

Low-grade Inflammation and the Phenotypic Expression of Myocardial Fibrosis in Hypertrophic Cardiomyopathy

Johanna Kuusisto MD PhD¹, Vesa Kärjä MD PhD^{2*}, Petri Sipola MD PhD^{3*}, Ivana Kholová MD, PhD⁴,
Keijo Peuhkurinen MD PhD¹, Pertti Jääskeläinen MD PhD¹, Anita Naukkarinen PhD², Seppo Ylä-
Herttuala MD PhD⁴, Kari Punnonen MD PhD⁵, Markku Laakso MD PhD¹

Departments of Medicine¹, Clinical Pathology² and Clinical Radiology³, Kuopio University Hospital,
Kuopio, Finland and University of Eastern Finland, Kuopio, Finland; A.I. Virtanen Institute⁴,
University of Eastern Finland, Kuopio, Finland; Laboratory Center of Eastern Finland, Kuopio,
Finland⁵

*equal contribution

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Address for correspondence:

Johanna Kuusisto, MD, PhD
Kuopio University Hospital
Department of Medicine/ Center for Medicine and Clinical Research
Puijonlaaksontie 2
Post Office Box 1777
Kuopio FIN-70210
Finland
Tel: +358 17 173949
Fax: +358 17 173959
E-mail: johanna.kuusisto@kuh.fi

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Abstract

Objective - To investigate the role of inflammation in the phenotypic expression of myocardial fibrosis in hypertrophic cardiomyopathy (HCM).

Design - Clinical study.

Setting - Kuopio University Hospital and University of Eastern Finland, Kuopio, Finland.

Subjects: Twenty-four patients with a single HCM-causing mutation D175N in the α -tropomyosin gene and 17 control subjects.

Main outcome measures - Endomyocardial biopsy samples taken from the patients with HCM were compared to matched myocardial autopsy specimens. Levels of high-sensitivity C-reactive protein (hs-CRP) and proinflammatory cytokines were measured in patients and controls. Myocardial late gadolinium enhancement (LGE) in cardiac magnetic resonance imaging (CMRI) was detected.

Results - Endomyocardial samples in patients with HCM showed variable myocyte hypertrophy and size heterogeneity, myofiber disarray, fibrosis, inflammatory cell infiltration, and nuclear factor kappa B (NF- κ B) activation. Levels of hs-CRP and interleukins (IL-1 β , IL-1RA, IL-6, IL-10,) were significantly higher in patients with HCM compared to those in control subjects. In patients with HCM, there was a significant association between the degree of myocardial inflammatory cell infiltration, fibrosis in histopathological samples, and myocardial LGE in CMRI. Levels of hs-CRP were significantly associated with histopathological myocardial fibrosis. Hs-CRP, tumor necrosis factor α and IL-1RA levels had significant correlations with LGE in CMRI.

Conclusions - We demonstrated a variable myocardial and systemic inflammatory response in patients with HCM attributable to an identified sarcomeric mutation. Inflammatory response was associated with myocardial fibrosis, suggesting that myocardial fibrosis in HCM is an active process modified by inflammatory response.

Abbreviations and Acronyms

CMRI= cardiac magnetic resonance imaging

HCM = hypertrophic cardiomyopathy

hs-CRP = high-sensitivity C-reactive protein

IL-1 = interleukin-1

IL-6= interleukin-6

IL-10= interleukin-10

IL-1RA = interleukin-1 receptor antagonist

LGE = late gadolinium enhancement

LV= left ventricle, left ventricular

LVH =left ventricular hypertrophy

NF- κ B = nuclear factor kappa B

SI = signal intensity

TPM1-D175N = mutation D175N in the α -tropomyosin gene

TNF- α = tumor necrosis factor α

Introduction

Hypertrophic cardiomyopathy (HCM), most commonly caused by mutations in sarcomeric genes, is a myocardial disease characterized by left ventricular hypertrophy (LVH), myocyte disarray and myocardial fibrosis (1). The cardiac phenotype in HCM varies not only between unrelated individuals but also between family members with an identical disease-causing sarcomeric mutation, suggesting that HCM is a complex inherited disease modified by other genetic and environmental factors (1-5). The molecular events triggered by the genotype or other factors that induce the cardiac phenotype, particularly fibrosis, remain to be determined (4-6).

