Coronary flow reserve in hypertrophic cardiomyopathy: relation with microvascular dysfunction and pathophysiological characteristics

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Background. The decrease in coronary flow reserve (CFR) in hypertrophic cardiomyopathy (HCM) predisposes to myocardial ischaemia, systolic dysfunction and cardiac death. In this study we investigate to which extent haemodynamic, echocardiographic, and histological parameters contribute to the reduction of CFR.

Methods. In ten HCM patients (mean age 44 ± 14 years) and eight heart transplant (HTX) patients (mean age 51 ± 6 years) CFR was calculated in the left anterior descending coronary artery. In all subjects haemodynamic, echocardiographic and histological parameters were assessed. The relationship between these variables and CFR was determined using linear regression analysis.

Results. CFR was reduced in HCM compared with HTX patients (1.6 ± 0.7 vs. 2.7 ± 0.8 , $p < 0.01$). An increase in septal thickness ($p < 0.005$), indexed left ventricular (LV) mass ($p < 0.005$), LV end-diastolic pressure ($p < 0.001$), LV outflow tract gradient ($p < 0.05$) and a decrease in arteriolar lumen size ($p < 0.05$) were all related to a reduction in CFR.

Conclusion: In HCM patients haemodynamic (LV

end-diastolic pressure, LV outflow tract gradient), echocardiographic (indexed LV mass) and histological (% luminal area of the arterioles) changes are responsible for a decrease in CFR. (*Neth Heart J* 2007;15:209-15.)

Keywords: coronary flow reserve in hypertrophic cardiomyopathy, coronary flow reserve, cardiomyopathy (hypertrophic)

Hypertrophic cardiomyopathy (HCM) is characterised by a hypertrophied left ventricle (LV) in the absence of apparent causes for hypertrophy such as aortic valve stenosis and hypertension.¹ Angina is frequently present in patients with HCM despite the presence of normal coronary arteries. A reduction in coronary flow reserve (CFR) is a recognised feature in patients with HCM. Indeed, the inadequate increase in myocardial blood flow on demand in HCM predisposes patients to myocardial ischaemia, which in turn can result in syncope, an abnormal blood pressure response to exercise, LV systolic and diastolic function and sudden death.²⁻⁶ Recent studies have shown that severe impairment of the coronary vasodilator response to dipyridamole is strongly associated with 'end stage' disease, long-term clinical deterioration and cardiac death in patients with HCM.^{7,8} A number of mechanisms are associated with the decrease in CFR, including diastolic dysfunction,^{9,10} systolic compression of the septal coronary branches,^{11,12} the existence and degree of LV outflow tract gradient,¹³ small-vessel disease of the intramyocardial arterioles¹⁴⁻¹⁶ and inadequate capillary density relative to the increase in myocardial mass.¹⁷ Although all mechanisms have a role in creating an imbalance between oxygen supply and demand, their relative contribution to the decrease in CFR has not yet been evaluated in HCM patients. The aim of the present study is to investigate to which extent each of the aforementioned variables contribute to the reduction of CFR in HCM patients.

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Methods

Patient population

Haemodynamic, echocardiographic, and histological changes in the coronary vascular bed in symptomatic obstructive (LV outflow tract gradient >50 mmHg at rest or provocation) HCM patients ($n=10$, mean age 44 ± 14 years, range 20-63 years) were compared with a control group of asymptomatic HTX patients ($n=8$, mean age 51 ± 6 years, range 40-61 years). In HCM patients septal myocardium was obtained at the time of Morrow myectomy.¹⁸ In HTX patients septal myocardium was obtained at the time of routine follow-up right ventricular biopsy one to five years after HTX. These data were compared with left-sided septal myocardial tissue from victims of traffic accidents ($n=10$, mean age 38 ± 19 , range 8-58 years) without apparent cardiovascular disease. The institutional review board approved the study and patients gave oral and written informed consent.

Cardiac catheterisation

Cardiovascular therapy was continued during the study in all patients. After intravenous administration of 10,000 IU heparin and 250 mg acetylsalicylic acid right heart catheterisation was carried out with a 7F balloon-tipped flow-directed thermodilution catheter. Left heart catheterisation was performed with an 8F double-sensor high fidelity pigtail catheter (Cordis 811-180, Roden, the Netherlands). In each patient, baseline mean pulmonary wedge pressure, LV systolic and end-diastolic pressure, peak LV-aortic gradient, and cardiac index were noted. In addition, coronary angiography was performed according to standard Judkins techniques.

