioles, which are crucial for the regulation of the coronary resistance and blood flow, are only detectable in endomyocardial biopsies. These are taken from the subendocardial layer of the myocardium, which is known to be exceptionally prone to ischaemia [12, 33]. According to Strauer [48], Brush et al. [7] and Antony et al. [2] the coronary flow reserve is diminished in patients with hypertension even in the absence of left ventricular hypertrophy. Owens et al. [40] described a hypertrophic process of the arterial wall, while other studies [6, 32] revealed that a decrease in the luminal diameter was induced by remodelling without the addition of wall material. However, these studies were done on small arteries with an outer diameter exceeding 150 μm and done in vascular beds other than the myocardium, so that the relevance of these findings to myocardial vasculature is doubtful [25]. In hypertensive patients Schwartzkopff et al. [46] observed an increase in the medial area of intramyocardial arterioles as an independent predictor for an increased minimal coronary resistance in HHD. In this study, we found a considerable increase in arteriolar wall thickness in terms of medial hypertrophy and in periarteriolar collagen deposition in hypertensive patients. This is in accordance with Tanaka et al. [49], who found considerable hypertrophy of arterioles and small arteries in HHD. Even in the absence of marked myocytic hypertrophy an increase in cross-sectional medial area was observed. Hence, in the absence of LVH, patients with HHD may already undergo a disproportionate vascular hypertrophy, which indicates an independent growth process in different compartments of the myocardium [36]. Since the resistance vessels are prone to an increased coronary perfusion pressure in HHD, the arteriolar hypertrophy might first be adaptive. However, in the course of prolonged arterial hypertension the alteration in the vascular wall architecture might become pathogenic as vasodilatation is decreased [18]. It is not yet clear to what extent different mechanisms are involved in the growth process, and if mechanical stretching of blood vessels is itself a growth stimulus. Certain endocrine, paracrine and auto-

crine factors might act as growth promoters. Vasoconstricting factors such as angiotensin II, endothelins or catecholamines have been found to stimulate the growth of vascular smooth muscle cells (VSMC) and with haemodynamic stimuli such as stretch or wall tension these vasoconstricting factors may induce hypertrophy as well as hyperplasia of the VSMC via mediation of such auto-/paracrine factors as platelet-derived growth factor or the fibroblast growth factor family [1, 20]. Furthermore, these factors are fibrogenic and may lead to an increase in intramural and periarteriolar collagen matrix, as was noted in the studies of Schwartzkopff et al. [46] and Tomanek et al. [52]. A recent study by Reddy et al. [43] suggests that hyperpermeability of the coronary vasculature in response to angiotensin II might be involved in the initiation of perivascular fibrosis. In addition, evidence obtained from animal studies by Isoyama et al. [28] suggests that with the deposition of periarteriolar collagen the ability of coronary resistance vessels to dilate is reduced and coronary vascular resistance is increased. Furthermore, only in those animals with complete regression of periarteriolar collagen was there any benefit of antihypertensive therapy (normalization of the coronary vascular resistance). With regard to the numerical density of intramyocardial arterioles, we found no significant reduction in HHD. It is unlikely that in the mildly hypertrophied myocardium in hypertension a decreased coronary blood flow is due to inadaquate growth in length or loss of intramyocardial arteriolar segments. This is in keeping with the results of animal studies by Rakusan et al. [42] and Tomanek et al. [53], who found adequate arteriolar growth in length in the hypertrophied heart with hypertension. However, Vitullo et al. [55] described a reduction in arteriolar length in the early phase of hypertension in spontaneously hypertensive rats. Stimuli that may contribute to adequate angiogenesis include mechanical factors, inflammatory processes or energy imbalance [41]. Important endogenous angiogenic factors are involved for example in the complex process of vascular growth (fibroblast growth factors, transforming growth factor β, vascular endothelial growth factor and platelet-derived endothelial cell growth factor; for review see [17]).

In HCM reduced coronary flow reserve has been found in different clinical studies [10, 11]. In contrast to HHD, in HCM the reduction in the numerical density of arterioles is the predominant finding. The reduction in the arteriolar density correlates inversely with myocytic hypertrophy. We speculate that inadequate growth, rather than rarefication of the preformed vascular segments, predominates in HCM. As hypertrophy of the septum and of the myocytes was excessive in HCM in comparison with controls and HHD, it is reasonable to assume that critical hypertrophy with a left ventricular weight above 220 g might be associated with an reduced arteriolar density since the vasculaturization might lag behind myocytic growth, as pointed out by Hort [26]. However, as angiogenesis or loss of arterioles is a dynamic process [17, 27] a definite interpretation of the mechanisms leading to a reduced arteriolar density in HCM cannot be derived from this study. Another explanation might be a genetically linked inborn error of arteriolar growth in HCM, which might lead to an arteriolar deficient myocardium a priori, by way of lack of angiogenic factors in the myocardium of patients with HCM. Furthermore, loss of preformed arteriolar segments is possible. In the presence of increased endothelin concentrations, as described by Hasegawa et al. [24], and dysplastic small arteries [38], which were seen in the myocardium in up to 80% of patients with HCM investigated after death, it seems possible that combination of both these changes predisposes to vasospasm and loss of arterioles. However, we detected neither dysplastic small arteries nor medial hypertrophy of arterioles in the majority of patients with HCM. Since our study missed arteries over 100 μm in diameter we are not able to make a final statement on dysplastic small arteries, which are described as having a segmental or focal character. HCM is a genetic disease of the sarcomere, and the mechanisms by which the vasculature is involved are still obscure. Molecular studies stress the genetic heterogeneity of HCM [47], which might be an explanation for the variety of morphological findings in HCM in contrast to HHD in our study. Further investigations on the link between genetic mutations and morphological alterations of the microvascular structure will help to understand the pathomechanisms.

With the aid of multivariate discrimination analysis we defined the predominant structural alterations in the two different forms of hypertrophic heart diseases. The most sensitive single discriminant factor was the cross sectional medial area of the arteriole. It was possible to group patients according to their clinical diagnosis correctly in 72,5% of cases by using medial area, collagen content and periarteriolar collagen area/medial area as morphological variables (Table 4). The sensitivity of the analysis was 81%, the specificity 100%. The ability to discriminate between HHD, HCM and controls on structural data derived from REMCB underlines the significance of the different remodelling in the two examined forms of pathological hypertrophy.

End note

The endomyocardial biopsies were taken from the right side of the septum. Since the left ventricle is mainly involved in the hypertrophic process the data presented for morphological quantification may differ from the reported data when left-sided specimens are investigated. However, it is known that the right side of the septum is involved in the hypertrophic process in HHD and HCM. Moreover, in HCM this region is affected secondarily by the intraventricular pressure gradient leading to pressure overload and the systolic anterior movement of the mitral valve. Since the morphological data in our study was derived from REMCB it seems unlikely that haemodynamic factors such as an elevated left ventricular systolic and/or diastolic pressure in the absence of pulmonary venous hypertension would interfere with the results.

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