Previous studies have shown that chronic inflammation decreases myocardial contractility, induces hypertrophy and promotes apoptosis and fibrosis, thus contributing to myocardial remodelling (7-9). Inflammatory cytokines, tumor necrosis factor α (TNF- α), interleukin-1 (IL-1) and interleukin-6 (IL-6), are not constitutively expressed in the normal heart, but are up-regulated and produced as a stress response to myocardial injury or mechanical stress (8). TNF- α and IL-6 can directly attenuate myocardial contractility and induce the development of myocyte hypertrophy, collagen deposition and fibrosis (8). IL-1 β has been correlated with myocardial collagen deposition, cardiomyocyte apoptosis and inflammation (10). The expression of both inflammatory and anti-inflammatory cytokines is increased in heart failure (9). In addition, nuclear factor kappa B (NF- κ B), a transcription factor regulating inflammatory genes, has been shown to be activated in patients with heart failure of various etiologies (11).

Circulating inflammatory cytokines are elevated in HCM (12-14), but none of the previous studies has investigated the association of cytokine levels with cardiac phenotype determined by cardiac

magnetic resonance imaging (CMRI). Furthermore, there are no previous studies on NF- κ B activation in HCM. Even more importantly, there are little data on myocardial histopathological findings or inflammatory response in patients with HCM caused by a specific sarcomeric gene mutation. Therefore, to investigate the role of inflammation in the phenotypic expression of myocardial fibrosis in HCM, we investigated cardiac histopathology, inflammatory cytokine and hs-CRP levels, and cardiac phenotype by CMRI in patients with HCM attributable to an identical HCM-causing mutation D175N in the α -tropomyosin gene (*TPM1-D175N*).

Patients and Methods

The study protocol was approved by the Ethics committee of the Kuopio University Hospital. All subjects gave written informed consent. Consent to use cadaver myocardial samples was obtained from the Finnish Center for Legal Protection in Health Care. Data of endomyocardial biopsy and cytokine findings has not been published in any form. Previously, we have published studies on CMRI derived myocardial perfusion, myocardial LGE, myocardial contractile impairment, and inducibility of life-threatening arrhythmias in this same patient population (15, 16,2,17).

Patients with HCM

Twenty-four patients from five Finnish families (11 men, 13 women; mean age, 42 ± 13 years; age range, 17–68 years) with HCM attributable to the mutation D175N in the α -tropomyosin gene (*TPM1-D175N*) (18,19) were included in the study. In Finland, *TPM1-D175N* is one of the two founder mutations causing about 11 % of all and 25 % of familial HCM cases in eastern Finland (19). Of 24 subjects with *TPM1-D175N*, two did not meet the diagnostic criteria for clinical HCM,

and were regarded as healthy mutation carriers. All subjects with *TPM1-D175N*, however, are called HCM patients in the subsequent analysis. The study protocol described below was performed during one two-day visit at the Kuopio University Hospital.

Control Subjects

Seventeen healthy volunteers not related to patients with HCM and without a previous cardiac disease or medications, and with race, gender and age similar to the HCM patients, were included into our study. Study protocol was identical to that of the patients with HCM, except for cardiac catheterization and endomyocardial biopsy.

For histopathological analyses, control myocardial specimens were obtained from 20 age, gender and race matched cadavers without known cardiac disease (no history of a cardiac disease, normal macroscopic myocardial findings, and no clear evidence of a specific myocardial disease in microscopic examination).

Clinical and Echocardiographic Evaluation

All patients with HCM and control subjects underwent an interview, physical examination, 12-lead ECG recording, and echocardiography as previously described (2, 15-17).

CMRI Protocol

CMRI cine, perfusion and LGE imaging were performed in patients with HCM and controls as previously described (2, 15, 16). CMRI cine imaging was performed in all patients, perfusion imaging in 17 HCM patients, and LGE imaging in 22 HCM patients.

CMRI Image Analysis

Left ventricular (LV) characteristics and myocardial perfusion by CMRI were evaluated. LGE image analysis was performed in LV short-axis images at the levels of tips of the mitral valve leaflets and papillary muscles (16). In statistical analyses, the maximal value of the six segmental LGE heterogeneity values was used. Figure 1 shows LGE images of one control subject and three patients with HCM.