Coronary flow velocity measurements and analysis

After right and left cardiac catheterisation, a 0.014-inch Doppler guide wire with a floppy distal end (Cardiometrics, Inc., Mountain View, USA) was introduced through an 8F guiding catheter and positioned at the midsegment of the left anterior descending coronary artery (LAD) to measure Doppler flow velocity at rest and after hyperaemia. In both groups, hearts were paced at a constant heart rate of 100 beats/min to avoid metabolic vasodilatation during CFR determination. After optimisation of the settings of the velocity signal, baseline recordings of flow velocity and perfusion pressure were collected and digitised at a sample rate of 125 Hz for off-line analysis. Maximal hyperaemia was induced by an intracoronary bolus of 18 μ g adenosine.¹⁹ The sample volume from the Doppler wire was positioned at a distance of 5.2 mm from the transducer and has an approximated width of 2.25 mm due to a divergent ultrasound beam. After real-time processing of the quadrature audio signal, a fast-Fourier algorithm was used to increase the reliability of the analysis.²⁰ The flow velocity measurements obtained with this system have been

validated in vitro and in an animal model using simultaneous electromagnetic flow measurements for comparison.²¹ CFR was defined as hyperaemic divided by basal velocity.

Quantitative angiographic measurements

A validated on-line system operating on digital images (ACA-DCI, Philips, Eindhoven, the Netherlands) was used during the catheterisation procedure. With this system, the end-diastolic and end-systolic diameter of the LAD and the proximal part of the first septal branch²² were determined in the segment of the LAD in which the sample volume of the Doppler wire was located. The derived vessel diameters at the tip of the Doppler guidewire were used to calculate the vessel lumen area assuming a circular shape of the vessel. This vessel area was multiplied by the spatially averaged Doppler velocity (assuming a parabolic profile) to obtain coronary flow (ml/min).

Echocardiography

Two-dimensional echocardiographic studies were performed with commercially available equipment (Toshiba, Tokyo, Japan). Septal and posterior LV wall thickness was measured in end-diastole from both the parasternal short-axis and long-axis views.²³ LV mass was calculated according to the Devereux method and subsequently indexed for body surface area.²⁴

Morphometric analysis of arterioles

The myocardial specimens from HCM patients, HTX patients and victims of car accidents were fixed by immersion in 4% phosphate-buffered formaldehyde. Care was taken that only specimens from the endocardial septal site were utilised for analysis in all three groups. After fixation, the myocardial tissue was processed for paraffin sectioning and staining. For identification and analysis of intramyocardial small arteries an elastic von Giesson staining was used according to Tanaka et al.¹⁴ In brief, each preparation was magnified by a light microscope (Zeiss, Oberkochen, Germany) and digitised for further analysis with a quantitative morphometric system (IBAS system, Kontron, Germany). Only vessels with a round appearance and without side branches were studied. For each arteriole, the wall (intima plus media) and total vessel area were traced. The adventitia was not included in the measurements since the distinction between adventitia and perivascular fibrosis was difficult to visualise. The % luminal area was calculated as $L / (W+L) \times 100\%$, where L = lumen area and W = wall area. The external diameter (D) of the intramyocardial coronary arterioles was calculated by the formula: $D = 2 \times \sqrt{((\text{wall} + \text{lumen area}) / \pi)}$. In each subject at least ten arterioles were identified and used for analysis.

