CRISTINA GAVINA

# DETERMINANTS OF LEFT VENTRICULAR HYPERTROPHY BEFORE AND AFTER SURGICAL TREATMENT OF AORTIC STENOSIS:

## CLINICAL, MORPHOFUNCTIONAL, GENETIC AND MOLECULAR CORRELATIONS

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## List of acronyms:

AS = Aortic stenosis
ASE = American Society of Echocardiography
AUC = Area under the curve
AV = Aortic valve
AVA = Aortic valve area
AVR = Aortic valve replacement
BNP = Brain natriuretic peptide
BSA = Body surface area
CKD = Chronic kidney disease
CVF = Collagen volume fraction
DM = Diabetes mellitus
EAE = European Association of Echocardiography
ECM = Extracellular matrix
EF = Ejection fraction
EOA = Effective orifice area
GFR = Glomerular filtration rate
HF = Heart failure
HT = Hypertension
LA = Left Atrium
LV = Left ventricle
LVH = Left ventricular hypertrophy
LVM = Left ventricular mass
MMP = Metalloproteinase
NYHA = New York Heart Association
PIP = Procollagen type I carboxy-terminal peptide
PPM = Patient prosthesis mismatch
ROC = Receiver operating characteristic
RWT = Relative wall thickness
SAP = Systolic arterial pressure
SAVR = Surgical aortic valve replacement
SV = Stoke volume
TAVI = Transcatheter aortic valve implantation
TIMP = Tissue inhibitor of MMP
Zva = Valvuloarterial impedance

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*Success is not final, failure is not fatal:*
*it is the courage to continue that counts.*

***— Winston Churchill***

*To my mother Elisabete, who inspired this work*

# 1

# ABSTRACT / RESUMO

# 1. ABSTRACT / RESUMO

Aortic stenosis is the most common type of valvular heart disease and its recent increase in prevalence is related to aging. The progressive reduction in aortic valve area imposes a chronic systolic pressure overload to the left ventricle (LV). In response, the LV hypertrophies in an attempt to normalize the increased wall stress, and changes in cardiomyocytes and extracellular matrix connective tissue occur, being some of them irreversible. This helps to explain the deterioration of diastolic and systolic function that takes place after longstanding overload. At histopathological level, LV fibrosis has been implicated in the progression to heart failure and has been associated with lower long-term survival rates. Indeed, late outcome after AVR depends mainly on the stage of myocardial disease before surgery, besides prosthetic related complications, and co-morbidities.

However, the LV hypertrophic response is not uniform under similar degrees of stenosis and its regression after surgical correction is variable, suggesting that non-hemodynamic factors can help to explain these differences. This work intended to better understand LV hypertrophy in aortic stenosis, find load-independent factors that could influence hypertrophic response variability, and identify markers of more advanced myocardial disease with potential impact in clinical outcomes.

For that purpose, we followed for eight years a cohort of 132 patients with isolated severe aortic stenosis, referred for aortic valve replacement at Centro Hospitalar S. João, between January 2006 and December 2009. Comprehensive clinical and echocardiographic characterization was done before and after surgery. In a subgroup of 56 patients myocardial biopsies were also performed at the time of valve replacement.

We have found that an excessive LV hypertrophy, by LV mass predicted according to load, gender and height, is associated with a maladaptive response, with a more advanced form of myocardial disease and worse prognosis. Likewise, the lack of normalization of LV mass after surgery helps to identify patients with worse long-term prognosis. Specific subgroups of patients, such as women and hypertensive were particularly vulnerable to this maladaptive response.

Some specific subgroups of patients are particularly vulnerable to this maladaptive response, such as women and hypertensive patients. At the structural and molecular level, we have described differences in ECM remodeling and higher degrees of interstitial fibrosis in those with impaired reverse remodeling and those with worse outcomes. Moreover, we found more severe preoperative fibrosis and evidence of a specific ECM remodeling in women, which raises the hypothesis that ECM may be a determinant of gender differences in LV remodeling and may justify gender-specific therapeutic interventions.

Early surgery and the use of pharmacological anti-fibrotic therapies after valve replacement, particularly in patients with non-invasive evidence of preoperative fibrosis and maladaptive response, might help to improve prognosis in aortic stenosis.

A estenose aórtica é a doença valvular mais frequente no mundo ocidental e a sua crescente prevalência está associada ao envelhecimento populacional. A progressiva redução da área valvular aórtica leva à sobrecarga crónica de pressão sobre o ventrículo esquerdo, sendo que a resposta hipertrófica resulta de uma tentativa de normalização do stress de parede para preservar a contractilidade miocárdica. Concomitantemente, ao nível histológico e molecular, ocorrem alterações nos cardiomiócitos e matriz extracelular, com hipertrofia miocitária e fibrose intersticial, por vezes irreversíveis, que, a longo prazo, levam à deterioração da função diastólica e sistólica, comprometendo a sobrevida. De facto, um dos principais determinantes do prognóstico após substituição valvular aórtica é a gravidade do atingimento miocárdico, para além das comorbilidades e das complicações relacionadas com a prótese.

No entanto, a resposta hipertrófica para níveis semelhantes de sobrecarga na estenose aórtica não é uniforme e há grande variabilidade na regressão de massa após cirurgia, sugerindo que este processo depende também de factores independentes da carga. Este trabalho pretendeu melhorar a compreensão da resposta hipertrófica na estenose aórtica, encontrar determinantes da hipertrofia ventricular, e identificar potenciais marcadores de doença miocárdica mais avançada, com potencial impacto nos eventos clínicos.

Foi estudada uma coorte prospectiva de 132 doentes com estenose aórtica grave isolada e indicação para cirurgia de substituição valvular aórtica. Os doentes foram selecionados de Janeiro de 2006 a Dezembro de 2009 e seguidos durante 8 anos. Dados clínicos e ecocardiográficos foram recolhidos antes e após substituição valvular aórtica. Num subgrupo de 56 doentes foram recolhidas biopsias miocárdicas durante a cirurgia.

Os nossos resultados mostraram que doentes com hipertrofia inapropriada e excessiva para aquilo que seria esperado de acordo com a carga, o sexo e a altura, apresentam uma resposta inadequada com uma forma mais grave de atingimento miocárdico e pior prognóstico. Para além disso, a hipertrofia residual após cirurgia, provavelmente um indicador de doença irreversível, identifica um subgrupo de doentes com pior prognóstico.

Alguns doentes são particularmente vulneráveis a este tipo de resposta, como as mulheres e os hipertensos, e podem merecer tratamento diferenciado. O nosso grupo descreveu diferenças na remodelagem da matriz extracelular e fibrose no sexo feminino, levantado hipóteses sobre os mecanismos responsáveis pelas diferenças entre sexos na remodelagem ventricular com potenciais consequências no tratamento.

A indicação de cirurgia mais precoce e o uso de terapêutica farmacológica com efeito anti-fibrótico após o alívio da carga, em particular em doentes com evidência não invasiva de fibrose e resposta inadequada, poderão contribuir para melhorar o prognóstico na estenose aórtica.

# 2

# INTRODUCTION

## Left Ventricular Hypertrophy in Isolated Aortic Stenosis: Primetime for the Ventricle

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# Left Ventricular Hypertrophy in Isolated Aortic Stenosis: Primetime for the Ventricle

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**Abstract:** Aortic stenosis is the most common type of valvular heart disease and its recent increase is related to aging. The decreased aortic valve area imposes a chronic systolic pressure overload to the left ventricle. In response, the ventricle hypertrophies in an attempt to normalize the increased wall stress, but this response is not uniform in patients with similar degrees of stenosis and its regression after surgical correction is variable, suggesting that several factors, other than load, can explain these differences. These findings are particularly important since the presence of left ventricular hypertrophy after aortic valve replacement is an independent predictor of worse outcome, probably because it indicates irreversible remodeling. Age, gender, hypertension, patient-prosthesis mismatch and interstitial remodeling also play an important role in this setting, raising the possibility of intervention beyond valve replacement. The possibility of combining estrogen treatment, antihypertensive agents, antioxidants and modulators of the renin-angiotensin-aldosterone system with surgical treatment to promote reverse remodeling is very appealing. On the other hand, a preventive strategy to intervene earlier in patients with significant left ventricular mass and avoid patient-prosthesis mismatch, especially in the younger and those with systolic dysfunction, can have a significant impact on prognosis. Further evidence, with well designed clinical trials, is needed but the spotlight must be in the ventricle, not the valve.

**Keywords:** Left ventricular hypertrophy, isolated aortic stenosis, aortic valve replacement, remodeling, prognosis.

## INTRODUCTION

Aortic stenosis (AS) is the most prevalent of all valvular diseases in developed countries. Its increase has a direct relation with population aging. In the Cardiovascular Health Study, in which 5201 men and women older than 65 years were analyzed, 26% had aortic sclerosis while 2% had frank stenosis [1].

Once considered a degenerative process, it is now believed to be similar to atherosclerosis, sharing the same risk factors and presenting evidence of active inflammation [2,3].

Progressive narrowing of the aortic orifice, to less than half of its usual size, leads to a significant pressure overload to the left ventricle (LV). The onset of symptoms like heart failure, syncope and angina, mark the decline in prognosis and, together with LV dysfunction, is the major determinant for aortic valve replacement [4,5]. Indeed, treatment of AS has been a mechanistic one, based on the relief of pressure overload. Only recently has attention been directed to the valve, considering the possibility of prevention of progression with drugs used in atherosclerosis, namely statins [6]. Small retrospective and prospective trials have shown that statins could delay the progression of AS [6,8], but large prospective studies have not confirmed these findings [9,10]. No specific medical therapy is recommended for protection of the ventricle before or after aortic valve replacement (AVR), except if there is systolic dysfunction or hypertension.

## LEFT VENTRICULAR HYPERTROPHY IN PRESSURE OVERLOAD DUE TO AS

In chronic pressure overload states, like systemic hypertension (HT) and AS, the LV responds with hypertrophy and altered geometry as an adaptative mechanism that helps to maintain contractile performance despite abnormal loading conditions. LV hypertrophy (LVH) allows for normalization of systolic wall stress and has been considered as compensatory [11,13], but it is also associated with impaired coronary blood-flow reserve [14,15] and changes in cardiomyocytes and extracellular matrix connective tissue, some of them irreversible. The later might explain the deterioration of diastolic and systolic function that take place after longstanding overload [16].

In epidemiological studies and in landmark hypertension clinical trials the increase in indexed left ventricular mass [17-20] is accompanied by an increase in cardiovascular events and overall mortality. In aortic stenosis, preoperative LVM has been identified as a strong predictor of poor outcome after AVR [21-24]. Increased preoperative LVM index is a strong independent predictor of operative morbidity and in-hospital mortality, with a striking incidence of low cardiac output syndrome despite normal ejection fraction [22, 24]. These patients are mostly older women and typically have a concentric remodeling with a marked increased in relative wall thickness and small LV cavity, associated with a supranormal systolic function [21]. With the sudden unloading of

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the LV after AVR some develop abnormal intra-cavitary flow acceleration and hypotension. The use of intra-aortic ballon pump and inotropics increase contractility and wor sens the intra-ventricular gradient, resulting in a low cardiac output syndrome, the main cause of death in this subgroup [22]. Hypotension in this setting should be treated with volume and alfa agonists, avoiding the routine use of positive inotropic support.

Several mechanisms can explain the association between preoperative LVH and morbimortality after AVR: (1) effective cardioprotection of the hypertrophied LV during ischemic arrest is challenging and can lead to perioperative ischemia, (2) diminished coronary flow reserve, even in the presence of normal epicardial coronary arteries [14,15], (3) transient exacerbation of diastolic dysfunction due to superimposed ischemia [25], (4) propensity for cardiac arrhythmias [26].

But not all ventricles respond with the same degree of LVH under identical loading conditions [27,28]. Severity of left ventricular hypertrophy in the presence of aortic stenosis is multifactorial and not affected only by the hemodynamic severity as assessed by aortic valve area or pressure gradient estimation. Patient age, gender, systolic blood pressure, ventricular function and genetics should also be considered when evaluating the degree of left ventricular hypertrophy in these patients [28-33].

About 10 % of patients with severe AS do not have LVH [34]. This challenges the paradigm that hypertrophy is needed to maintain wall stress and contractility. Experimental studies in animal models of aortic stenosis have shown that LVH is not necessary to maintain LV function [35,36] and that deficient hypertrophy can, in fact, prevent rather than promote systolic dysfunction. In a mouse model of cardiorestricted deficiency in Gq signaling with blunted hypertrophic response, after 1 week of aortic constriction control mice showed completed normalization of wall stress, while gene modified mice were unable to reduce wall stress. After 8 weeks, control mice had an increase in chamber dimension and progressive deterioration of LV function, whilst mice with a blunted hypertrophy and persistent elevation of wall stress had only limited LV dilatation and preserved LV function [36]. Similar findings were observed after inhibition of calcineurin-NFAT pathway [37]. These findings suggest that the LV was better off "stressed out" than being hypertrophied. In a prospective cohort of patients with isolated AS, increased LV mass (LVM) alone predicted systolic dysfunction and heart failure, regardless of the severity of the obstruction [38]. Even in patients with critical AS (valvular area < 0,4 $cm^2/m^2$), one fifth did not have LVM increase and had better preserved ejection fraction and less heart failure when compared to those with increased LVM [38]. But the lack of LVH does not imply persistent elevation of LV wall stress. Patients without LVH had, nevertheless, concentric remodeling with augmented relative wall thickness (RWT) due to small LV cavities in response to pressure overload. LV ejection fraction was related directly to RWT and inversely with LVM, raising the idea that concentric LV remodeling may be the key compensatory mechanism in AS, and multifactorial LVH a maladaptive response. More recently, concentric hypertrophy and concentric remodeling had similar early mortality risk after AVR and both were associated with worse outcomes than nonconcentric remodeling [39]. Unfortunately, since there was no data on LV wall stress, the paradigm that wall stress normalization is needed for maintaining LV function has yet to be clinically tested.

In a single-center observational study involving more than 3000 patients who underwent AVR with a single type of bioprosthesis, severe preoperative LVH (defined as LVM index ≥ 185 $g/m^2$), which preceded symptoms in 17% of patients, decreased long-term survival [40].

Given the clinical and experimental evidence of increased morbimortality in patients with higher LVM index, the indication for early AVR in asymptomatic patients should take into consideration significant LVH. So far, recent guidelines advice AVR in asymptomatic patients with severe AS if there is rapid disease progression, very severe stenosis or abnormalities in exercise testing [5, 41]. Only the European guidelines refer to excessive LV hypertrophy (≥15 mm), unless this is due to hypertension, as a class IIb indication for AVR [41].