Coronary Angiography

Coronary angiography was performed on 21 of 24 HCM patients using standard angiographic techniques by the same cardiologist (J.K.).

Endomyocardial Biopsy and Autopsy Myocardial Samples

Myocardial specimen for histological analysis and immunohistochemistry were obtained from 20 patients with HCM. Endomyocardial biopsies were obtained under fluoroscopic guidance from the right ventricle side of the interventricular septum with the standard endomyocardial biptome. Control cadaver samples were selected from the archives of the Department of Clinical Pathology of the Kuopio University Hospital. Representative myocardial routine autopsy specimens from the anterior, posterior or septal wall of the heart were taken from 20 matched cadavers.

Histological and Immunohistochemical Methods

Histological methods and immunochemistry analyses are described in detail in Supplementary information.

Laboratory Determinations of Cytokines and hs-CRP

Plasma concentrations of TNF- α , IL-6, IL-10, IL-1 β and IL-1RA were measured using assay kits from R&D Systems (Minneapolis, USA). Hs-CRP was measured using an Immulite analyzer and a DPC High Sensitivity CRP assay (DPC, Los Angeles, CA) in all 24 patients with HCM and in 17 controls.

Statistical Analysis

Data are given as mean \pm SD. Statistical analyses were performed with a statistical software package (SPSS Win 11.5., SPSS Inc., Chicago, IL, USA). Because of skewed distribution, LV mass and all cytokines were analyzed after logarithmic transformation. The differences between the patients and controls were assessed by Student's *t*-test. Mann-Whitney test was used to investigate the association between endomyocardial findings and proinflammatory cytokines. Pearson's correlation coefficients were calculated to investigate the association of cytokines with LGE in CMRI. Spearman's correlation coefficient was used to investigate the association of different **histopathological** features, the association between histopathological inflammation and fibrosis, and between histopathological inflammation/fibrosis and LGE in CMRI.

Results

Clinical, Echocardiographic and CMRI Characteristics

Clinical, echocardiographic and CMRI characteristics of the patients with HCM and controls have been published before (2, 15-17) and are summarized in Table 1. Patients with HCM had stable mild to moderate symptoms (90 % of patients had NYHA functional class I-II). None of the subjects with HCM had a history or clinical symptoms or signs of **decompensated heart failure**, myocarditis, systemic infection, chronic inflammatory disease, or life-threatening arrhythmias.

None of the patients had intracardiac defibrillator. About one third of the patients used cardiac medication, mostly β -blocking agents. **None of the patients was taking ACE inhibitors or AT1 receptor antagonists, or medication for heart failure.**

None of the subjects with HCM had a significant LV outflow tract obstruction (>30 mmHg) at rest. In CMRI, LV maximal wall thickness was increased in HCM patients compared to controls. No difference was seen in global LV ejection fraction between patients with HCM and control subjects, but the number of hypokinetic segments was increased in patients with HCM compared to controls (2). The left ventricular perfusion reserve was lower and the maximal LV LGE increased in patients with HCM compared to controls (15, 16).

Coronary Angiography in patients with HCM

HCM patients had normal coronary arteries except for one patient with HCM, who had <50% stenosis in the left anterior descending coronary artery, and another patient who had <50% stenosis in the left anterior descending, intermediate and right coronary arteries.

Histological Findings in Endomyocardial Samples

Table 2 shows the histopathological findings in hematoxylin-eosin stained endomyocardial samples in patients with HCM. Sufficient endomyocardial biopsy samples for histology were available for 16 of 20 patients with HCM. Histological samples showed variable amounts of heterogeneity of myocyte size, myocyte hypertrophy, myofiber disarray, myocardial fibrosis, inflammatory cell infiltration and intramyocardial small artery narrowing. Interstitial and perivascular fibrosis was found in about 90% of cases. Inflammatory cell infiltration, including mainly mononuclear inflammatory cells and eosinophilic granulocytes, was found in 37 % of the

patients. Narrowed intramyocardial small arteries were found in one fourth of the cases. Figures 2 A-C show typical, mild and marked histopathological findings in patients with HCM, respectively.