Morphometric analysis of capillaries

In order to elucidate capillary density in the myocardial specimens, sections (4 μ m) from formalin-fixed,

paraffin-embedded tissue were stained with antibodies directed against the CD-34 epitope (Biogenex, San Ramon, USA) as described below. After deparaffinisation and rehydration, the sections were rinsed with phosphate-buffered saline and incubated with normal goat serum (DAKO, Glostrup, Denmark) antibodies directed against the CD-34 epitope, biotinylated secondary antibodies (Multilink, Biogenex, San Ramon, USA) and peroxidase-labelled streptavidin (Biogenex, San Ramon, USA), respectively. The subsequent immunoperoxidase product was visualised with 3,3'-diaminobenzidine. In negative controls the primary antibody was replaced by normal mouse serum (DAKO, Glostrup, Denmark). In our experience, staining by Jones' silver methanamine, which was used by Rakusan et al.²⁵ to analyse capillary density in human myocardium, provided suboptimal results because discrimination of individual capillaries was more difficult. Quantitative morphometric analysis of the sections with an area of 62,500 μm^2 was performed with a specially developed software programme applied to a morphometric system (Clemex Techn Inc.,

Quebec, Canada). We paid special attention that cross-sections were originating from the endo- or mid-myocardium, only regions with transversely cut cardiomyocytes were selected and no or only minimal fibrosis was present. For each patient at least five different regions were selected. The number of cardiac myocytes and therefore the capillary-myocyte ratio was not determined because of the inability to outline myocytes correctly.

Statistical analysis

Data are presented as mean $\pm$ SD. To evaluate differences between groups unpaired student t-test was utilised. The relationship between CFR and haemodynamic, echocardiographic and morphometric variables was evaluated by linear regression analysis. A p value of <0.05 was considered significant.

Interobserver variability

The % luminal area of the arterioles and the capillary density were independently quantified by two different observers, the variance was $<3\%$ and $<5\%$, respectively.

Table 1. Demographic, haemodynamic, echocardiographic, and morphometric data of patients with hypertrophic cardiomyopathy and cardiac transplant recipients.

	Age/sex	NYHA	Therapy	IVS (mm)	LVPW (mm)	LV mass index (g/m ²)	Cardiac index (l/min/m ²)	LVEDP (mmHg)	LVOT gradient (mmHg)	% lumen IMSA	Capillaries (n/mm ²)	CFR
HCM												
# 1	45 F	II	-	20	11	168	2.8	12	32	-	-	3.10
# 2	59 M	III	B	23	13	202	2.4	28	100	14.9	1379	1.33
# 3	35 F	II	V	30	18	276	3.1	23	124	18.9	849	1.12
# 4	27 F	III	B	25	12	205	2.9	26	100	16.0	864	1.20
# 5	44 F	II	V	20	11	122	3.2	14	100	22.1	1541	2.10
# 6	20 M	II	V	23	11	182	2.9	23	58	27.6	1768	1.22
# 7	41 F	III	B,V	24	18	199	1.8	37	124	19.7	969	0.97
# 8	63 M	III	B	17	13	147	2.3	16	74	18.4	1686	1.13
# 9	62 M	III	V	22	12	184	3.4	20	88	30.7	1496	1.41
# 10	43 M	III	B	22	12	186	2.0	13	84	23.4	2451	1.68
HTX												
# 1	49 M	I	A,C,N,P	11	10	91	3.4	9	-	26.1	1308	3.38
# 2	61 M	I	C,D,N,P	12	10	88	3.2	6	-	26.6	1747	4.01
# 3	40 M	I	A,C,N,P	11	10	82	4.8	10	-	35.3	2079	2.00
# 4	57 M	I	C,D,N,P	11	11	119	4.1	11	-	31.2	2373	2.70
# 5	51 M	I	C,D,N,P	11	10	84	3.4	5	-	31.2	1511	2.25
# 6	55 M	I	A,C,N,P	10	10	73	3.2	7	-	33.7	2050	3.13
# 7	50 M	I	A,C,D,V	10	9	71	2.6	11	-	28.0	2245	1.97
# 8	52 M	I	A,C,N,P	11	10	100	3.9	13	-	31.5	1279	1.72

A=aspirin, B= β -blocker, C=cyclosporin, D=dipyridamole, N=nifedipin, V=verapamil, CFR=coronary flow reserve, IMSA=intramycardial small arteries, IVS=inter-ventricular septum, LVEDP=left ventricular end-diastolic pressure, LV mass=left ventricular mass, LVOT gradient=left ventricular outflow tract gradient, LVPW=left ventricular posterior wall, NYHA=New York Heart Association functional class, HCM=hypertrophic cardiomyopathy, HTX=heart transplant recipients.