More recently, much has been published regarding risk stratification in asymptomatic patients for early AVR. Proposed parameters include jet velocity, progression of valvular narrowing, response to exercise testing, comorbidity, abnormally raised biomarkers and ventricular dysfunction [42,43]. But one should also take into consideration significant LVH.

## RESIDUAL LEFT VENTRICLE HYPERTROPHY AFTER AVR

Aortic valve replacement increases long-term survival, which becomes similar to age-matched population, reduces symptoms and improves quality of life in patients with aortic valve stenosis [44,45]. Late outcome after AVR depends mainly on the stage of heart disease before surgery, prosthetic related complications, and co-morbidities. Late deaths after AVR are mostly due to sudden cardiac death and congestive heart failure [46]. Risk factors for poor post-operative outcomes include age, co-morbidities, severe functional limitation, irreversible myocardial damage such as a large myocardial scar, severe LV hypertrophy, more severe AS, ventricular arrhythmias, and untreated co-existing coronary disease [47]. In addition, poor post-operative outcomes may result from prosthesis-related complications or suboptimal prosthetic valve hemodynamic performance [48,49].

After successful AVR, LV pressure and wall stress are significantly reduced [50] and, as a consequence, LVH regression is expected. But since LV wall stress is not the only predictor of LVH, reduction of intraventricular pressure cannot solely predict regression after AVR. Significant LVM regression seems to occur as early as 5 days after AVR [51], is maximal after the first year, and has only non significant slight decrease after 18 months to 10 years [52-56]. Early regression of LVM reflects pressure unloading and is a consequence of reduction of LV diameters while wall thickness is maintained [57]. Additional regression of LVM occurs in the late period, mainly as a consequence of a reduction in wall thickness, probably as a result of normalization of car-

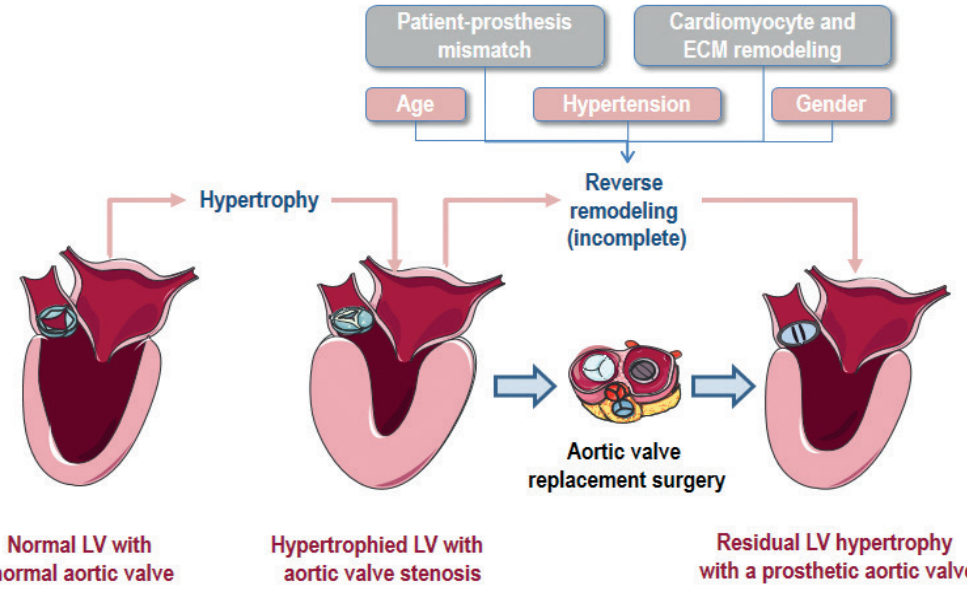


**Fig. (1).** Left ventricular hypertrophy and reverse remodeling before and after aortic valve replacement (AVR) surgery, respectively. The grey boxes show some factors that contribute to residual hypertrophy after AVR. LV – left ventricle.

diomyocyte hypertrophy and reduction of the fibrous content in the extracellular matrix [57,58].

Nearly half of patients with aortic stenosis have residual LVH late after surgery [16, 59]. This persistent increase in LVM is an independent predictor of cardiac-related morbidity [59] and mortality [46, 60].

The worse long-term outcome of patients with residual LVH after AVR can be explained by the existence of more extensive preoperative disease and persistent diastolic and/or systolic dysfunction [57, 61]. Indeed, an important independent predictor of incomplete regression of LVH is LVM before AVR [62,63]. These patients have more severe symptoms and worse pre and postoperative ventricular function [16]. At the histological level, these findings correlate with the degree of myocyte abnormalities (higher nucleus volume), higher muscle cell mass index and increased amount of fibrous tissue at the time of surgery, altogether suggesting the presence of irreversible remodeling [16].

Regression of LVH after AVR is mostly the result of pressure overload relief. Hemodynamic factors, such as type of valve and residual gradients, hypertension, are not the only determinants of incomplete regression. Age, gender, genetic polymorphisms and irreversible interstitial remode ling also play an important role in this setting.

## DETERMINANTS OF LEFT VENTRICULAR REMODELING AFTER AVR

### Age

Given the increase in calcific AS prevalence with age, most patients undergoing AVR are older than 65 years. In the large multicenter Cardiovascular Health Study, LVM increased with age but, after adjustment for body weight, the age effect was attenuated (less than one gram per year increase) [64]. Although this may be due to the highly narrow age range (all subjects were 65 years or older) these results are in accordance with those obtained in a healthy subgroup of 862 participants aged 18 to 79 years from the Framingham cohort (n=177 subjects) and offspring (n=685 subjects), where only minimal changes in LVM were noted with advancing age. Multivariate analyses in this normotensive, nonobese subgroup with no clinical evidence of cardiovascular disease revealed that age was not significantly associated with LVM in men and only weakly associated with LVM in women [65].

In conclusion, the relation of incomplete LVH regression after AVR with age is controversial. Some authors advocate that older patients have higher LVM index after surgery [55, 59] and speculate that older age could negatively affect collagen turnover and lead to less fibrous degradation with slower remodeling. Most studies, however, do not support this hypothesis and found no relation between age and indexed post-operatory LVM [62,63, 66].

### Gender

It has been long recognized that men have higher left ventricular mass when compared to women, even though these differences are attenuated after indexation to body surface area [67-69]. In epidemiological studies, ventricular mass increases with age in healthy women, whereas it remains constant in men [65]. In an autopsy series, which excluded primary cardiac pathology, LVM decreased in men but remained constant in women [68]. In women aging did not lead to myocyte cell loss or reactive hypertrophy, whereas in men there was a significant decrease in myocyte number associated with an increase in volume [68]. This

suggests an age-related involution of the heart in men, but LVH was not addressed in this study since hypertrophic hearts were excluded.

In pressure overload states, such as hypertension and aortic stenosis, many studies have reported different remodeling responses between sexes, with similar LVM index but higher prevalence of LVH in women, according to sex specific criteria [31, 70,71]. Women have smaller LV chamber size, higher relative wall thickness, higher LVM/volume ratio, supernormal LV function and less wall stress, while men exhibit ventricular dilatation and earlier ventricular dysfunction [31, 71-73]. These data in humans are consistent with animal studies showing higher depression of contractile reserve in male animals, despite similar magnitude of LVH and systolic load [74]. At molecular level, this translated into a greater expression of beta-myosin heavy chain and atrial natriuretic factor, as well as, depressed levels of SERCA-2a in male hearts compared to female [74], which could help explain the early signs of ventricular dysfunction.

The effect of gender-related differences in LVH regression is less well established. Some have found the association of female sex with more complete regression [48, 63], but others described similar normalization of LVM 1 year after surgery [62]. Male gender was found an independent predictor of regression after AVR with stentless bioprosthesis, but patients with previous myocardial infarction were not excluded and may have influenced results [75]. The clinical impact of gender-related LV geometry is still under debate since most authors failed to identify sex as an independent predictor of adverse outcome after AVR [23, 73, 76].

These distinctive LV remodeling responses to pressure overload can be explained by the effect of sex hormones. This is supported by evidence of the presence of myocardial estrogen and androgen receptors in animals and humans [74, 77]. In sinoaortic denervated rats, Cabral *et al.* have found that testosterone exerts a facilitator and estradiol an inhibitory action in the development of LVH [78]. Estrogens also seem to have antiproliferative effects on cardiac fibroblasts [79] and vascular smooth–muscle cells, while androgens have opposite effects [80]. Given that older patients have relative hypogonadal hormone concentrations, with a decrease in estrogens and ovarian production of androgens in postmenopausal women and decrease in androgens in men, there is a hypothetical explanation for the gender differences in LVM with aging. Hormone replacement therapy (HRT) in postmenopausal women seems to be associated with lower LVM [81-83] arguing in favor of this hypothesis.

The beneficial effect of estrogens at myocardial level is supported by the presence of functional estrogen receptors (ER) α and β in humans [84] and evidence of local synthesis in animals [85]. Estrogen receptors belong to the steroid hormone receptor superfamily, and estrogen binding has both genomic and nongenomic effects [86]. They can act as a transcription factor on downstream genes and also exert non genomic effects, inducing intracellular signaling cascades, such as activation of protein kinase C, extracellular signal-regulated kinase, and modulating signaling by growth factors such as insulin-like growth factor (IGF)-1, epidermal growth factor or transforming growth factor [87]. In patients with aortic valve stenosis, Nordmeyer *et al.* have shown that ER α and β expression is increased in comparison with controls, and found an inverse correlation between ER β and calcineurin A-β expression [88]. The isoform calcineurin A-β mRNA of the phosphatase calcineurin is particularly relevant for the development of cardiac hypertrophy since isolated knockout of this gene prevents the development of an hypertrophic response [89]. These findings suggest an association between the increase in ER β and the suppression of the calcineurin-induced hypertrophic stimuli, although the underlying mechanism is not yet completely understood.

The possibility of combining estrogen treatment with antihypertensive and surgical treatment to prevent/regress LVH is very appealing. Further evidence with well designed clinical trials is needed.

**Hypertension**

When investigating hemodynamic factors that influence LVM regression after AVR, most studies focused solely on pressure gradient and valve related parameters. But the trigger stimulus for LVH is not pressure gradient itself but the elevated LV pressure, which depends also of systemic blood pressure.

Around 30-60% of patients with aortic stenosis are hypertensive [33, 90]. Data on systemic blood pressure before and after AVR is absent in most studies on incomplete LVH regression and only recently some authors have reported its relation to postoperative LVM [55, 66, 91]. In a retrospective observational study with 79 patients with pure aortic stenosis, all underwent valve replacement with bileaflet mechanical values. The only independent predictors of postoperative LVM index were preoperative LVM and postoperative systolic blood pressure (defined as normal if < 130 mmHg) [91]. None of the prosthesis related variables had significant influence, although it is controversial if indexed orifice area provided by manufacturers should be used as an indicator of patient-prosthesis mismatch [91]. Uncontrolled hypertension is not only related to higher LVM after AVR but also with worse survival, with higher incidence of heart failure and bleeding as causes of death [66].

Blood pressure control, which can be reduced more effectively than transprosthetic gradients, has a significant impact in LVM regression. This can be particularly important in patients with large residual pressure gradients and severe LVH.

**Patient-Prosthesis Mismatch**

The immediate expected result of AVR is the reduction of transvalvular pressure gradients (TPG) with improvement in afterload. This is thought to allow for normalization of LVM and function, but the impact of hemodynamic variables on the extent of LVM regression is controversial. In the Strong Heart Study, in American Indians demographic and hemodynamic factors could account for only about half the LVM variance [92].

Patient-prosthesis mismatch (PPM) was first described in 1978 by Rahimtoola as being present when the effective prosthetic valve area was less than that of a normal human valve [93]. This is a very broad concept and it is well known that most of the prosthetic valves are, at least, mildly steno-

tic. In a more restrict definition, it implies the existence of a smaller than needed effective orifice area (EOA) in relation to the patient's body surface area (BSA) and, therefore, cardiac output requirements. Given that TPG is directly related to the square of transvalvular flow and inversely related to the square of the valve area, PPM will ultimately result in persistent abnormally high TPGs [94] despite normal prosthesis function.

Several parameters have been used to define PPM. Internal geometric area (IGA) is a static manufacturer specification based in ex vivo measurement of the diameter of the prosthesis. It differs from one type of prosthesis to another, tends to overestimate the EOA, especially in bioprosthesis, and does not predict postoperative gradients or functional improvement after AVR [95-97]. In contrast, indexed EOA (patients EOA divided by BSA) is the only parameter that has consistently correlated with postoperative gradients [95] and has become the most used in recent studies. It has been demonstrated that, in order to avoid any significant gradient at rest and during exercise, the indexed EAO of a prosthetic valve should ideally be no less than 0.85 to 0.9 $cm^2/m^2$, under which gradients increase exponentially. The threshold for PPM in the aortic position is an indexed EOA $\leq$ 0.85 $cm^2/m^2$. Values between 0.65-0.85 $cm^2/m^2$ are considered moderate PPM and those < 0.65 cm2/m2 as severe PPM [98]. Thus, the objective of AVR should be to ensure an indexed EOA above these levels to avoid residual stenosis. Even so, the prevalence of moderate PPM varies from 10-70% and that of severe mismatch from 2-28%, reflecting great heterogeneity in these studies [99]. The high number of patients with PPM reported result, most of the times, from surgical difficulties in implanting adequate sized prosthesis in small and severely calcified aortic roots.

There has been great controversy regarding the impact of PPM on postoperative outcomes. Several studies reported that PPM is an independent predictor of cardiac events and early and late mortality after AVR [100-108], while others failed to demonstrate a significant impact on outcomes [97, 109-113]. These apparently contradictory results may be explained by differences in baseline characteristics of patient populations included, valve substitutes used and compared, parameters for defining mismatch (indexed IGA vs indexed EOA) and different surgical approaches. Theses discrepancies make it impossible to compare results or perform meta-analysis.

Despite studies discrepancies, some consensus might be drawn from the existing evidence. First, severe PPM is a predictor of early and mid-term mortality irrespective of LV function [99-101, 108, 114], especially for patients under 70 years and body mass index below 30, and should be always avoided [101]. In moderate PPM evidence is weak for patients with normal ejection fraction, but in the presence of LV dysfunction it is clear that moderate mismatch is associated with increased operative and late mortality [99, 101, 103, 106]. This can be explained by the greater vulnerability of the failing heart to increased afterload, particularly in the immediate postoperative period.