In control cadaver myocardial samples, mild heterogeneity of myocyte size was found in one of 20 specimens, mild myocyte hypertrophy in 7 samples, mild myofiber disarray in 1 sample, mild interstitial fibrosis in 5 samples, mild inflammatory cell infiltration of mononuclear cells in one sample, and eosinophilic granulocytes and intramyocardial small artery narrowing in none of the samples.

Immunohistochemical Findings in Endomyocardial Samples

Using immunostaining with rabbit anti-human antibody, 7 of 11 (63%) patient endomyocardial biopsy samples studied showed CD3 positivity implicating T-lymphocytes (2 with moderate and 5 with weak positive staining). We could, however, replicate the CD3 positivity with NCL-CD3-PS1 in only one patient endomyocardial biopsy sample showing marked inflammatory response (Figure 2Cc). In 20 control cadaver myocardial samples, weak CD3 positivity with rabbit antihuman antibody was found in 2 samples, and CD3 positivity with NCL-CD3-PS1 in none of the samples. B-lymphocytes with M755 staining were not found in cases or controls. Occasional macrophages with MO814 were found in one patient sample only.

Picrosirius collagen staining was positive in 12 of 15 HCM samples. Extensive fibrosis was found in 2 cases (Figure 3), moderate fibrosis in 4 cases and mild fibrosis in 6 cases. In 20 control cadavers, mild fibrosis in Picrosirius staining was found in 2 cases.

NF- κ B nuclear positivity was detected in 8 of **15 patient endomyocardial biopsy samples**. NF- κ B nuclear activity was found in cardiomyocytes in 4 cases (3 cases showed NF- κ B positivity in 20-50% of nuclei, 1 case in 5% of nuclei) (Figure 4). In 3 other cases, nuclear positivity of NF- κ B was found in inflammatory cells. Endothelial NF- κ B nuclear positivity was found in 1 case. In control cadaver myocardial specimens, no nuclear NF- κ B positivity was detected.

Cytokines

Levels of hs-CRP, IL-1 β , IL-1RA, IL-6 and IL-10 were significantly higher in patients with HCM than in control subjects (Table 3). There was a trend towards higher TNF- α levels in patients with HCM compared to control subjects (P=NS).

Associations between Histopathological Myocardial Fibrosis, LGE and Inflammatory Response

In patients with HCM, the degree of histopathological myocardial fibrosis significantly correlated with LGE in CMRI ($r=0.568$, $p=0.034$). The grade of myocardial inflammatory cell infiltration correlated with fibrosis in histopathological samples ($r=0.614$, $p=0.011$), and with LGE in CMRI ($r=0.541$, $p=0.046$).

Levels of hs-CRP were significantly associated with histopathological myocardial fibrosis ($p<0.05$).

All other cytokine levels tended to be higher in patients with moderate or marked histopathological findings compared to those with no or mild findings (P=NS, data not shown).

Cytokine levels did not correlate significantly with NF- κ B activation (data not shown).

Levels of hs-CRP, TNF - α and IL -1RA significantly correlated with maximal LGE in patients with HCM (Table 4). There were no significant associations with IL-1, IL-6, IL-10, and CMRI derived

maximal LGE. There **were** no significant associations of cytokine levels with CMRI derived maximal LV thickness, LV mass, LV diastolic or systolic volumes, global ejection fraction, or myocardial perfusion (data not shown).

Discussion

Our study demonstrates that a low grade myocardial and general inflammatory response is present in HCM attributable to a single well-documented causative sarcomeric mutation (*TPM1-D175N*). Variable low grade myocardial inflammation in the patients with HCM was indicated by the presence of myocardial inflammatory cell infiltration and enhanced nuclear NF- κ B activity in the myocardium, and by increased levels of hs-CRP and circulating inflammatory cytokines. Myocardial inflammatory cell infiltration and levels of hs-CRP significantly correlated with **histopathological** myocardial fibrosis and LGE in CMRI, and TNF - α and IL -1RA correlated with LGE, suggesting that myocardial fibrosis in HCM may be an active process modified by inflammatory response.