Results

Patient characteristics

Demographic, haemodynamic, echocardiographic, and histological data of individual HCM and HTX patients are presented in table 1. As seen in table 2, compared with HTX patients, HCM patients had increased thickness of the septal (2.3 ± 0.3 vs. 1.1 ± 0.1 cm, $p < 0.0001$) and posterior wall (1.3 ± 0.3 vs. 1.1 ± 0.1 cm, $p < 0.01$), and increased indexed LV mass (187 ± 41 vs. 89 ± 15 g/m², $p < 0.0001$).

HCM and HTX patients had comparable stroke volume. However, because of lower resting heart rates (70 ± 13 beats vs. 97 ± 13 beats/min, $p < 0.005$), HCM patients had a lower cardiac index (2.7 ± 0.5 vs. 3.5 ± 0.7 l/min/m², $p < 0.01$). LV end-diastolic pressure was significantly higher in HCM patients (21 ± 8 mmHg vs. 9 ± 3 mmHg, $p < 0.001$).

None of the HCM and HTX patients had epicardial coronary artery disease or systolic septal perforator artery compression. End-systolic and end-diastolic luminal diameters of the LAD and first septal branch were comparable in HCM and HTX patients.

Histological analysis

As seen in table 2, the external calibre of the intramyocardial arterioles in HCM patients (42.0 ± 7.8 µm) and HTX patients (46.1 ± 12.7 µm) were not significantly different from those in victims of car accidents (49.9 ± 13.4 µm). In contrast, the % luminal area in HCM patients ($21.3 \pm 5.2\%$) was significantly reduced, both compared with HTX patients ($30.4 \pm 3.3\%$, $p < 0.001$) and victims of car accidents

($30.9 \pm 5.7\%$, $p < 0.001$). In victims of car accidents the number of capillaries (3883 ± 798 /mm²) was higher, both compared with HCM patients (1445 ± 514 /mm², $p < 0.0001$) and HTX patients (1824 ± 425 /mm², $p < 0.0001$). Figure 1 shows an example of capillary density in an HCM patient and a victim of a car accident, respectively. Capillary density in HCM patients tended to be lower than in HTX patients (1445 ± 514 /mm² vs. 1824 ± 425 /mm², $p = 0.11$).

Variables related to CFR

CFR in HCM patients was significantly reduced compared with HTX patients (1.6 ± 0.7 vs. 2.6 ± 0.8 , $p < 0.01$). An increase in LV septal thickness ($p < 0.005$), indexed LV mass ($p < 0.005$, figure 2A), LV end-diastolic pressure ($p < 0.001$, figure 2B), LV outflow tract gradient ($p < 0.05$), and a decrease in % luminal area of the arterioles ($p < 0.05$) were all related to the reduction in CFR. The reduction in capillary density in HCM patients was not significantly related to the reduction in the vasodilatory reserve ($p = 0.10$). Multivariate analysis was not performed because of the small patient number.

Discussion

The decrease in CFR is an important phenomenon in HCM, since it predisposes patients to myocardial ischaemia and, moreover, decrements in CFR are a strong predictor of clinical deterioration and death.²⁻⁵ In previous studies the reduction of CFR in HCM patients has been related to single haemodynamic,¹³ echocardiographic^{9,10} and histological characteristics.¹⁴⁻¹⁷ This is the first study to relate a combination of haemo-

Table 2. Comparison of patient characteristics between hypertrophic cardiomyopathy, cardiac transplant patients and victims of car accidents.

	HCM patients (n=10)	HTX patients (n=8)	Car accidents (n=10)
Age (years)	44±15	51±6	38±19
Septal wall thickness (cm)	2.3±0.3	1.1±0.1 [#]	
Posterior wall thickness (cm)	1.3±0.3	1.0±0.1 ^{\$}	
LV mass index (g/m ²)	187±41	89±15 [#]	
Cardiac index (l/min/m ²)	2.7±0.5	3.5±0.7 ^{\$}	
LV end-diastolic pressure (mmHg)	21±8	9±3 [#]	
End-diastolic LAD (mm)	3.3±1.0	3.1±0.5	
End-systolic LAD (mm)	3.1±0.9	3.1±0.5	
End-diastolic SA (mm)	1.5±0.4	1.3±0.2	
End-systolic SA (mm)	1.3±0.4	1.2±0.2	
External diameter of IMSA (mm)	42.0±7.8	46.1±12.7	49.9±13.4
% Luminal area	21.3±5.2	30.4±3.3	30.9±5.7
Capillary density (number/mm ²)	1445±514 [#]	1824±425 [#]	3883±798
Coronary flow reserve	1.6±0.7	2.7±0.8 ^{\$}	