When LVH regression after AVR is analyzed, some authors have found that persistent PPM results in less regression [48, 75, 107], but even patients with PPM or small prosthesis can have significant reduction in LVM, suggesting that PPM is not a determinant issue [115-117]. The extent of regression varies from patient to patient in the presence of PPM, and is largely dependent on the extent of EOA increase after AVR [49]. Given the curvilinear relation of indexed EOA and TPG, the degree of regression seems to be dependent on the original and final positions of an individual patient on the indexed EOA-gradient curve [98]. The extent of preoperative LVM is also important for regression in PPM. Fuster *et al.* have found that, in the group with higher LVM, only those with evidence of moderate or severe mismatch had impaired regression after AVR [107]. Moreover, PPM was not found as an independent predictor of mortality by itself, but was a promoter of the impact of LVM index in in-hospital mortality. In the presence of mismatch, increased LVM was the strongest predictor of mortality [107].

The possibility of avoiding PPM with the use of a preventive strategy at the time of surgery [96] makes it an important factor, particularly in younger patients (with higher basal metabolic rate and physically more active) [101, 105], in those with higher preoperative LVM and those with LV dysfunction.

### Cardiomyocyte and Extracellular Matrix Remodeling

The normal adult heart consists of highly differentiated parenchymal cells, cardiomyocytes, and stroma formed by the extracellular matrix (ECM), tissue fluid and undifferentiated multipotent mesenchymal cells. About one third of the cellular compartment is made of cardiomyocytes, the rest being mainly fibroblasts. The major ECM proteins are type I and III collagen, but type IV and V collagen, elastin fibers, proteoglycans and integrins are also present. Fibrillar collagen serves as a structural framework, connecting the contractile elements of adjacent cardiomyocytes to translate into ventricular pump function, and maintaining the alignment of the myofibrils within the myocytes through a collagen-integrin-cytoskeletal myofibril relation.

In the end of the seventies and early eighties, many stu dies established a relationship between myocardial structure and left ventricular function in aortic stenosis [118-120]. Pressure overload results more frequently in concentric hypertrophy, with an increase in relative wall thickness and mass, but with little or no change in chamber volume. In this pattern of remodeling myocyte hypertrophy (with addition of sarcomeres in parallel and lateral growth of individual cardiomyocytes) and perivascular and interstitial fibrosis are the histological hallmark [58, 121]. The rate of regression of LV hypertrophy after AVR is different with regard to the muscular and nonmuscular compartments of the LV [58, 120]. This behavior of the different structures of the myocardium influences systolic and diastolic function differently.

In AS, myocardial stiffness is increased before surgery when compared with controls, increases even further early after AVR, but tends to normalize late after surgery [120, 122]. It appears not to be influenced by left ventricular muscle mass or muscle fiber size, but by the presence of massive left ventricular interstitial fibrosis [121]. Interestingly, in contrast to diastolic heart failure (DHF) patients, myofilaments stiffness from AS patients was not increased [123]. The In DHF high $F_{passive}$ was positively correlated both with

hypertrophy and concentric remodeling [124], being corrected by *in vitro* administration of protein kinase A (PKA) acting directly on the phosphorylatable cytoskeletal protein titin [125]. This giant protein determines $F_{passive}$ through isoform shifts [126,127], phosphorylation status [125, 128] and via $Ca^{2+}$ interaction [129].

Postoperative changes in myocardial structure are characterized by an initial decrease in muscle fiber diameter and a relative increase in interstitial fibrosis, whereas total ventricular fibrous content remains unchanged [58, 120]. This could explain why myocardial stiffness is increased early after AVR [120, 122]. In a later phase, LVM regression continues more slowly, with no further change in muscle fiber diameter but an additional reduction in percentual fibrosis [58], thus allowing for the normalization of myocardial stiffness. Nevertheless the impact of fibrosis in myocardial stiffness is dependent on LV geometry, being particularly important in small ventricles with concentric hypertrophy [120].

In the progression to HF, there is a significant correlation between myocyte degeneration and fibrosis, and both have shown an inverse relation with ejection fraction [130,131]. With worsening of fibrosis, LVEDP increases and, later on, EF decreases. This suggests a close association between structure and function in aortic stenosis [131]. However EF may not be the best parameter to evaluate systolic function in AS, since it is mainly related to global radial function, which is reduced only in end-stage disease [132]. Fibrotic changes in AS hypertrophic hearts are initially subendocardial and affect basal segments (where regional wall stress is highest). This will impact mainly longitudinal function, which is not well represented by ejection fraction [133]. In a recent study, in patients with severe symptomatic AS, radial function was relatively preserved, even in patients with severe fibrosis, while mitral ring displacement, a surrogate of overall longitudinal function of the septum, was reduced in the presence of severe fibrosis. This finding is relevant once only parameters of longitudinal systolic function predicted functional improvement [133].

Fibrosis is an early morphological alteration in patients with AS and has been pointed as one of the reasons for impaired LVH regression after AVR [58]. It is a major determinant of diastolic and systolic dysfunction and it is one of the structural substrates for arrhythmogenicity, thus playing a major role for sudden death and the progression of HF [122, 131]. While myocyte hypertrophy is dependent on load, fibrosis seems also to be regulated by non-hemodynamic factors such as neurohormones [134].

Myocardial fibrosis is the result of both increased synthesis of collagen types I and III and unchanged or decreased extracellular collagen degradation [135-137].

Involved in the regulation of collagen turnover is a highly complex enzymatic system of proteolysis and antiproteolysis of the extracellular matrix (ECM). Matrix metalloproteinases (MMPs), the major proteolytic system, and their tissue inhibitors (TIMPs) are determinant for cardiac remodeling, and their activity varies in different cardiac diseases. Besides inhibiting MMPs, TIMPs seem to have direct profibrotic activity, stimulating collagen production by cardiac fibroblasts [138].

In pathological conditions, fibroblasts assume a profibrotic phenotype with transformation into myofibroblasts [139,140]. Myofibroblasts stain positive for smooth muscle alfa actine and, with the exception of heart valve leaflets, are not found in normal cardiac tissue. They have the ability of autocrine and paracrine regulation, producing growth factors, cytokines, ECM proteins and proteases [139-141].

In LVH associated with aortic stenosis, there seems to be an increased production of collagen and a shift towards inhibition of collagen degradation [136,137, 142]. When compared with controls, myocardial biopsies of aortic stenosis patients have higher expression of collagens and transcripts of collagen synthesis [137]. When evaluating the levels of MMPs and their inhibitors (TIMPs), there is an upregulation of TIMP 1 and 2 mRNA, which significantly related to the degree of fibrosis, while MMP 1, 2 and 9 mRNA did not differ significantly. Also the ratio between TIMP1/MMP2, TIMP2/ MMP2 and TIMP2/MMP9 were significantly increased in aortic stenosis patients, favoring inhibition of collagen degradation [137].

In animal models of hypertension, the progression from compensated LVH to LV dysfunction is accompanied by a change in the balance between MMPs and their inhibitors, now favoring MMPs activity [143]. This appears also to be the case in pressure overload due to aortic stenosis, where several MMPs levels (including MMP1 and 9) are progressively higher with decreasing EF, with insufficient increase in TIMP1 when compared to collagen type I and MMP1 increase [144]. Thus, collagen turnover in aortic stenosis is a highly dynamic process and varies depending on the degree of hypertrophy and dysfunction progression. Changes in MMPs and TIMPs expression can serve as early markers of disease progression in pressure overloaded myocardium. Also in reverse remodeling, the ECM seems to play an important role. Such has been demonstrated in several studies that described collagen shift from stiff collagen type I, which transient increases in early phase of reverse remodeling, to the extensible types III and VIII a few days later [83]. Other experimental studies have described total regression of MMP and TIMP gene expression as well as an association between changes in LVMI and MMP/TIMP gene expression after corrective surgical therapy and LV hypertrophy regression [84].

Several stimuli contribute to myocardial fibrosis, such as cytokines (TGF-β1, TNF-α, interleukin family), angiotensin II, aldosterone, endothelin-1 (ET-1) and catecholamines [141, 145]. The most important of these systems is the renin-angiotensin-aldosterone (RAA). Mechanical stretch induces local production of angiotensin II, which in turn stimulates the release of multiple growth factors and cytokines from cardiac fibroblasts that act in an autocrine and paracrine fashion, affecting the progression of hypertrophy and remodeling [146-148]. Although the paracrine factors released by cardiac fibroblasts are required for induction of cardiomyocyte hypertrophy, there is a crosstalk between both cell types, with cardiomyocytes being also crucial for fibroblasts regulation [149]. In an animal model of chimeric mice that expressed both AT1a receptor intact and null cells, after angiotensin II infusion, mice developed mild cardiac hypertrophy and fibrosis. Interestingly, most proliferating fibroblasts

were found around cardiomyocytes with intact AT1a gene, while fibroblasts adjacent to AT1a null cardiomyocytes showed a lesser degree of cell proliferation [149].

In isolated human cardiac fibroblasts, angiotensin II does not directly increase collagen or fibronectin expression [150], but it contributes to the phenotype switch into myofibroblasts, which is followed by the secretion of local mediators (TGF-β1, ET-1; IL-6; osteopontin) that induce collagen and tissue metalloproteinases production [141,150,151]. The main mediator of angiotensin II effects on ECM synthesis is the transforming growth factor β1 (TGF-β1) [150,152,153]. Angiotensin II upregulates TGF-β1 levels in human AS with normal and impaired systolic function, and this increase is correlated with the upregulation of ACE mRNA [136]. In animal models, an early increase in TGF-β1 mRNA precedes the increase in collagen and fibronectin mRNA, suggesting it mediates the profibrotic effect of angiotensin II [154,155].

Other signaling pathways, such as NOS uncoupling and oxidative stress, have been linked to LV hypertrophy progression. An experimental study has recently demonstrated that tetrahydrobiopterin (BH4) levels, a nitric oxide synthase (NOS) cofactor, decline in pressure-overload remodeling in conjunction with NOS uncoupling [156]. Additionally, its supplementation was able to recouple eNOS and reverse advanced hypertrophy/dilation more effectively than a less specific antioxidant Tempol. These data highlight the importance of myocyte NOS uncoupling in hypertrophic heart disease and support BH4 as a potential new approach to treat this disorder [157].

The understanding of the molecular mechanisms underlying LVH and fibrosis is relevant because it opens avenues for future intervention in order to prevent/regress these events. In a sheep model of supracoronary aortic banding and debanding, after development of LVH, there was a significant increase in ACE and AT1 receptor mRNA expression, with complete reversal of these changes after surgical treatment [158]. Also the inhibition of the angiotensin converting enzyme (ACE) has been shown to prevent the deve lopment of LVH in rats [159,160] and to reduce its extent in humans with pressure-overload hypertrophy [161,162]. Local inhibition of the cardiac renin-angiotensin system, achieved through the infusion of enalaprilat for 15 minutes into the left coronary arteries during coronary angiography, results in improvement in abnormal LV diastolic properties in patients with severe concentric pressure-overload hypertrophy due to AS. In contrast, in patients with nonischemic dilated cardiomyopathy, intracoronary enalaprilat does not affect LV relaxation, distensibility, or stiffness [163]. This is evidence of the importance of local RAA system for reverse remodeling in AS.

After AVR for aortic stenosis, additional medical therapy modulating the RAA system (ACE inhibitor or AT1 receptor antagonists) may be an attractive strategy to promote ventricular reverse remodeling.

## CONCLUSION

Residual LVH late after AVR, as a marker of irreversible myocardial disease, is responsible for increased cardiovascular morbidity and mortality. Given the ominous significance of residual LVH after AVR, its regression is an important endpoint. Early surgery could ensure a more complete hypertrophy regression and, therefore, improve long term survival. An effort should be made to ensure the conduction of prospective randomized clinical trials with medical treatment aimed to regression of LVH. It is time to look attentively at the ventricle.

## CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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## ABBREVIATIONS

| | | |
|---|---|---|
| AS | = | Aortic Stenosis |
| AVR | = | Aortic Valve Replacement |
| BSA | = | Body Surface Area |
| DHF | = | Diastolic Heart Failure |
| EOA | = | Effective Orifice Area |
| ER | = | Estrogen Receptors |
| ECM | = | Extracellular Matrix |
| HRT | = | Hormone Replacement Therapy |
| HT | = | Hypertension |
| IGF-1 | = | Insulin-like Growth Factor |
| IGA | = | Internal Geometric Area |
| LV | = | Left Ventricle |
| LVH | = | LV Hypertrophy |
| LVM | = | LV Mass |
| PPM | = | Patient-Prosthesis Mismatch |
| RWT | = | Relative Wall Thickness |
| TPG | = | Transvalvular Pressure Gradients |

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# 3

# AIMS

# 3. AIMS

Our aim was to achieve clinical, functional and molecular characterization of patients with isolated aortic stenosis before and after aortic valve replacement (AVR). In addition, we intended to better understand the prognostic importance of LV hypertrophy and determine its predictors.

For that purpose we established the following objectives:

1. Clinical characterization of patients with isolated aortic stenosis referred to AVR.
2. Evaluation of clinical outcomes after AVR.
3. Evaluation of echocardiographic parameters of left ventricular (LV) remodeling, before and after AVR.
4. Echocardiographic evaluation of systolic and diastolic function, before and after AVR.
5. Evaluation of left ventricular mass regression after AVR.
6. Evaluation of the changes induced by chronic cardiac pressure overload (aortic stenosis) in myocardial expression of genes related to neurohormoral regulation and extracellular matrix (ECM) remodeling.
7. Histologic evaluation of myocardial fibrosis induced by aortic stenosis, and its correlation with LV function and prognosis.
8. Determination of plasma biomarkers of extracellular matrix remodeling.

# 4

# METHODS

# 4. METHODS

## *Patient selection and follow-up*

Between January 2006 and December 2009 we included 141 consecutive patients over 18 years old with severe symptomatic AS (aortic valve area <1 $cm^2$ or mean transaortic gradient ≥40 mmHg) referred for AVR at the Cardiothoracic Surgery Department of Centro Hospitalar São João, Porto, Portugal. We excluded patients with aortic regurgitation >II/IV or other significant valve diseases (>mild), significant coronary artery disease (lesions >50% on coronary angiography) or previous cardiac surgery. All patients were in sinus rhythm at the time of inclusion. From the initial 141 patients included, 132 were considered for this prospective analysis: one was refused for surgery, other died before surgery from non-cardiovascular reason, and there was incomplete clinical data in seven of them. All patients had clinical follow-up up-to 98 months (8.2 years), and echocardiographic follow up was achieved in 123 (93.2%) patients. Mean follow-up was 6.0±1.5 years.