Potential pathogenic mechanism for myocardial fibrosis formation in HCM

Based on our findings we suggest a pathogenic mechanism for myocardial fibrosis formation in HCM. We propose that myocardial fibrosis in HCM is likely to be an active process, in which primary injury, e.g mechanical stress (20), due to disorganized sarcomeric and cellular architecture (21), myocardial ischemia (22), or neuroendocrinological activation (7) induces NF- κ B up-regulation in the myocardium. NF- κ B, in turn, activates production of proinflammatory cytokines, inflammatory cell invasion into the myocardium, and activation of fibroblasts finally leading to myocardial fibrosis (11, 7, 8).

Histopathological myocardial phenotype and the causative mutation in HCM

Endomyocardial samples in our patients with HCM showed variable amounts of myocyte hypertrophy, myocyte size heterogeneity, myofiber disarray, myocardial fibrosis, low grade inflammatory cell infiltration, and intramyocardial small artery narrowing. Transgenic mice carrying a single missense mutation in codon 403 of the myosin heavy chain gene exhibit variable amounts of histopathological hypertrophy, myocyte disarray, fibrosis, and susceptibility to induced arrhythmias (23). In humans, only limited information on histopathological myocardial findings in patients with HCM attributable to an identified sarcomeric mutation has been published (24). As histopathology appears to vary to a great extent as well in humans as in transgenic animals with identical disease-causing mutations, other factors than the causative mutation necessarily contribute to histopathology in HCM.

Chronic myocardial inflammatory cell infiltration in HCM

Mild to marked interstitial and perivascular inflammatory cell infiltration of mononuclear inflammatory cells, **showing CD3 positivity in immunostaining with rabbit anti-human antibody**, and of eosinophilic granulocytes, was found in over one third of histological endomyocardial specimens of the patients with HCM. In one patient with severe HCM inflammatory cell infiltration was extensive (**Figure 2C**). In contrast, practically no inflammatory cells were recognized in control myocardial samples. Three previous studies have reported that there is mild chronic inflammatory cell infiltration in the myocardium of HCM patients (3, 25, 26), but none of them has included genotyped subjects.

Myocardial NF- κ B activity in HCM

A novel finding in our study was that immunohistochemical staining for nuclear NF- κ B activity was positive in half of the endomyocardial samples of patients with HCM. NF- κ B activation was detected particularly in cardiomyocytes, but also in inflammatory and endothelial cells. None of the control myocardial samples showed NF- κ B activation. NF- κ B is a pivotal intracellular mediator of inflammatory response, inducing the pro-inflammatory cytokine expression (11). NF- κ B activation has been previously implicated in cardiac dysfunction and heart failure (11). **NF- κ B activation leads to proinflammatory phenotype including up-regulation of TNF- α , which activates inflammatory cell invasion and fibroblasts, resulting in perivascular fibrosis formation in the myocardium (27).** Our finding of NF- κ B activation in the myocardium of patients with HCM supports the concept that inflammatory response may play an integral part in the phenotypic expression of myocardial fibrosis in HCM.

Pro-inflammatory and anti-inflammatory cytokines in HCM

In the present study, both pro-inflammatory and anti-inflammatory cytokines were elevated in patients with HCM. TNF- α levels have been reported to be increased in HCM in some (12, 14) but not in all previous studies (13). IL-6 levels have been shown to be elevated in HCM in two previous studies (13, 14). Decreased myocardial TNF- α expression has been reported after nonsurgical septal reduction in patients with obstructive HCM (28). There are, however, no previous studies evaluating systematically the levels of both proinflammatory and anti-inflammatory cytokines in patients with HCM, and particularly, there are no studies correlating levels of cytokines with LGE and other LV characteristics in CMRI.

Clinical implications of the association of inflammatory response with myocardial fibrosis in HCM

CMR derived LGE reflects collagenous scar formation in HCM (29). The notion that myocardial fibrosis is a potentially modifiable inflammatory process opens interesting clinical implications in preventing cardiovascular events in HCM, as myocardial fibrosis is a major determinant of malignant arrhythmias and end-stage systolic heart failure in HCM, and consequently, increases the risk of cardiac death (30,5).

Strengths and limitations of the study

We demonstrated for the first time, in a well-defined genotyped patient population with HCM, consistent signs of low-grade myocardial inflammation by serological, histopathological and immunohistochemical methods. We also showed that inflammatory response is associated with myocardial fibrosis, documented by histopathological methods and LGE in CMRI. Yet, there are some limitations in the present study. First, the patient population with HCM is of limited size.