Data are presented as mean values ± SD. [#]= $p < 0.0001$, ^{\$}= $p < 0.01$, HCM=hypertrophic cardiomyopathy, HTX=heart transplant, LV=left ventricular, LAD=left descending coronary artery (diameter), SA=septal artery (diameter).

Figure 1. The capillary density of a victim of a car accident is represented in the upper panel. In the lower panel, the capillary density of a patient with hypertrophic cardiomyopathy is displayed. Note the decrease in the number of capillary vessels in hypertrophic cardiomyopathy (magnification 40 $\times$).

dynamic (LV end-diastolic pressure, LV outflow tract gradient), echocardiographic (indexed LV mass) and histological (% luminal area of the arterioles) characteristics to the reduction in CFR.

Histological analysis: the role of small-vessel disease

In HCM patients post-mortem histological analysis of LV myocardium has revealed the presence of abnormal intramyocardial arterioles with markedly thickened walls and narrowed lumens.²⁵ Abnormal intramyocardial arteries are considered one of the possible mechanisms for a reduction in CFR in these patients.^{13,14} Indeed in the present study a positive relationship between the degree of arteriolar dysplasia and the decrement in the CFR was present. Since myocardial tissue was obtained from different sites (LV septal endocardium for HCM patients, right ventricular septal endocardium in HTX patients), this could have affected our results. Nevertheless, Tanaka et al.¹⁴ demonstrated that the % luminal area of the intramyocardial arterioles was identical in the right, middle and left

Figure 2A. The relation between the coronary flow reserve (CFR) and left ventricular mass corrected for body surface area (LV mass/BSA, g/m²) in patients with hypertrophic cardiomyopathy (♦) and cardiac transplant recipients (■).

Figure 2B. The relation between the coronary flow reserve (CFR) and left ventricular end-diastolic pressure (LVEDP, mmHg) in patients with hypertrophic cardiomyopathy (♦) and cardiac transplant recipients (■).

thirds of the ventricular septum in HCM hearts and in normal hearts. To evaluate the role of small-vessel disease in HTX patients the % luminal area of intramyocardial arterioles in this group was compared with that of normal hearts and no significant differences were found.

Histological analysis: the role of capillary density

Although small arteries and arterioles mainly control the blood flow of the coronary circulation, the capillary density and distribution are of crucial importance in the process of exchange between blood and tissue.²⁶ In HCM patients ischaemia might be due to an increased oxygen diffusion distance caused by an inadequate growth of capillaries. The existence of this phenomenon has been confirmed in animals with pressure-overload LV hypertrophy.²⁷ However, there are only few studies that have investigated the coronary vascular microcirculation in the normal human population and in patients with pressure-overload LV hypertrophy. Rakusan et al.²⁵ showed that human LV capillary density is comparable with that of other mammalian

Figure 3. Mechanisms underlying the reduction in coronary flow reserve in hypertrophic cardiomyopathy. LVOT=left ventricular outflow tract, LVEDP=LV end-diastolic pressure, CFR=coronary flow reserve.

species, and that pressure-overload LV hypertrophy in children is associated with proportional capillary angiogenesis, whereas in adults, LV hypertrophy appears to be associated with failure of compensatory angiogenesis. To the best of our knowledge, there are no studies concerning the role of capillary angiogenesis in HCM. In our study, we showed that there was a clear reduction in capillary density in HCM patients compared with patients without cardiovascular disease and HTX patients, although the latter comparison did not reach statistical significance. There was only a weak relation between the reduction in CFR and the decrease in capillary density ($p=0.10$). However, the patient population could have been too small for differences to appear.