Clinical endpoints were defined as all cause of death or non-fatal cardiovascular hospitalization (heart failure, myocardial infarction, re-operation for prosthesis dysfunction, new-onset atrial fibrillation or advanced AV block requiring hospitalization) and by a composite endpoint of both events.

The diagnosis of hypertension was defined by clinical records. Chronic Kidney disease (CKD) was determined when estimated glomerular filtration rate (GFR) <60 ml/min/1.73$m^2$ by the Cockcroft-Gaul formula and perioperative acute renal lesion if there was an increase in serum creatinine >25% the preoperative value. Medical therapy was at the discretion of assistant physician.

This investigation conforms to the Declaration of Helsinki, had institutional ethical review board approval and each study participant signed an informed consent before enrolment.

## *Surgical technique*

All surgeries were performed using standard procedure for AVR. The patients were placed on cardiopulmonary bypass and cardiac arrest was induced and maintained with cold blood cardioplegia. The majority of patients received a bioprosthesis (73.3%). Two patients also had ascending aorta aneurism and underwent aortic root replacement with valved composite grafts (Bentall technique). At the time of surgery, 56 patients underwent myocardial biopsy from the LV interventricular septum. In 9 mitral stenosis patients undergoing mitral valve replacement, excised papillary muscles were collected and used as control myocardial biopsies. In both cases, excised myocardium was immediately snap-frozen in liquid nitrogen and stored at -80ºC.

## *Echocardiographic studies*

All patients had echocardiographic assessment one month before surgery and final echocardiographic evaluation was performed 5.0±2.2 years after surgery in 123 patients. The reason for not having postoperative echocardiographic information was early death in 5 of them (2 perioperative deaths and 3 sudden deaths in the first 30 days after surgery) and refusal to come to our hospital to do a follow-up examination in 4 (3 were alive at the end of follow-up but one had died 4 months after surgery from non-cardiovascular cause).

Echocardiographic examinations were performed by a trained cardiologist and recorded on digital support. All recordings were examined by an experienced echocardiographer in an accredited independent echocardiography laboratory (Hospital Clínico San Carlos in Madrid, Spain), blinded to patient details. Studies were performed using Phillips IE-33 equipment with a S5-1 transducer and M-mode, two dimensional, pulsed, continuous, color-flow and tissue Doppler capabilities.

Correct orientation of imaging planes, cardiac chambers dimensions and function measurements were performed according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations[1].

LV mass (LVM) was estimated according to the joint recommendations of the ASE and EAE [1] using Devereux's formula for ASE measurements in diastole: LV mass=0.8 x (1.04 x ([LV internal dimension + posterior wall thickness + interventricular septal thickness]$^3$ – [LV internal dimension]$^3$) + 0.6 g . Left ventricular hypertrophy was defined by LV mass index greater than 115 g/m$^2$ in men and greater than 95 g/m$^2$ in women.

Appropriateness of LVM was evaluated as the deviation from the value predicted individually from hemodynamic load, body size and sex, as previously described [2]. LVM was predicted from stroke work, gender and body size (as height (m) to the 2.7 power) by the following equation [3]:

Predicted LVM = 55.37 + (6.63 x height 2.7) + (0.64 x stroke work) - (18.1 x gender)

Stroke work was estimated from brachial systolic blood pressure (measured at the beginning of the echo exam) plus continuous wave (CW) Doppler transaortic peak gradient times stroke volume and converted to grammeters by multiplying by 0.0144. Gender was assigned the value of 1 for men and 2 for women. Using this equation, measured echocardiographic LVM could be expressed as the deviation (excess) from the predicted value (for convenience expressed as % of predicted, %LVMobs/pred). An excess of LVM was considered present when the ratio between the observed and predicted value >100%.
Relative wall thickness (RWT) was calculated for the assessment of LV geometry using the formula 2x posterior wall thickness/ LV diastolic diameter. Increased RWT was present when this ratio was greater than 0.42 [1]. Left atrial (LA) volume was measured in LV end systole in the frame preceding mitral valve opening.The volume was measured using the biplane area length method and corrected

for body surface area. Peak transvalvular gradient was estimated using the simplified Bernoulli equation. Aortic valve area (or effective orifice area, EOA) was estimated using quantitative Doppler by the continuity equation. The EOA values were then indexed to body surface area (EOAI). Patient prosthesis mismatch was considered present if EOAI ≤0.85 $cm^2/m^2$ and severe when EOAI≤ 0.65 $cm^2/m^2$ [4].

Peak wall stress (WS) was estimated using a previously validated formula: 0.86 x (0.334 x SAP x EDD / [PWTd x (1 + PWTd / EDD)] - 2) x 103 dynes/$cm^2$, where SAP=systolic arterial pressure, MaxG=maximal transvalvular pressure gradient, LVID=LV internal diameter, and PWTd=posterior wall thickness in diastole [5].

As a measure of global LV load, we calculated the valvuloarterial impedance: Zva=(SAP+MG)/SVI, where SAP is the systolic arterial pressure, MG is the mean transvalvular pressure gradient and SVI is stroke volume index. Blood pressure was measured before echocardiography with patients in supine position, and a mean of 3 measurements was considered.

Mitral inflow was assessed in the apical 4-chamber view using pulsed wave Doppler with the sample volume placed at the tips of mitral leaflets during diastole. From the mitral inflow profile, the peak flow velocity of early filling (E wave), peak flow velocity of atrial contraction (A wave), the E/A ratio, and early filling deceleration time (DT) were measured. Doppler tissue imaging (DTI) of the mitral annulus was obtained from the apical 4-chamber using a sample volume placed in the septal mitral valve annulus. Peak systolic annular velocity (Sm) and early diastolic septal velocity (e') were determined, and the E/e' ratio was derived. Patients with an E/e'septal >15 were considered to have increased filling pressure, whereas patients with E/e'septal <8 were considered to have normal filling pressure. In the remaining patients with an indeterminate E/e', those with LAVI ≥34mL/$m^2$ were considered to have increased filling pressure [6]. The presence of increased filling pressures was considered indicative of diastolic dysfunction.

All indexed values were obtained by dividing by body surface area according to the method of Mosteler.

### *Histological determination of fibrosis*

Light microscopic quantification of fibrosis has previously been described and validated. Fibrosis analysis of myocardial biopsies was performed using picrosirius-red–stained, 4-µm-thick-sections of tissue (± 5 sections of each sample). Images of these sections were acquired with a projection microscope (x50). Subsequent image analysis with Slidebook 4.0 software (3I, Denver, Colo) was performed to determine the extent of reactive interstitial fibrosis, which was expressed as collagen volume fraction (%). Areas of reparative and perivascular fibrosis were excluded. Myocardial fibrosis was calculated as the sum of all connective tissue areas divided by the sum of connective tissue and muscle areas averaged over 4 to 6 representative fields of the section of 56 random AS patients (18 male and 38 female). In our laboratory, normal values of fibrosis for LV myocardial biopsy material are 5.4±2.2% [7].

### *mRNA quantification*

For gene expression evaluation, RNA was extracted with TriPure (Roche) according to manufacture instructions. RT-PCR was performed with total RNA, followed by real time PCR analyses using the SYBR Green method, in a LightCycler 2.0 (Roche) as previously described (13). Results are normalized for GAPDH and expressed in arbitrary unit. Specific PCR primer pairs for the studied genes were: hGAPDH – fw 5′- GGT GGT CTC CTCTGA CTT CAA CA -3′ and rev 5′- GTTGCTGTAGCCAAATTCGTTGT -3′; hMMP2 – fw 5′ – GGCGCGCTCACGGGT – 3′ and rev 5′ – TGTTCAGGTATTGCACTGCCAACT – 3′; hTIMP2 – fw 5′ – ATCTACACGGCCCCCTCCTCG – 3′ and rev 5′ – CCCCTCGGCCTTTCCTGCAATG – 3′; hCollagen type I – fw 5′- GAGCGGACGCTAACCCCCTC – 3′ and rev 5′ –TCCTCTTGGCCGTGCGTCAG – 3′; hCollagen type III – fw 5′- CCCGTCGGCATTCCTGGAGC – 3′ and rev 5′ – GGCTCACCTGCACCACCTCG – 3′; hCTGF – fw 5′ –TGCCCGGGAAATGCTGCGAG – 3′ and rev 5′ – CAGTCGGTAAGCCGCGAGGG – 3′; hTGF-β1 – fw 5′ – GGCTTTCGCCTTAGCGCCCA – 3′ and rev 5′ – CGGCCGGTAGTGAACCCGTTG – 3′.

### *Serum determination of biomarkers of extracellular matrix remodeling*

Blood samples from forty AS patients were collected prior to AVR surgery in ethylenediamine-tetra-acetic acid-containing tubes. The samples were centrifuged at 5000 rpm for 15 min at 4 °C and plasma separated and frozen at -80 °C until analysis. Endogenous plasma levels of connective tissue growth factor (CTGF, USCN), metalloproteinase-1 (MMP1, Amersham), MMP9 (Amersham), procollagen type I carboxy-terminal peptide (PIP, Takara), tumor growth factor-β (TGF-β, Biorbyt), tissue inhibitor of metalloproteinase-1 (TIMP1, Amersham) and TIMP2 (Amersham) were quantified using an ELISA Kits accordingly to manufacturer's instructions. Each sample was analyzed in duplicate. Absorbance was recorded at 450nm using an ELISA plate reader (Perkin-Elmer, Wellesley, Massachusetts) and standard logarithmic curve was plotted and used to calculate PIP, MMP1, TIMP1 and TIMP2 concentration in the plasma samples.

### *Statistical analysis*

Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation or median and interquartile range, unless otherwise specified. Continuous variables were compared between groups using an unpaired t-test (for normally distributed variables) or the Mann–Whitney U-test (for non-normally distributed variables). For comparison between baseline and follow-up a paired Student's t-test was applied (normally distributed variables).

In the study of gender differences in hypertrophic response to aortic stenosis, continuous variables were expressed as mean (standard deviation), after logarithm transformation, as most of them proved to have non-normal distribution (Kolmogorov-Smirnov test). Continuous variables (after logarithm transformation in order to obtain normal distributed variables; mean and standard deviation were obtained using the resulting statistics after inverting the transformation used) were compared between

groups using the independent t-test (non-adjusted) or the ANCOVA (for age adjusted comparison). For comparison between baseline and follow-up a paired sample t-test was used (non-adjusted) and repeated measures ANCOVA was applied (for age adjusted comparison).

Chi-square test or the Fisher exact test (small samples), were used to compare proportions. In the chapter/study of gender differences in hypertrophic response to aortic stenosis, chi-square test was used to compare proportions and age adjusted proportion with a Z-test (adjustment by Direct Standardization, done with the following age groups: <50, 50-59, 60-69 and ≥70 years).

Spearman's rank correlation was used for the assessment of correlations between LVM index and clinical, echocardiographic and molecular continuous variables.

Cut-off points for several continuous variables were calculated using the point of highest sensitivity and specificity obtained under the receiver operating characteristic (ROC) curve for a particular outcome. In these cases, the probability function used for the ROC curve was produced by the logistic regression model, using the continuous variables as independent variables.

The stepwise binary logistic multivariate regression model (Wald backward stepwise method, p = 0.05 for covariate inclusion and 0.2 for exclusion) was performed in several occasions: for LVM index regression analysis 1 year after AVR; for NYHA improvement regression analysis 6 months after AVR; for independent predictors of gender-specific residual LVH after surgery; and for predicting residual LVH and the outcome of all-cause death and cardiovascular hospitalization.

The models validity was evaluated through the determination coefficients of Cox and Snell, NagelKerke, and the Hosmer and Lemeshow test. Furthermore, it was assessed using the area under the ROC curve (AUC).

The Kaplan-Meier and Cox models were used to evaluate survival times after surgery for all-cause death, for non-fatal cardiovascular hospitalization, and for all-cause death and cardiovascular hospitalization, and the log-rank test was used to compare survival curves.

All reported probability values are two-tailed, and p<0.05 was considered statistically significant. Analyses were performed with the IBM® SPSS® Statistics software package versions 19.0, 20.0 and 21.0 (along this thesis) (IBM Corporation, USA).

# 5

# RESULTS

# RESULTS

## 5.1. Characterization of the total cohort

# 5. RESULTS

## 5.1 Characterization of the total cohort

The study group included 132 patients with severe symptomatic AS referred for AVR, 58.3% women, with a mean age of 66.6±12.0 years.

At baseline, patients had a mean indexed effective orifice area (EOAI) of 0.42±0.12 $cm^2/m^2$ and 23.7% were in NYHA class III. Mean indexed left ventricular mass (LVMI) was 131.0±32.5 $g/m^2$ and 80.6% of patients had sex-specific criteria for left ventricular hypertrophy (LVH) before surgery. Most patients had preserved ejection fraction (EF) with a mean value of 61.7±10.3%.

Detailed demographic, clinical and echocardiographic (before and after AVR) characterization can be found in tables 1 and 2.

A bioprosthesis was used in 73.3% of patients and the most used valve size was 21 mm (0.8% 17 mm, 9.8% 19 mm, 0.8% 20 mm, 46.2% 21 mm, 28% 23 mm, 14.4% 25 mm). All mechanical valves were bileaflet. There were 2 perioperative deaths (one fatal stroke and one severe sepsis), and the median time of hospitalization was 6 days (P25-75: 6-8 days, minimum 4 days and maximum 76 days). Perioperative complications included atrial fibrillation (23.3%), acute renal lesion (18.3%), AV-block requiring permanent pacemaker implantation (6.9%), stroke (2.3%), re-operation for bleeding (1.5%), pneumonia (1.5%) and death (1.5%).

Mean follow-up was 6.0±1.5 years and, on the top of perioperative deaths, fifteen (11.5%) additional patients died during this period.

Six years after AVR, 22.4% patients were in NYHA class II, and the remainder were in NYHA class I. There was a significant increase in EOAI and a decrease in transprosthetic gradients, wall stress and total hemodynamic load evaluated by valvuloarterial impedance (table 2). Patient-prosthesis mismatch was present in 62.3% of patients and it was severe in 10.8%. Left ventricular geometry was improved, with a significant decrease in LVMI and relative wall thickness (RWT). Nevertheless, 54 (43.9%) of patients still had left ventricular hypertrophy (LVH) at echocardiographic evaluation 5.0±2.2 years after surgery. There was a median absolute left ventricular mass (LVM) regression of 23.7 $g/m^2$ (P25-75: 44.8-2.6 $g/m^2$), and relative mass regression of 18.5% (P25-75: 31.6-2.3%).