Human studies including patients with genotype-verified diagnosis of HCM, especially those with a single causative mutation, are, however, few and generally small, as shown by recent studies (31, 32). Second, it is possible that the findings of the present study may not be applicable to all HCM patients with different causative mutations in sarcomeric genes. However, myocardial fibrosis is a common manifestation of the HCM, LGE presenting in 80 % of cases (1). Furthermore, according to current knowledge, no particular clinical HCM phenotype is mutation specific (5). Probably, the findings of the present study apply to HCM caused by other sarcomeric mutations as well, but confirmation of the findings of the present study in large genotyped patient populations is warranted. Third, in our CMRI method, severity and not the extent of LGE was measured. However, the method used in the present study has been regarded as scientifically valid and shown to be associated with serum amino-terminal propeptide of type III collagen (16). Fourth, cadaver myocardial specimens were used as controls and compared to endomyocardial biopsy

findings of patients with HCM, since it is unethical to obtain endomyocardial samples from healthy controls. **Fifth, there was a variable number of patient samples in different immunohistochemical analyses, because the second best endomyocardial biopsy sample designated for immunohistochemical stainings was not sufficient for all microscopic slides in every patient (see Supplementary information). Yet, the present study is, to our knowledge, the first human study to perform both endomyocardial biopsy and CMRI in genotyped subjects with HCM.**

Conclusions

In conclusion, we demonstrate that in patients with HCM, attributable to an identical disease-causing mutation in the α -tropomyosin gene, myocardial inflammatory cells and enhanced NF- κ B activity are present, and circulating cytokine and hs-CRP levels are increased. This inflammatory response is associated with myocardial fibrosis. Our findings suggest that a low grade inflammatory response plays a significant role in the phenotypic expression of myocardial fibrosis in hypertrophic cardiomyopathy.

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Authors' contributions

All authors have contributed to the article by participating in the design (JK, VK, PS,KPe,KPu,AN,ML,IK,SY), performance of genetic studies (JK, PJ, ML), clinical and echocardiographic studies (JK,KPe), magnetic resonance studies (PS,JK), histological and immunohistochemical studies (JK,VK,AN,IK,SY), laboratory measurements (KPu), drafting the manuscript and approving the final manuscript (all authors).

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Conflict of Interest: None declared

No additional data available.

Figure Legends

Figure 1. Contrast-enhanced T1-weighted inversion recovery images in (a) a 52-year-old control subject, (b) a 45-year-old male HCM patient, (c) 37-year-old female HCM patient , and (d) 19-year-old male HCM patient. Patients with HCM (b-d) show intramyocardial focal high signal areas (arrows) with increased segmental LGE.

Figure 2A. Typical histopathology in an endomyocardial biopsy of a patient with hypertrophic cardiomyopathy. (a) A general view, HE 200x; (b-c) moderate fiber disarray, interstitial fibrosis (stars), moderate myocyte size heterogeneity and hypertrophy, and scattered mononuclear inflammatory cells (arrows), HE 400x, 630x. **2B. Mild histopathological findings in a patient with hypertrophic cardiomyopathy.** (a) A general view, HE 200x; (b-c) mild fiber disarray, fibrosis (star), myocyte hypertrophy, and occasional mononuclear inflammatory cells (arrow), HE 400x, 630x. **2C. Severe hypertrophic cardiomyopathy.** (a) A general view: Weigert van Gieson staining highlights marked fibrosis (red, shown by star), 200x; (b) multiple mononuclear inflammatory cells (arrow); (c) showing CD 3 positivity in immunohistochemistry (staining in brown, arrow). Original magnification 400x. The patient had severe symptoms, marked LVH and inducible ventricular arrhythmia in ventricular stimulation. Intracardiac defibrillator was subsequently implanted.

Figure 3. Endomyocardial fibrosis in hypertrophic cardiomyopathy. Increased fibrosis is shown by Picosirius Red staining (in red, star). Cardiomyocytes are pale yellow stained. Original magnification 400x.

Figure 4. Nuclear NF- κ B activation is detected in cardiomyocytes (arrows) in hypertrophic cardiomyopathy. 400x.