Limitations of the study

The use of HTX patients as control group is debatable; nevertheless Vassali et al. showed that CFR is comparable with the normal population one to six years after transplantation.²⁸ Importantly, none of our HTX patients had signs of acute allograft rejection or epicardial CAD, conditions which are known to diminish CFR.^{29,30} Of the parameters we studied which could influence CFR, the reduction in capillary density in HTX patients was the most striking difference in

comparison with the normal situation. Also, the sample size of the patient populations we studied was relatively small, as a consequence only univariate analysis could be performed and only clear differences in patient characteristics showed significance.

Conclusion

In HCM patients haemodynamic (LV end-diastolic pressure, LVOT gradient), echocardiographic (indexed LV mass) and histological (% luminal area of the arterioles) changes are responsible for a decrease in CFR (figure 3). ■

References

- 1 Maron BJ. Hypertrophic cardiomyopathy: a systematic review. *JAMA* 2002;287:1308-20.
- 2 Maron BJ, Epstein SE, Roberts WC. Hypertrophic cardiomyopathy and transmural myocardial infarction without significant atherosclerosis of extramural coronary arteries. *Am J Cardiol* 1979;43:1086-102.
- 3 Koga Y, Yamaguchi R, Ogata M, Kihara K, Toshima H. Decreased coronary vasodilatory capacity in hypertrophic cardiomyopathy determined by split-dose thallium-dipyridamole myocardial scintigraphy. *Am J Cardiol* 1990;65:1134-9.
- 4 Camici P, Chiriaci G, Lorenzoni R, Bellina RC, Gistri R, Italiani G, et al. Coronary vasodilatation is impaired in both hypertrophied and non-hypertrophied myocardium of patients with hypertrophic cardiomyopathy: A study with nitrogen-13 ammonia and positron emission tomography. *J Am Coll Cardiol* 1991;17:879-86.