Parameters of LV systolic function showed a trend for an increase in EF and a significant enhancement in longitudinal systolic function, evaluated by peak systolic annular velocity (Sm) (table 2). The number of patients with increased left ventricular filling pressures had no significant change after

surgery (71.6% before surgery vs 70.2% after AVR, p=0.788) and there was a worsening in parameters of diastolic function like left atrium volume index (LAVI) and E/e' (table 2), despite an improvement in E/A ratio and e' velocity.

*Table 1. Clinical characterization of aortic stenosis patients.*

| | |
|---|---|
| **Age** (years) | 66.6±12.0 |
| **BSA** ($m^2$) | 1.8±0.2 |
| **Euroscore II** | 1.6±1.4 |
| **HT** [n (%)] | 74 (56.5%) |
| **DM** [n (%)] | 27 (20.6%) |
| **CKD** [n (%)] | 48 (36.4%) |
| **GFR** (ml/min) | 68.9±18.5 |
| **NYHA≥3** [n (%)] | 31 (23.7%) |
| **LVH baseline** [n (%)] | 104 (80.6%) |

Values are presented as mean±SD, unless otherwise indicated.
BSA= body surface area; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate (MDRD); NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy.

*Table 2. Baseline and follow-up echocardiographic characterization of aortic stenosis patients.*

| | Baseline | Final | p* |
|---|---|---|---|
| **LV geometry** | | | |
| **Interventricular septum (cm)** | 1.46±0.25 | 1.33±0.2 | **<0.001** |
| **Posterior wall (cm)** | 1.09±0.18 | 0.99±0.16 | **<0.001** |
| **Relative wall thickness** | 0.47±0.1 | 0.43±0.08 | **<0.001** |
| **LV mass index ($g/m^2$)** | 130.9±32.5 | 107.8±31.2 | **<0.001** |
| **LV end-diastolic volume index ($ml/m^2$)** | 51.5±16.1 | 50.6±17.9 | 0.282 |
| **LV end-systolic volume index ($ml/m^2$)** | 20.8±11.9 | 20.1±11.8 | 0.530 |
| **Aortic/Prosthesis stenosis severity** | | | |
| **Maximal transaortic velocity (cm/s)** | 463.8±60.9 | 261.7±59.1 | **<0.001** |
| **Medium transaortic gradient (mmHg)** | 54.3±14.3 | 15.9±7.1 | **<0.001** |
| **Effective orifice area index ($cm/m^2$)** | 0.42±0.12 | 0.86±0.24 | **<0.001** |
| **Increase in effective orifice area (%)** | 121.8±85.4 | | |
| **Hemodynamic load** | | | |
| **Valvuloarterial impedance (mm Hg/$ml/m^2$)** | 6.43 ±2.29 | 5.45±1.92 | 0.003 |
| **Peak LV wall stress ($dynes/cm^2$)** | 226.2±78.6 | 175.6±44.5 | **<0.001** |
| **Diastolic function** | | | |
| **LA volume index ($ml/m^2$)** | 35.4±12.9 | 38.7±10.1 | **<0.001** |
| **E/A** | 0.85±0.36 | 1.15±1.20 | 0.075 |
| **e' (cm/s)** | 5.42 ±2.23 | 5.81±1.90 | **<0.001** |
| **E/e'** | 15.8 ±6.4 | 16.4±6.7 | **<0.001** |
| **Systolic function** | | | |
| **Ejection fraction (%)** | 61.7±10.3 | 63.6±7.7 | 0.062 |
| **Peak systolic annular velocity (cm/s)** | 5.49±1.24 | 6.32±1.31 | **<0.001** |

Values are presented as mean±SD unless otherwise indicated. Bold values indicate statistical significance. * Wilcoxon test

## *Analysis of clinical outcomes*

At the end of 8.2 years of follow-up, the overall mortality was 12.9% (17 patients). The cause was cardiovascular in twelve (1 perioperative fatal stroke, 6 sudden deaths and 5 due to heart failure) and non-cardiovascular in five (1 perioperative sepsis, 3 from cancer and 1 with no information). There were 12 non-fatal cardiovascular hospitalizations (9.2%, after excluding perioperative deaths), 5 for heart failure, 3 re-operations for prosthesis dysfunction, 2 for symptomatic new-onset atrial fibrillation, and 2 for advanced AV block requiring pacemaker implantation.

Patients who died were older, had higher operative risk, worse longitudinal systolic function (evaluated by Sm) and higher levels of collagen volume fraction (CVF) in myocardial biopsies (table 3). In univariate analysis, age and baseline value of Sm were associated with an increased risk of death. In multivariate analysis, after adjustment for age, Euroscore II and baseline E/e' and Sm, %LVMobs/pred was the only independent predictor of all-cause mortality (HR 1.020; 95%CI: 1.005-1.036 for each 1% increase), although age had marginal statistical significance (HR 3.023; 95%CI: 0.986-9.269) (table 4).

*Table 3. Characterization of aortic stenosis patients according to death status.*

| | Death | | |
|---|---|---|---|
| | **No (n=115)** | **Yes (n=17)** | **p** |
| **Age** | 65.4±12.2 | 75.3±5.3 | **0.001*** |
| **Glomerular filtration rate** (MDRD) | 68.9±18.1 | 68.8±22.4 | 0.992 |
| **Euroscore II** | 1.60±1.46 | 2.14±1.43 | **0.039*** |
| **Relative wall thickness** | 0.51±0.10 | 0.54±0.12 | 0.221* |
| **LA volume index** ($ml/m^2$) | 35.7±13.1 | 38.1±11.3 | 0.245* |
| **LV mass index** ($g/m^2$) | 129.6±32.0 | 141.0±38.6 | 0.307* |
| **LVMobs/pred** (%) | 149.3±46.3 | 171.3±54.7 | 0.131 |
| **Maximal aortic velocity** (m/s) | 4.59±0.59 | 4.64±0.54 | 0.812 |
| **Effective oriffice area index** ($cm^2/m^2$) | 0.41±0.11 | 0.41±0.12 | 0.991 |
| **Stroke volume index** ($ml/m^2$) | 31.1±8.1 | 30.2±7.1 | 0.654 |
| **Valvuloarterial impedance** (mm Hg/$ml/m^2$) | 6.43±2.00 | 6.51±1.63 | 0.598* |
| **Sm** (cm/s) | 5.54±1.23 | 4.24±0.72 | **0.009*** |
| **E/e'** | 16.4±6.5 | 19.1±8.3 | 0.225* |
| **LV end-diastolic volume index** ($ml/m^2$) | 52.3±16.1 | 51.0±17.7 | 0.452* |
| **LV end-systolic volume index** ($ml/m^2$) | 21.2±11.5 | 21.5±16.1 | 0.847* |
| **Ejection fraction** (%) | 61.3±10.4 | 60.1±11.5 | 0.468* |
| **BNP** (pg/ml) | 392.0±498.6 | 774.9±1055.8 | 0.344* |
| **Decrease in LVMI** (g) | 22.9±37.6 | 25.5±27.6 | 0.778* |
| **Gender** (female) | 65 (66.5%) | 12 (70.6%) | 0.272 |
| **Hypertension** | 49 (42.6%) | 8 (47.1%) | 0.752 |
| **Diabetes** | 22 (19.1%) | 5 (29.4%) | 0.336 |
| **Chronic kidney disease** | 40 (34.8%) | 8 (47.1%) | 0.326 |
| **NYHA ≥ 3** | 27 (23.5%) | 4 (23.5%) | 0.989 |
| **Patient Prosthesis Mismatch** | 56 (48.7%) | 5 (29.4%) | 0.443 |
| **Collagen volume fraction** (%) ** | 15.4±11.8 | 27.1±20.7 | **0.035*** |

Values are presented as mean (±SD) unless otherwise indicated. Bold values indicate statistical significance.
* Mann-Whitney test ** Measured in 56 patients

*Table 4. Univariate and multivariate associations with all-cause death.*

| | | Univariate analysis | | Multivariate Analysis |
|---|---|---|---|---|
| | p | Unadjusted HR (95% C.I.) | p | Adjusted HR (95% C.I.) |
| **Sex** (female) | 0.319 | 1.700 (0.598-4.831) | | |
| **Age** (10 y increment) | **0.002** | 2.813 (1.482-5.34) | 0.053 | 3.023 (0.986-9.269) |
| **HT** | 0.830 | 0.901 (0.347-2.34) | | |
| **DM** | 0.262 | 1.818 (0.640-5.167) | | |
| **GFR** (MDRD) | 0.877 | 0.998 (0.971-1.026) | | |
| **Euroscore II** | 0.195 | 1.146 (0.932-1.410) | | |
| **NYHA ≥ 3** | 0.922 | 0.945 (0.308-2.901) | | |
| **LVMI** | 0.224 | 1.008 (0.995-1.022) | | |
| **LVMobs/pred** (%) | 0.131 | 1.008 (0.998-1.019) | **0.009** | 1.020 (1.005-1.036) |
| **EOAI** | 0.813 | 1.682 (0.023-123.273) | | |
| **SVI** | 0.567 | 0.981 (0.917-1.049) | | |
| **EF** | 0.630 | 0.988 (0.941-1.037) | | |
| **Sm** | **0.017** | 2.456 (1.175-5.131) | | |
| **E/e'** | 0.122 | 1.065 (0.983-1.154) | | |

Variables entered in Cox regression multivariate model: age, Euroscore II, LVMobs/pred (%), and baseline E/e' and Sm
HT= hypertension; DM= diabetes mellitus; GFR= glomerular filtration rate (MDRD formula); NYHA= functional class of New York Heart Association; LVMI= left ventricular mass index; EOAI= effective orifice area index; SVI= stoke volume index; Sm= peak systolic annular velocity; EF= ejection fraction.

Patients with a non-fatal cardiovascular hospitalization had more concentric geometry, evaluated by RWT and lower baseline BNP levels, but with great overlap of values between groups. These patients also tended to have lower stroke volume (SV) index (table 5). In multivariate Cox regression analysis, after adjustment for Euroscore II and baseline values of RWT, EOAI, EF, E/e', SVI, Zva and BNP, %LVMobs/pred (HR 1.021, 95%CI: 1.003-1.039) was the only independent predictor of this event (table 6).

*Table 5. Characteristics of aortic stenosis patients, according to the outcome of non-fatal cardiovascular hospitalization.*

| | Non-fatal cardiovascular hospitalization | | |
|---|---|---|---|
| | **N=120** | **Y=12** | **p** |
| **Age** | 66.6±11.8 | 67.5±14.1 | 0.695 |
| **Glomerular filtration rate** (MDRD) | 68.6±18.1 | 71±23.1 | 0.633 |
| **Euroscore II** | 1.53±1.06 | 2.78±3.25 | 0.142 |
| **Relative wall thickness** | 0.48±0.10 | 0.41±0.07 | **0.024** |
| **LA volume index** (ml/m2) | 35.8±13.2 | 36.7±11.2 | 0.714 |
| **LV mass index** (g/m2) | 130.6±32.5 | 135.7±39.3 | 0.752 |
| **LVMobs/pred** (%) | 148.6±45.0 | 176.4±60.9 | 0.114 |
| **Maximal aortic velocity** (m/s) | 459.7±58.1 | 460.2±66.2 | 0.890 |
| **Effective oriffice area index** ($cm^2/m^2$) | 0.41±0.12 | 0.47±0.12 | 0.069 |
| **Stroke volume index** ($ml/m^2$) | 31.4±7.7 | 26.9±9.1 | 0.053 |
| **Valvuloarterial impedance** (mm Hg/$ml/m^2$) | 6.32±1.83 | 7.41±2.84 | 0.200 |
| **Sm** (cm/s) | 5.43±1.18 | 5.04±1.50 | 0.215 |
| **E/e'** | 16.4±6.0 | 19.2±10.6 | 0.518 |
| **LV end-diastolic volume index** ($ml/m^2$) | 52.3±15.6 | 50.2±20.3 | 0.557 |
| **LV end-systolic volume index** ($ml/m^2$) | 21.0±11.4 | 23.3±16.5 | 0.834 |
| **Ejection fraction** (%) | 61.6±10.0 | 56.8±13.6 | 0.334 |
| **BNP** (pg/ml) | 461.9±595.8 | 141.1±126.6 | **0.039** |
| **Decrease in LVMI** (g) | 23±37.1 | 24.7±33.8 | 0.919 |
| **Gender** (female) | 70 (58.3%) | 7 (58.3%) | ≈1.000 |
| **Hypertension** | 66 (55.5%) | 8 (66.7%) | 0.456 |
| **Diabetes** | 23 (19.3%) | 4 (33.3%) | 0.268 |
| **Chronic kidney disease** | 43 (35.8%) | 5 (41.7%) | 0.757 |
| **NYHA** ≥ 3 | 27 (22.7%) | 4 (33.3%) | 0.477 |
| **Patient Prosthesis Mismatch** | 56 (52.8%) | 5 (45.5%) | 0.641 |
| **Collagen volume fraction** (%) ** | 16.3±13.2 | 24.8±17.7 | 0.227 |

Values are presented as mean±SD unless otherwise indicated. Bold values indicate statistical significance.
* Mann-Whitney test; ** Measured in 56 patients

*Table 6. Univariate and multivariate associations with non-fatal cardiovascular hospitalization.*

| | | Univariate analysis | | Multivariate Analysis ** |
|---|---|---|---|---|
| | P | Unadjusted HR (95% C.I.) | p | Adjusted HR (95% C.I.) |
| **Sex** (female) | 0.944 | 0.96 (0.304-3.03) | | |
| **Age** (10 y increment) | 0.543 | 1.174 (0.705-1.953) | | |
| **HT** | 0.430 | 1.622 (0.488-5.387) | | |
| **DM** | 0.211 | 2.152 (0.647-7.152) | | |
| **GFR** (MDRD) | 0.919 | 1.002 (0.971-1.033) | | |
| **Euroscore II** | **0.009** | 1.264 (1.061-1.505) | | |
| **NYHA ≥ 3** | 0.482 | 1.538 (0.463-5.112) | | |
| **RWT** (1 unit decrease) | **0.036** | 500 (1.506-250000) | | |
| **LVMI** | 0.688 | 1.003 (0.987-1.02) | | |
| **LVMobs/pred** (%) | **0.070** | 1.01 (0.999-1.021) | **0.020** | 1.021 (1.003-1.039) |
| **EOAI** | **0.049** | 52.927 (1.022-2741.138) | | |
| **SVI** | **0.077** | 0.935 (0.867-1.007) | | |
| **ZVA** | **0.138** | 1.17 (0.951-1.44) | | |
| **EF** | **0.140** | 0.968 (0.928-1.011) | | |
| **Sm** (1 unit decrease) | 0.279 | 1.289 (0.814-2.045) | | |
| **E/e'** | **0.110** | 1.064 (0.986-1.149) | | |
| **BNP** (1 pg/ml increase) | **0.125** | 0.996 (0.991-1.001) | 0.054 | 0.991 (0.983-1.000) |

Variables entered in Cox regression multivariate model: Euroscore II, RWT, LVMobs/pred, EOAI, EF, E/e', SVI, ZVA, BNP
HT= hypertension; DM= diabetes mellitus; GFR= glomerular filtration rate (MDRD formula); NYHA= functional class of New York Heart Association; RWT= relative wall thickness; LVMI= left ventricular mass index; EOAI= effective orifice area index; SVI= stoke volume index; Sm= peak systolic annular velocity; EF= ejection fraction.