Table 1. Clinical, echocardiographic and CMRI characteristics in control subjects and patients with hypertrophic cardiomyopathy

Characteristics	Control Subjects (n=17)	Patients with HCM (n = 24)
Men / Women	8 / 9	11 / 13
Age, y	38 \pm 12	42 \pm 13
V _{max} , m/sec	1.3 \pm 0.2	1.4 \pm 0.5
LV magnetic resonance imaging findings		
Maximal wall thickness, mm	9.7 \pm 1.7 [†]	19.5 \pm 4.9*
Mass, g	123 \pm 32	151 \pm 57
End-diastolic volume, ml	146 \pm 25	122 \pm 45*
End-systolic volume, ml	56 \pm 15	52 \pm 29
Hypokinetic segments, %	12 \pm 12	37 \pm 20*
Ejection fraction, %	61 \pm 7	58 \pm 7
Perfusion reserve	1.80 \pm 0.58	1.12 \pm 0.35 [†]
The heterogeneity of late-enhancement, %	12 \pm 3	18 \pm 11 [†]

V_{max} indicates maximum velocity in the Doppler signal from the jet in left ventricular outflow tract.

Data are means \pm SD.

* $P < 0.001$; [†] $P < 0.05$.

Table 2. Endomyocardial biopsy findings in patients with hypertrophic cardiomyopathy (hematoxylin- eosin staining)

	Number (%)
Heterogeneity in myocyte size	
No	3 (19%)
Moderate	6 (38%)
Marked	7 (44%)
Myocyte hypertrophy	
No	1 (6%)
Mild	11 (69%)
Moderate	4 (25%)
Myofiber disarray	
No	2 (13%)
Mild	6 (38%)
Moderate	6 (38%)
Marked	2 (13%)
Myocardial fibrosis	
No	2 (13%)
Mild	6 (38%)
Moderate	6 (38%)
Marked	2 (13%)
Inflammatory cell infiltration*	
No	10 (63%)
Mild	4 (25%)
Moderate	1 (6%)
Marked	1 (6%)
Intramyocardial small artery narrowing	
No	12 (75%)
Mild	2 (13%)
Moderate	1 (6%)
Marked	1 (6%)

*Eosinophilic granulocytes and mononuclear inflammatory cells.

Table 3. Circulating levels of cytokines in control subjects and in patients with hypertrophic cardiomyopathy

	Control Subjects (n=17)		Patients with HCM (n=24)	
	Mean \pm SD	Range	Mean \pm SD	Range
hs-CRP, mg/L	1.29 \pm 1.50	0.09 - 5.69	4.93 \pm 8.38*	0.33 - 38.90
TNF - α , pg/mL	2.33 \pm 1.22	1.38 - 6.43	3.24 \pm -2.98	1.56 - 16.50
IL-1 β , pg/mL	0.18 \pm 0.08	0.09 - 0.34	0.32 \pm 0.25 [†]	0.09 - 1.00
IL-1RA, pg/mL	216 \pm 76	101 - 391	490 \pm 577*	194 - 3023
IL - 6, pg/mL	1.11 \pm 0.72	0.38 - 2.80	2.15 \pm 1.67*	0.75 - 7.55
IL - 10, pg/mL	0.86 \pm 0.55	0.49 - 2.04	2.98 \pm 4.04 [‡]	0.49-20.86

hs-CRP, high sensitivity C-reactive protein; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin 1 β ;

IL- 1RA, Interleukin 1 receptor antagonist; IL-6, Interleukin 6; IL-10, Interleukin 10.

* P <0.01; [†] P < 0.05; [‡] P < 0.001.

TABLE 4. The association of circulating cytokine levels with myocardial maximal LGE heterogeneity at mid-ventricular level in patients with HCM (Pearson's correlation coefficients).

	LGE in CMRI
hs-CRP, mg/L	0.517*
TNF – α , pg/mL	0.486*
IL-1 β , pg/mL	0.032
IL-1RA, pg/mL	0.593 [†]
IL – 6, pg/mL	0.387
IL – 10, pg/mL	0.038

Abbreviations of cytokines as in Table 3.

Analysis is performed using log 10 transformed values.

* $P < 0.05$; [†] $P < 0.01$.