- 5 Misawa K, Nitta Y, Matsubara T, Oe K, Kiyama M, Shimizu M, et al. Difference in coronary blood flow dynamics between patients with hypertension and those with hypertrophic cardiomyopathy. *Hypertens Res* 2002;**25**:711-6.
- 6 Kawada N, Sakuma H, Yamakado T, Takeda K, Isaka N, Nakano T, et al. Hypertrophic cardiomyopathy: MR measurement of coronary blood flow and vasodilator flow reserve in patients and healthy subjects. *Radiology* 1999;**211**:129-35.
- 7 Olivetto I, Cecchi F, Gistri R, Lorenzoni R, Chiriatti G, Girolami F, et al. Relevance of coronary microvascular flow impairment to long-term remodeling and systolic dysfunction in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2006;**47**:1043-8.
- 8 Cecchi F, Olivetto I, Gistri R, Lorenzoni R, Chiriatti G, Camici P. Coronary microvascular dysfunction and prognosis in hypertrophic cardiomyopathy. *N Engl J Med* 2003;**349**:1027-35.
- 9 Cannon RO, Rosing DR, Maron BJ, Leon MB, Bonow RO, Watson RM, et al. Myocardial ischemia in patients with hypertrophic cardiomyopathy: contribution of inadequate vasodilator reserve and elevated left ventricular filling pressures. *Circulation* 1985;**71**:234-43.
- 10 Yamakado T, Okano H, Higashiyama S, Hamada M, Nakano T, Takezawa H. Effects of nifedipine on left ventricular diastolic function in patients with asymptomatic or minimally symptomatic hypertrophic cardiomyopathy. *Circulation* 1990;**81**:593-601.
- 11 Akasaka T, Yoshikawa J, Yoshida K, Maeda K, Takagi T, Miyake S. Phasic coronary flow characteristics in patients with hypertrophic cardiomyopathy: A study by coronary doppler catheter. *J Am Soc Echocardiogr* 1994;**7**:9-19.
- 12 Pichard AD, Meller J, Tiechholz LE, Lipnick S, Gorlin R, Herman MV. Septal perforator compression (narrowing) in idiopathic hypertrophic subaortic stenosis. *Am J Cardiol* 1977;**40**:310-4.
- 13 Kyriakidis MK, Dernelis JM, Androulakis AE, Kelepeshis GA, Barbetseas J, Anastasakis AN, et al. Changes in phasic coronary blood flow velocity profile and relative coronary flow reserve in patients with hypertrophic obstructive cardiomyopathy. *Circulation* 1997;**95**:834-41.
- 14 Tanaka M, Fujiwara H, Onodera T, Wu DJ, Matsuda M, Hamashima Y, et al. Quantitative analysis of narrowings of intramyocardial small arteries in normal hearts, hypertensive hearts, and hearts with hypertrophic cardiomyopathy. *Circulation* 1987;**75**:1130-9.
- 15 Schwartzkopff B, Mundhenke M, Strauer BE. Alterations of the architecture of subendocardial arterioles in patients with hypertrophic cardiomyopathy and impaired coronary vasodilator reserve: a possible cause for myocardial ischemia. *J Am Coll Cardiol* 1998;**31**:1089-96.
- 16 Krams R, Kofflard MJM, Duncker DJ, von Birgelen C, Carlier S, Kliffen M, et al. Decreased coronary flow reserve in hypertrophic cardiomyopathy is related to remodelling of the coronary microcirculation. *Circulation* 1998;**97**:230-3.
- 17 Pasternac A, Noble J, Streulens Y, Elie R, Henschke C, Bourassa MG. Pathophysiology of chest pain in patients with cardiomyopathies and normal coronary arteries. *Circulation* 1982;**65**:778-89.
- 18 Morrow AG, Reitz BA, Epstein SE, Henry WL, Conkle DM, Itscoitz SB, et al. Operative treatment in hypertrophic subaortic stenosis: techniques, and the result of pre- and postoperative assessment in 83 patients. *Circulation* 1975;**52**:88-102.
- 19 Wilson RF, Wyche K, Christensen BV, Zimmer S, Laxson DD. Effects of adenosine on human coronary arterial circulation. *Circulation* 1990;**82**:1595-606.
- 20 Di Mario C, Roelandt JRTC, de Jaegere P, Linker DT, Oomen J, Serruys PW. Limitations of the zero-crossing detector in the analysis of intracoronary Doppler: a comparison with fast Fourier transform of basal, hyperemic and transstenotic blood flow velocity measurements in patients with coronary artery disease. *Cathet Cardiovasc Diagn* 1992;**28**:56-64.
- 21 Doucette JW, Corl DP, Payne HP, Flynn AE, Goto M, Nassi M, et al. Validation of a Doppler guide wire for intravascular measurement of coronary artery flow velocity. *Circulation* 1992;**85**:1899-911.
- 22 Haase J, Di Mario C, Slager CJ, van der Giessen WJ, den Boer A, de Feyter PJ, et al. In-vivo validation of on-line and off-line geometric coronary measurements using insertion of stenosis phantoms in porcine coronary arteries. *Cathet Cardiovasc Diagn* 1992;**27**:16-27.
- 23 Maron BJ, Gottdiener JS, Epstein SE. Patterns and significance of distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy: A wide-angle, two-dimensional echocardiographic study of 125 patients. *Am J Cardiol* 1981;**48**:418-28.
- 24 Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986;**57**:450-8.
- 25 Rakusan K, Flanagan MF, Geva T, Southern J, Van Praagh R. Morphometry of human coronary capillaries during normal growth and the effect of age in left ventricular pressure-overload hypertrophy. *Circulation* 1992;**86**:38-46.
- 26 Turek Z, Hoofd L, Rakusan K. Myocardial capillaries and tissue oxygenation. *Can J Cardiol* 1986;**2**:98-103.
- 27 Marcus ML, Harrison DG, Chilian WM, Koyanagi S, Inou T, Tomanek RJ, et al. Alterations in the coronary circulation in hypertrophied ventricles. *Circulation* 1987;**75**:I-19-I-25.
- 28 Vassali G, Gallino A, Kiowski W, Jiang Z, Turina M, Hess OM. Reduced coronary flow reserve during exercise in cardiac transplant recipients. *Circulation* 1997;**95**:607-13.
- 29 Nitenberg A, Tavolaro O, Benvenuti, Loisanse D, Foulst JM, Hittinger L, et al. Recovery of a normal coronary vascular reserve after rejection therapy in acute human cardiac allograft rejection. *Circulation* 1990;**81**:1312-8.
- 30 McGinn AL, Wilson RF, Olivari MT, Homans DC, White CW. Coronary vasodilator reserve after human orthotopic cardiac transplantation. *Circulation* 1988;**78**:1200-9.