The combined outcome all-cause death or non-fatal cardiovascular hospitalization was more frequent in those who were older, had higher Euroscore II, an excessive increase in LVM in response to AS (%LVMobs/pred), worse longitudinal systolic function (evaluated by Sm), residual LVH and higher levels of collagen volume fraction (CVF) (table 7). In multivariate analysis, after adjustment for age, DM, Euroscore II, and baseline EOAI, SVI, EF, E/e' and Sm, %LVMobs/pred (HR 1.008, 95%CI: 1.001-1.016) and E/e' (HR 1.057, 95%CI: 1.001-1.116) were independent predictors of all-cause death or non-fatal cardiovascular hospitalization (table 8). Diabetes also increased the risk of combined events but had a borderline significance and wide confidence intervals.

*Table 7. Characterization of aortic stenosis patients according to the outcome of all-cause death or non-fatal cardiovascular hospitalization.*

| | All-cause death and non-fatal cardiovascular hospitalization | | |
|---|---|---|---|
| | **No (n=104)** | **Yes (n=28)** | **P** |
| **Age** | 65.3±12.1 | 71.6±10.3 | **0.006** |
| **Glomerular filtration rate** (MDRD) | 68.3±17.7 | 71.0±21.9 | 0.520 |
| **EuroSCORE II** | 1.53±1.00 | 2.30±2.31 | **0.017*** |
| **Relative wall thickness** | 0.51±0.13 | 0.52±0.10 | 0.562 |
| **LA volume index** (ml/m$^2$) | 35.7±13.4 | 36.6±10.8 | 0.428* |
| **LV mass index** (g/m$^2$) | 128.9±31.1 | 138.9±39.0 | 0.157 |
| **LVMobs/pred** (%) | 146.3±43.4 | 171.7±56.9 | **0.022** |
| **Maximal aortic velocity** (m/s) | 4.59±0.59 | 4.64±0.60 | 0.857* |
| **Effective oriffice area index** (cm$^2$/m$^2$) | 0.42±0.10 | 0.44±0.12 | 0.233 |
| **Stroke volume index** (ml/m$^2$) | 31.5±7.8 | 28.7±8.2 | 0.124 |
| **Valvuloarterial impedance** (mm Hg/ml/m$^2$) | 6.30±1.81 | 6.91 ±2.30 | 0.278* |
| **Sm** (cm/s) | 5.53±1.20 | 4.81±1.44 | **0.021** |
| **E/e'** | 16.1±5.7 | 18.9±9.3 | 0.259* |
| **LV end-diastolic volume index** (ml/m$^2$) | 52.4±15.4 | 51.1±18.8 | 0.430* |
| **LV end-systolic volume index** (ml/m$^2$) | 20.8±10.7 | 22.7±16.2 | 0.859* |
| **Ejection fraction (%)** | 61.8±9.8 | 58.4±12.6 | 0.226* |
| **BNP** (pg/ml) | 425.4±520.3 | 436.9±770 | 0.368* |
| **Decrease LVMI** (g) | 22.7±38.0 | 25.0±30.8 | 0.938* |
| **Gender** (female) | 59 (56.7%) | 18 (64.3%) | 0.472 |
| **Hypertension** | 58 (55.8%) | 16 (%57.1) | 0.937 |
| **Diabetes** | 18(17.3%) | 9 (32.1%) | 0.089 |
| **Chronic kidney disease** | 36 (34.6%) | 12(42.9%) | 0.326 |
| **NYHA** ≥ 3 | 24 (23.1%) | 7 (25.0%) | 0.421 |
| **PPM** | 51 (49.0%) | 10 (41.7%) | 0.486 |
| **Collagen volume fraction** (%) ** | 15.3±12.0 | 24.0±18.2 | **0.038*** |

Values are presented as mean±SD unless otherwise indicated. Bold values indicate statistical significance.
* Mann-Whitney test ** Measured in 56 patients

*Table 8. Univariate and multivariate associations with all-cause death or non-fatal cardiovascular hospitalization.*

| | | Univariate analysis | Multivariate Analysis | |
|---|---|---|---|---|
| | p | Unadjusted HR (95% C.I.) | p | Adjusted HR (95% C.I.) |
| **Sex** (female) | 0.576 | 1.247 (0.575-2.705) | | |
| **Age** (10 y increment) | **0.010** | 1.704 (1.137-2.551) | | |
| **HT** | 0.859 | 1.07 (0.506-2.265) | | |
| **DM** | 0.077 | 2.046 (0.925-4.524) | 0.056 | 2.320 (0.978-5.501) |
| **GFR (MDRD)** | 0.841 | 1.002 (0.981-1.024) | | |
| **Euroscore II** | **0.013** | 1.193 (1.038-1.371) | | |
| **NYHA ≥ 3** | 0.996 | 1.002 (0.426-2.359) | | |
| **LVMI** | 0.245 | 1.006 (0.996-1.017) | | |
| **LVMobs/pred** (%) | **0.046** | 1.008 (1-1.016) | **0.033** | 1.008 (1.001-1.016) |
| **EOAI** | 0.146 | 9.166 (0.462-181.68) | | |
| **SVI** | 0.158 | 0.964 (0.916-1.014) | | |
| **EF** | 0.164 | 0.977 (0.946-1.009) | | |
| **Sm** (1 unit decrease) | **0.030** | 1.562 (1.044-2.337) | | |
| **E/e'** | **0.045** | 1.061 (1.001-1.123) | **0.046** | 1.057 (1.001-1.116) |

Variables entered in Cox regression multivariate model: age, DM, Euroscore II, LVMobs/pred (%), and baseline EOAI, SVI, EF, E/e' and Sm
HT= hypertension; DM= diabetes mellitus; GFR= glomerular filtration rate (MDRD formula); NYHA= functional class of New York Heart Association; LVMI= left ventricular mass index; EOAI= effective orifice area index; SVI= stoke volume index; Sm= peak systolic annular velocity; EF= ejection fraction.

## *Kaplan-Meier Survival Analysis*

For event-free survival analysis the cutoff of 178% of %LVMobs/pred, correspondent to the 75$^{th}$ percentil in this cohort, was chosen after ROC curve analysis (AUC=0.63, 95%CI:0.49-0.77 for the combined event; AUC=0.76, 95%CI:0.66-0.85 for residual LVH). Those with %LVMobs/pred ≥178% were considered to have inappropriate LVM.

Patients with %LVMobs/pred ≥178% had worse survival after 8 years of follow-up, comparing with those with <178% (72.7% vs 90.8%, p=0.028) (Fig.1).

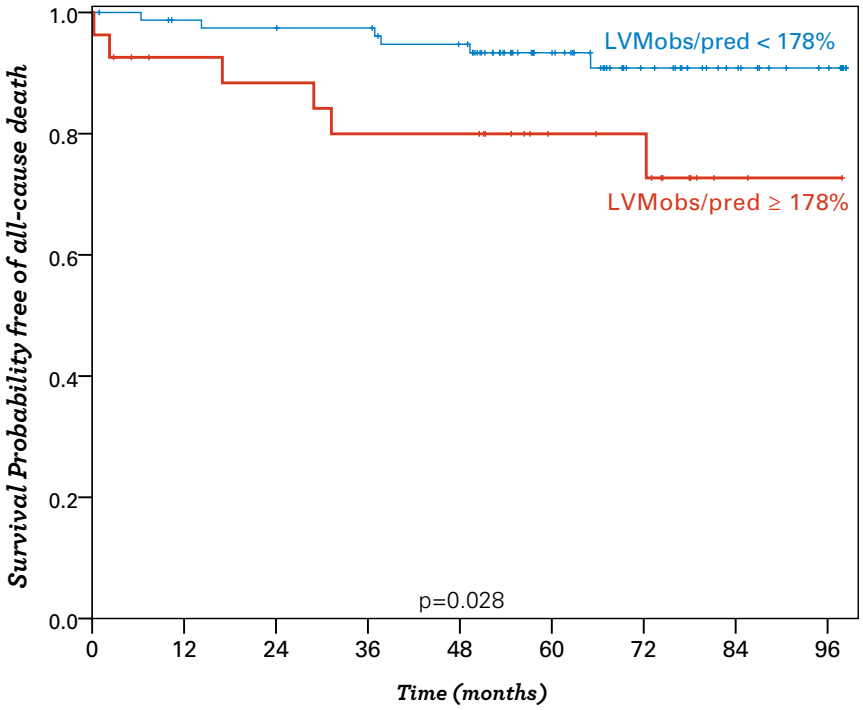


*Fig. 1 Relationship between inappropriate LVM (%LVMobs/LVMpred ≥178%) and long-term survival after aortic valve replacement.*

For the endpoint of non-fatal cardiovascular hospitalization, prognosis was also worse for those with higher excess in LVM (60.7% for %LVMobs/pred ≥178% vs 93.6% for %LVMobs/pred <178%, p=0.004) (Fig.2).

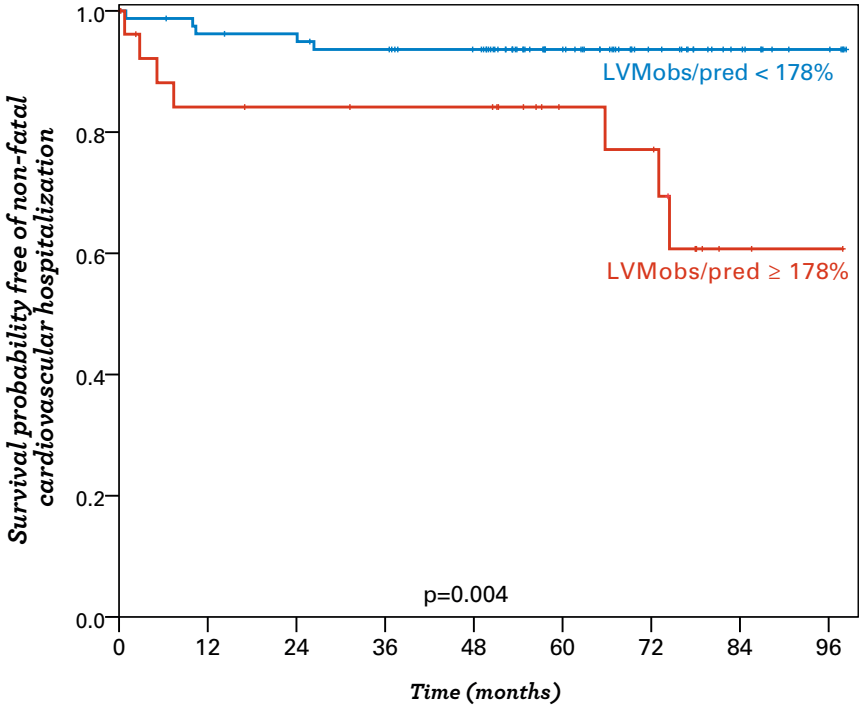


*Fig. 2 Relationship between inappropriate LVM (%LVMobs/LVMpred ≥178%) and long-term survival free of non-fatal cardiovascular hospitalization, after aortic valve replacement.*

Event-free survival analysis for the combined endpoint of all-cause mortality or non-fatal cardiovascular hospitalization also shows that patients with %LVMobs/pred ≥178% had a worse outcome after 8 years of follow-up, when comparing with those with a minor excess in LVM (46.2% vs 84.8%, p=0.001) (Fig.3).

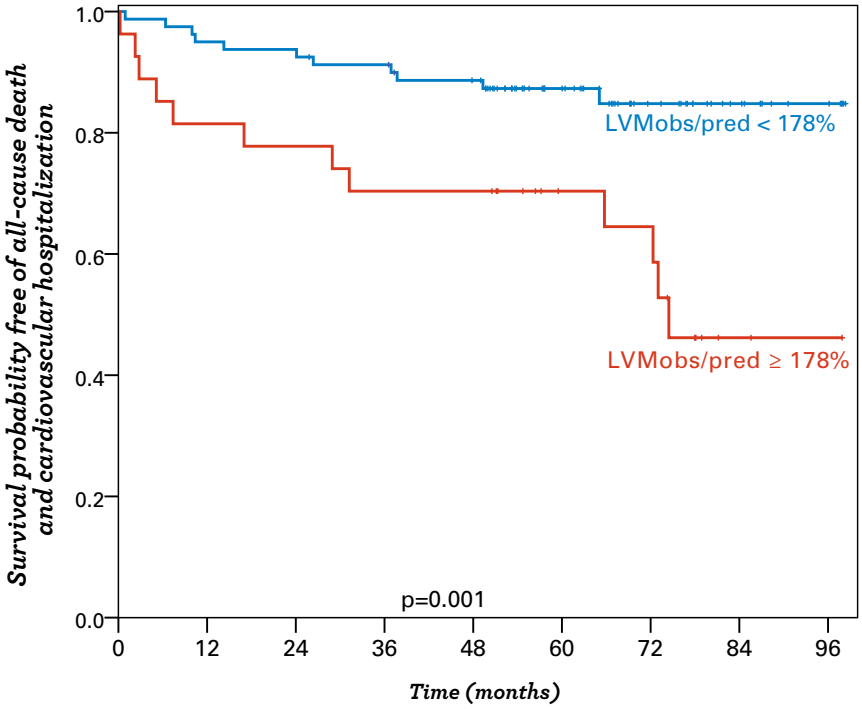


*Fig. 3 Relationship between inappropriate LVM (%LVMobs/LVMpred ≥178%) and long-term survival free of the composite of all-cause death and non-fatal cardiovascular hospitalization, after aortic valve replacement.*

## *Characterization of patients with inappropriate LVM*

In our cohort, inappropriate LVM was present in 25.2% patients. These patients tended to be older (table 9) and more frequently women (30.6% vs 17.8%, p=0.18), hypertensive (31.1% vs 17.7%, p=0.18) and diabetic (40% vs 22.1%, p=0.15). Moreover, they had more concentric geometry (evaluated by LVMI/LVEDVI), worse systolic function (lower EF and Sm) and lower values of e', indicating more impaired LV relaxation (table 9).

*Table 9. Characterization of aortic stenosis patients according to the existence of inappropriate LVM.*

| | AS patients | | |
|---|---|---|---|
| | **Appropriate LVM** | **Inappropriate LVM** | **p** |
| **Age** | 65.6±12.5 | 71.0±12.2 | 0.09 |
| **LV end-diastolic volume index** (ml/m²) | 88.5±23.8 | 93.0±39.9 | 0.89* |
| **LV end-systolic volume index** (ml/m²) | 32.3±12.0 | 48.9±34.4 | 0.17* |
| **LV mass index** (g/m²) | 119.7±26.7 | 161.9±31.0 | **<0.001*** |
| **LVMI/LVEDVI** | 2.40±0.68 | 3.31±0.91 | **<0.001** |
| **Relative wall thickness** | 0.48±0.10 | 0.45±0.12 | 0.42 |
| **Peak wall stress** (dynes/cm²) | 214.1±53.5 | 213.1±57.8 | 0.46 |
| **LA volume index** (ml/m²) | 36.2±14.2 | 37.8±10.1 | 0.30* |
| **Maximal aortic velocity** (m/s) | 4.55±0.56 | 4.66±0.68 | 0.34* |
| **Sm** (cm/s) | 5.6±1.1 | 4.4±1.0 | **<0.001** |
| **E/e'** | 16.2±6.8 | 18.8±7.3 | 0.15* |
| **Ejection fraction** (%) | 62.3±7.0 | 52.0±13.7 | **<0.001*** |

LVMI= left ventricular mass index; LVEDVI= left ventricular en-diastolic index. Values are presented as mean (±SD) unless otherwise indicated. Bold values indicate statistical significance. * Mann-Whitney test

# RESULTS

## 5.2. Gender differences in hypertrophic response to aortic stenosis

## 5.2 Gender differences in hypertrophic response to aortic stenosis

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## Abstract

**Objectives:** We aimed to evaluate gender differences in left ventricular (LV) remodeling, myocardial fibrosis and biomarkers of extracellular matrix (ECM) turnover in severe aortic stenosis (AS).

**Background:** Hypertrophic response to pressure overload is gender-specific. Women develop a more concentric geometry with supra-normal function while men more often have ventricular dilatation and earlier systolic dysfunction.

**Methods:** A prospective cohort of 132 severe AS patients, 77 females (58.3%), was evaluated at baseline and 6 months after surgical aortic valve replacement (AVR). Predicted LV mass (LVM) was calculated (according to gender, height and stroke work) and %observed/predictedLVM was derived. Quantification of fibrosis was performed by picrosirius-red staining of 56 myocardial biopsies. In 40 patients baseline ECM serum biomarkers procollagen type I carboxy-terminal peptide (PIP), metalloproteinase (MMP) 1 and 9, and tissue inhibitor of MMP (TIMP) 1 and 2 were measured.

**Results:** Women had similar indexed LV mass but, when comparing the observed with predicted LVM, they had a more excessive increase than men (%observed/predictedLVM: 166.45±54.28% vs 137.44±36.40%, age-adjusted p=0.016). Six months after AVR, LVM regression was similar between genders but women more often had residual hypertrophy (68.8% vs 39.6%, p=0.002). In multivariate analysis, female gender [OR:4.83(1.58-14.77)], hypertension [OR:3.39(1.23-9.36)] and baseline LVM [OR:1.04(1.02-1.06) per 1 $g/m^2$ increase] were independent predictors of residual hypertrophy. Histologically, women had more interstitial fibrosis than men (18.98±15.20% vs 12.41±7.61%, p=0.036). In women, but not in men, TIMP1/MMP1 levels positively correlated with LVM ($r_s$=0.681, p=0.003) and higher TIMP2 and TIMP1/MMP1 levels correlated with lesser 6 month LVM regression (TIMP2:$r_s$=-0.650, p=0.022; TIMP1/MMP1:$r_s$=-0.748, p=0.005).

**Conclusions:** In severe AS, women have a higher than predicted LVM increase compared to men, and female gender was an independent predictor of residual hypertrophy. A gender-specific ECM remodeling, might contribute to explain these differences.

## Introduction:

Hypertrophy in aortic stenosis (AS) is multifactorial and the degree of LVH varies under identical loading conditions (1,2). In a reference population of normotensive and normal weight individuals, the combination of stroke work, gender and body height could explain up to 82% of the variability in left ventricular mass (LVM) (3). Additionaly, a regression equation was derived for predicting LVM and evaluate the adequacy of left ventricular (LV) hypertrophic response to different load conditions (4). In hypertension and, more recently in AS, the comparison of the observed LVM with that predicted according to gender, height and load, has helped to identify those with a more "inappropriate" hypertrophy and worse prognosis (5,6). Moreover, cardiac hypertrophy in AS is accompanied by an increase in interstitial fibrosis and higher levels of fibrosis have been associated with the presence of irreversible remodeling and all-cause mortality (7).

In AS, many studies have reported different remodeling responses between men and women, with similar left ventricular mass (LVM) index but higher prevalence of left ventricular hypertrophy (LVH) in women, according to sex specific criteria (8-11). Women have smaller LV chamber size, higher relative wall thickness, higher LVM/volume ratio, supernormal LV function and less wall stress, while men exhibit ventricular dilatation and earlier ventricular dysfunction (8,10,12,13). At histological level, some authors have described a higher gene expression of collagen I and III in males (14), and it has been suggested that estrogens could have a protective role in the fibrotic response to pressure overload (15).

Our aim was to evaluate gender differences in LV remodeling, before and after aortic valve replacement (AVR), in patients with isolated severe aortic stenosis (AS).

## Methods:

### *Patient selection and follow-up*

Between January 2006 and December 2009 we included 141 consecutive patients over 18 years old with severe symptomatic AS (aortic valve area <1 $cm^2$ or mean transaortic gradient ≥40 mmHg), evaluated preoperatively by the same Cardiologist (CG) for aortic valve replacement (AVR) at the Cardiothoracic Surgery Department of Centro Hospitalar São João, Porto, Portugal. We excluded patients with aortic regurgitation >II/IV or other significant valve diseases (>mild), and significant coronary artery disease (lesions >50% on coronary angiography). All patients were in sinus rhythm at the time of inclusion. From the initial 141 patients, 132 were considered for this prospective analysis: one was refused for surgery, other died before surgery from non-cardiovascular reason (cholangitis with sepsis), and there was incomplete clinical data in seven of them. There were 2 perioperative deaths and one additional cardiovascular death at six months. At six months, clinical and echocardiographic follow up was achieved in 117 (88.6%) patients.

The diagnosis of hypertension was performed by the clinical records of the assistant physician. Renal insufficiency was determined when creatinine clearance <60 ml/kg by the Cockcroft-Gaul formula. The study was approved by local institutional review committee and all patients gave their informed consent.

### *Surgical technique*

All surgeries were performed using standard procedure for AVR. The patients were placed on cardiopulmonary bypass and cardiac arrest was induced and maintained with cold blood cardioplegia. The majority of patients received a bioprosthesis (73.3%). Two patients also had ascending aorta aneurysm and underwent aortic root replacement with valved composite grafts (Bentall technique). At the time of surgery, 56 patients underwent myocardial biopsies from the LV interventricular septum.

### *Echocardiographic studies*

Echocardiographic examination was performed by a trained cardiologist and recorded on digital support. All recordings were examined by an experienced echocardiographer in an accredited independent echocardiography laboratory (Hospital Clínico San Carlos in Madrid, Spain) blinded to patient details. Studies were performed using Phillips IE-33 equipment with a S5-1 transducer and M-mode, two dimensional, pulsed, continuous, color-flow and tissue Doppler capabilities. Correct orientation of imaging planes, cardiac chambers dimension and function measurements were performed according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations (16).

LV mass was estimated according to the joint recommendations of the ASE and EAE using Devereux's formula for ASE measurements in diastole(16): LV mass=0.8 x (1.04 x ([LV internal dimension + posterior wall thickness + interventricular septal thickness]$^3$ – [LV internal dimension]$^3$) + 0.6 g. Left ventricular hypertrophy was defined by LV mass index greater than 115 g/m$^2$ in men and greater than 95 g/m$^2$ in women. Appropriateness of LVM was assessed using the ratio between the observed and predicted value and an excess in LVM was considered present when > 100%. LVM was predicted from stroke work, gender and body size (as height (m) to the 2.7 power) by the following equation (5):

Predicted LVM = 55.37 + (6.63 x height $^{2.7}$) + (0.64 x stroke work) - (18.1 x gender)

Stroke work was estimated from brachial systolic blood pressure (measured at the beginning of the echo exam) plus continuous wave (CW) Doppler transaortic peak gradient times stroke volume and converted to grammeters by multiplying by 0.0144. Gender was assigned the value of 1 for men and 2 for women. Relative wall thickness (RWT) was calculated for the assessment of LV geometry using the formula 2x posterior wall thickness/ LV diastolic diameter. Increased RWT was present when this ratio was greater than 0.42 (16). LA volume was measured in LV end systole in the frame preceding mitral valve

opening. The volume was measured using the biplane area length method and corrected for body surface area. Aortic valve area was estimated using quantitative Doppler by continuity equation.

Peak wall stress (WS) was estimated using a previously validated formula: 0.86 x (0.334 x SAP x EDD / [PWTd x (1 + (PWTd / EDD)] - 2) x $10^3$ dynes/$cm^2$, where SAP=systolic arterial pressure, MaxG=maximal transvalvular pressure gradient, LVID=LV internal diameter, and PWTd=posterior wall thickness in diastole (17). As a measure of global LV load, we calculated the valvuloarterial impedance: Zva=(SAP+MG)/SVI, where SAP is the systolic arterial pressure and MG is the mean transvalvular pressure gradient and SVI is stroke volume index (18).

### *Histological determination of fibrosis*

Light microscopic quantification of fibrosis has previously been described and validated(19). Fibrosis analysis of myocardial biopsies was performed using picrosirius-red–stained, 4-µm-thick-sections of tissue (± 5 sections of each sample). Images of these sections were acquired with a projection microscope (x50). Subsequent image analysis with Slidebook 4.0 software (3I, Denver, Colo) was performed to determine the extent of reactive interstitial fibrosis, which was expressed as collagen volume fraction (%). Areas of reparative and perivascular fibrosis were excluded. Myocardial fibrosis was calculated as the sum of all connective tissue areas divided by the sum of connective tissue and muscle areas averaged over 4 to 6 representative fields of the section in 56 random AS patients (18 male and 38 female). In our laboratory, normal values of fibrosis for LV myocardial biopsy material are 5.4±2.2%.

### *Serum determination of biomarkers of extracellular matrix remodeling*

Blood samples from forty AS patients were collected prior to aortic valve replacement surgery in ethylenediamine-tetra-acetic acid-containing tubes. The samples were centrifuged at 5000 rpm for 15 min at 4 ºC and plasma separated and frozen at -80 ºC until analysis. Endogenous plasma levels of connective tissue growth factor (CTGF, USCN), metalloproteinase-1 (MMP1, Amersham), MMP9 (Amersham), procollagen type I carboxy-terminal peptide (PIP, Takara), tumor growth factor-β (TGF-β, Biorbyt), tissue inhibitor of metalloproteinase-1 (TIMP1, Amersham) and TIMP2 (Amersham) were quantified using an ELISA Kits accordingly to manufacturer's instructions. Each sample was analyzed in duplicate. Absorbance was recorded at 450nm using an ELISA plate reader (Perkin-Elmer, Wellesley, Massachusetts) and standard logarithmic curve was plotted and used to calculate PIP, MMP1, TIMP1 and TIMP2 concentration in the plasma samples.

### *Statistical analysis*

Categorical variables were expressed as percentages. Continuous variables were expressed as mean (standard deviation), after logarithm transformation as most of them proved to have non-normal

distribution (Kolmogorov-Smirnov test). Continuous variables (after logarithm transformation in order to obtain normal distributed variables; mean and standard deviation were obtained after inverting the transformation used) were compared between groups using the unpaired t-test (non-adjusted) or the ANCOVA (for age adjusted comparison). For comparison between baseline and follow-up a paired sample t-test was used (non-adjusted) and a repeated measures ANCOVA was applied (for age adjusted comparison). Chi-square test was used to compare proportions and age adjusted proportion with a Z-test (adjustment by Direct Standardization, done with the following age groups: <50, 50-59, 60-69 and ≥70). Spearman's rank correlation was used for the assessment of correlations between LVM index and its variation and clinical, echocardiographic and biomarker continuous variables. A multivariate logistic regression model (Wald backward stepwise method, p=0.05 for covariate inclusion and p=0.10 for exclusion) was built to detect independent predictors of gender-specific residual LVH after surgery. Variables that in univariate analysis showed to be associated with residual LVH ($p<0.05$) entered the multivariable model, and renal disease (CKD) was also included although it did not showed significantly association with the LVH outcome. For better clinical interpretation patterns, a second multivariable model was obtained, where the baseline LVM was categorized in tercils according to gender and, furthermore, the %observed/predicted LVM was dichotomized (≤142% and >142%) using cutoff value that produces the maximum sensitivity and specificity in relation with residual LVH outcome. All reported probability values are two-tailed, and $P< 0.05$ was considered statistically significant. Analyses were performed with the SPSS statistical software package (version 19.0) (SPSS Inc, Chicago, IL, USA).

## Results

The study group included 132 patients with severe symptomatic AS, 58.33% women, with a mean age of 66.5±11.8 years.

Demographic, clinical and echocardiographic characteristics according to sex are displayed at tables 1 and 2. Women were older and had smaller body surface area (BSA) but similar body mass index. Chronic kidney disease (CKD) was also significantly more frequent in women, but this difference disappeared after adjustment for age. Their operative risk, estimated by Euroscore II, was also higher, reflecting older age and worse creatinine clearance. When analyzing the age-adjusted preoperative echocardiographic data, maximal aortic velocity and mean aortic gradient were similar, but indexed aortic valve area tended to be smaller in women. Total hemodynamic load, evaluated by valvuloarterial impedance (Zva), was higher in women, but peak wall stress showed no differences between genders.

*Table 1: Clinical characterization of aortic stenosis patients according to gender.*

| | | Female (n=77) | Male (n=55) | p | Age-adjusted p |
|---|---|---|---|---|---|
| **Age** | Mean (st. dev) | 69.1 (11.3) | 63.2 (12.2) | **0.005** | |
| **BMI** | Mean (st. dev) | 28.7 (4.6) | 27.7 (3.9) | 0.183 | 0.204 |
| **BSA** | Mean (st. dev) | 1.67 (0.15) | 1.87 (0.16) | **<0.001** | **<0.001** |
| **Euroscore II** | Mean (st. dev) | 2.02 (1.69) | 1.11 (0.64) | **<0.001** | **0.003** |
| **HT** | n (%) | 48 (62.3%) | 26 (48.1%) | 0.107 | |
| | Age adjusted % | 36.5% | 36.9% | | 0.518 |
| **DM** | n (%) | 16 (20.8%) | 11 (20.4%) | 0.955 | |
| | Age adjusted % | 41.1% | 34.8% | | 0.233 |
| **CKD** | n (%) | 36 (46.8%) | 12 (21.8%) | **0.003** | |
| | Age adjusted % | 42.3% | 33.8% | | 0.164 |
| **GFR** (ml/min) | Mean (st. dev) | 64.5 (19.9) | 75.3 (14.3) | **0.001** | **0.005** |
| **NYHA** ≥3 | n (%) | 20 (26.0%) | 11 (20.4%) | 0.458 | |
| | Age adjusted % | 41.0% | 35.9% | | 0.279 |
| **LVH** | n (%) | 65 (85.5%) | 39 (73.6%) | 0.091 | |
| | Age adjusted % | 36.8% | 28.5% | | 0.164 |
| **LV mass** (g) | Mean (st. dev) | 216.7 (62.0) | 248.9 (58.6) | **0.003** | **0.005** |
| **LV mass index** (g/m$^2$) | Mean (st. dev) | 129.8 (35.1) | 132.8 (30.0) | 0.618 | 0.557 |

BSA= body surface area; BMI= body mass index; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate (MDRD formula); NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy. Results are presented as mean (standard deviation) unless otherwise noted.

When comparing age-adjusted ratio of observed LVM with the predicted LVM according to load, gender and height, women had a significantly higher excessive LVM increase than men (%observed/predicted LVM: 166.45±54.28% vs 137.44±36.40%, age-adjusted p=0.016, fig.1). There was a trend for a more concentric geometry in women, with a numerically higher relative wall thickness.

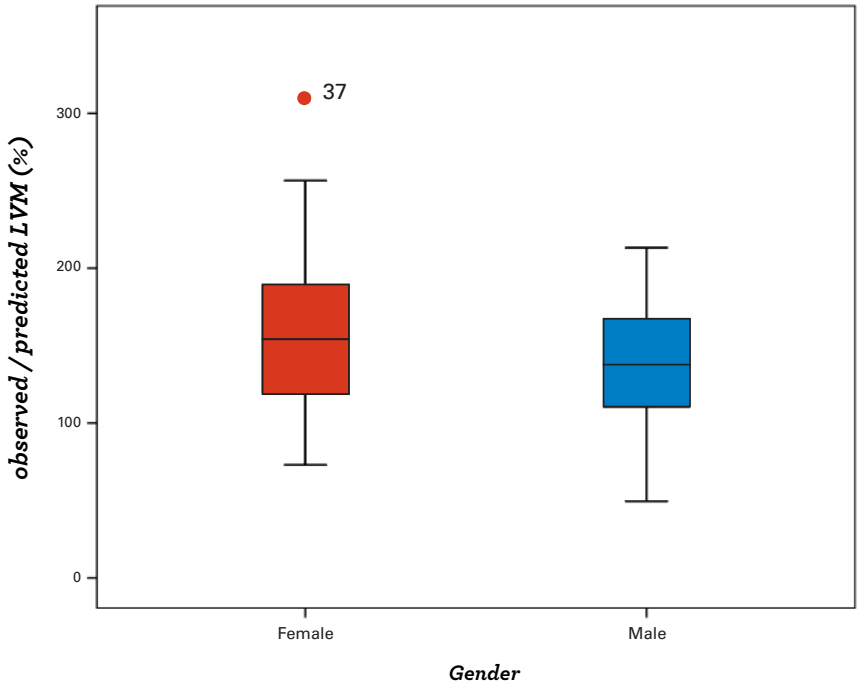


*Fig 1. Comparison by gender of the ratio of observed and predicted left ventricular mass (LVM) in aortic stenosis before valve replacement.*

*Table 2: Baseline and six months echocardiographic characterization of aortic stenosis patients according to gender, age adjusted comparisons.*

| | Female | | Age adjusted p | Male | | Age adjusted p | Age adjusted p (baseline F-M) | Age adjusted p (6 months F-M) |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 6 months | | Baseline | 6 months | | | |
| **LV geometry** | | | | | | | | |
| **LV end-diastolic diameter** (cm) | 4.53 (0.64) | 4.32 (0.48) | 0.748 | 4.91 (0.64) | 4.72 (0.51) | 0.132 | 0.007 | <0.001 |
| **LV end-systolic diameter** (cm) | 4.53 (0.64) | 4.32 (0.48) | 0.452 | 4.91 (0.64) | 4.72 (0.51) | 0.152 | 0.423 | 0.501 |
| **LV end-diastolic diameter index** | 2.75 (0.4) | 2.62 (0.31) | 0.754 | 2.64 (0.37) | 2.54 (0.36) | 0.127 | 0.143 | 0.292 |
| **LV end-systolic diameter index** | 1.80 (0.47) | 2.47 (3.43) | 0.459 | 1.77 (0.38) | 1.67 (0.34) | 0.147 | 0.558 | 0.378 |
| **Interventricular septum** (cm) | 1.44 (0.29) | 1.37 (0.26) | 0.733 | 1.43 (0.21) | 1.34 (0.19) | 0.122 | 0.818 | 0.665 |
| **Posterior wall** (cm) | 1.09 (0.17) | 1.00 (0.15) | 0.484 | 1.11 (0.17) | 1.07 (0.14) | 0.699 | 0.518 | 0.011 |
| **Relative wall thickness** | 0.49 (0.11) | 0.47 (0.09) | 0.284 | 0.46 (0.09) | 0.45 (0.08) | 0.139 | 0.137 | 0.523 |
| **LV mass index** ($g/m^2$) | 132.22 (33.79) | 110.15 (28.29) | 0.644 | 133.08 (31.73) | 115.32 (28.55) | 0.988 | 0.784 | 0.308 |
| **Observed/predicted LVM** (%) | 166.45 (54.28) | 170.15 (47.15) | 0.672 | 137.44 (36.40) | 141.36 (32.89) | 0.062 | 0.016 | 0.019 |
| **LV end-diastolic volume** (ml) | 84.31 (27.04) | 75.48 (25.2) | 0.611 | 105.74 (33.83) | 98.47 (31.13) | 0.355 | 0.002 | <0.001 |
| **LV end-systolic volume** (ml) | 36.42 (24.66) | 40.82 (16.27) | 0.190 | 42.16 (23.47) | 50.55 (18.08) | 0.147 | 0.370 | 0.019 |
| **LV end-diastolic volume index** | 51.07 (15.72) | 45.04 (13.84) | 0.591 | 55.38 (18.19) | 53.24 (17.62) | 0.296 | 0.267 | 0.015 |
| **LV end-sistolic volume index** | 21.96 (14.37) | 24.70 (9.38) | 0.227 | 22.48 (12.04) | 27.45 (10.54) | 0.182 | 0.964 | 0.268 |
| **Aortic/Prosthesis stenosis severity** | | | | | | | | |
| **Maximal transaortic velocity** (cm/s) | 473.02 (64.55) | 256.66 (68.52) | 0.012 | 458.21 (53.58) | 276.28 (72.47) | 0.014 | 0.609 | 0.646 |
| **Maximal transaortic gradient** (mmHg) | 90.30 (23.43) | 28.38 (16.31) | 0.001 | 85.64 (21.04) | 31.70 (19.11) | 0.012 | 0.567 | 0.991 |
| **Medium transaortic gradient** (mmHg) | 56.48 (15.15) | 15.63 (9.58) | 0.009 | 51.8 (12.34) | 17.4 (10.92) | 0.017 | 0.196 | 0.923 |
| **Effective orifice area index** ($cm/m^2$) | 0.39 (0.12) | 0.83 (0.25) | 0.608 | 0.45 (0.12) | 0.84 (0.24) | 0.045 | 0.094 | 0.549 |
| **Increase effective orifice area** (%) | | 136.87 (76.71) | | | 105.08 (55.44) | | | 0.070 |
| **Hemodynamic load** | | | | | | | | |
| **Valvuloarterial impedance** | 6.98 (2.47) | 6.16 (1.79) | 0.954 | 5.65 (1.51) | 4.98 (1.59) | 0.466 | 0.020 | 0.007 |
| **Peak LV wall stress** ($dynes/cm^2$) | 221.7 (84.91) | 169.22 (46.83) | 0.209 | 220.26 (46.53) | 168.07 (38.85) | 0.414 | 0.901 | 0.632 |
| **Systolic function** | | | | | | | | |
| **Ejection fraction** (%) | 60.6 (11.8) | 63.4 (8.2) | 0.370 | 61.6 (10.4) | 64.2 (9.3) | 0.227 | 0.553 | 0.449 |

Results are presented as mean (standard deviation)

## *Fibrosis and serum levels of biomarkers of extracellular matrix remodeling*

Intraoperative myocardial biopsy was performed in 56 patients (38 women), allowing for histological analysis of collagen volume fraction (CVF). The demographic data of this subgroup was similar to that of the overall group (table 3).

Women had significantly more fibrosis than men (18.98±15.20% vs 12.41±7.61%, p=0.036) at the time of valve replacement (fig.2). Since women were older, there could be the interference of age in these results. Although age tended to show a weak correlation with CVF (rs=0.25, p=0.064), when analyzing for gender, in women CVF increase was not associated with age (rs=0.13, p=0.443). Inversely, in men age positively correlates with CVF (rs=0.48, p=0.043).

*Table 3: Clinical characterization of aortic stenosis patients with collagen volume fraction measurement, according to gender.*

| | | Female (n=38) | Male (n=18) | p | Age-adjusted p |
|---|---|---|---|---|---|
| **Age** | Mean (st. dev) | 69.8 (9.8) | 58.9 (11.4) | 0.001 | |
| **BMI** | Mean (st. dev) | 28.6 (4.7) | 28.5 (2.8) | 0.900 | 0.981 |
| **BSA** | Mean (st. dev) | 1.65 (0.16) | 1.90 (0.13) | <0.001 | **<0.001** |
| **Euroscore II** | Mean (st. dev) | 1.90 (1.09) | 0.74 (0.27) | <0.001 | **0.004** |
| **HT** | n (%) | 48 (62.3%) | 26 (48.1%) | 0.107 | |
| | Age adjusted % | 35.2% | 28.6% | | 0.311 |
| **DM** | n (%) | 16 (20.8%) | 11 (20.4%) | 0.955 | |
| | Age adjusted % | 37.2% | 32.1% | | 0.355 |
| **CKD** | n (%) | 36 (46.8%) | 12 (21.8%) | 0.003 | |
| | Age adjusted % | 41.3% | 25.0% | | 0.117 |
| **GFR** (ml/min) | Mean (st. dev) | 59.3 (19.9) | 80.3 (14.9) | **<0.001** | 0.003 |
| **NYHA≥3** | n (%) | 20 (26.0%) | 11 (20.4%) | 0.458 | |
| | Age adjusted % | 41.1% | 29.3% | | 0.197 |
| **LVH** | n (%) | 65 (85.5%) | 39 (73.6%) | 0.091 | |
| | Age adjusted % | 38.4% | 27.9% | | 0.220 |
| **LV mass** (g) | Mean (st. dev) | 203.3 (55) | 262.8 (54.4) | <0.001 | **<0.001** |
| **LV mass index** ($g/m^2$) | Mean (st. dev) | 123.2 (30.7) | 138.5 (29.6) | 0.084 | **0.033** |

BSA= body surface area; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate (MDRD formula); NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy. Results are presented as mean (standard deviation) unless otherwise noted.

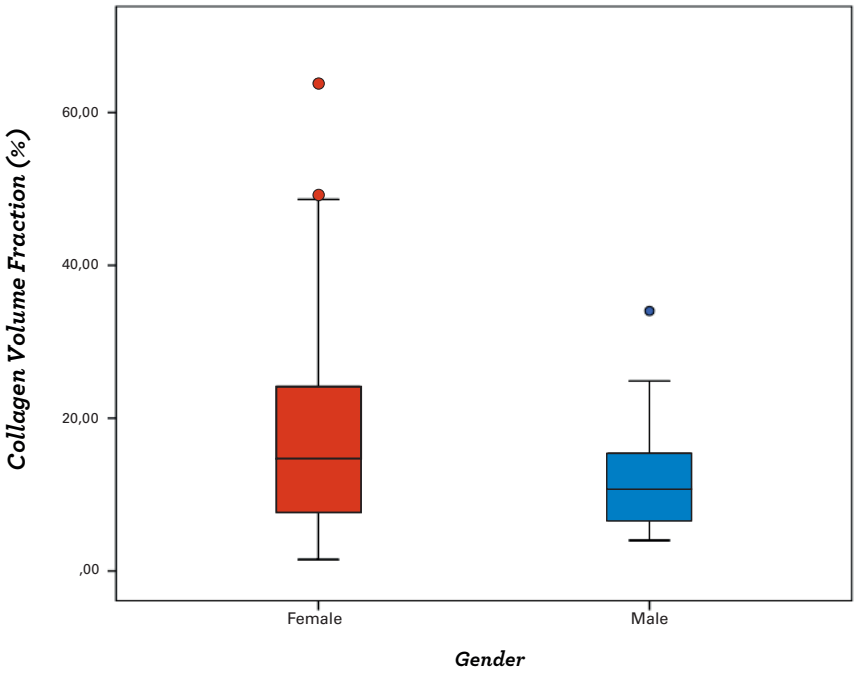


*Fig 2. Comparison by gender of collagen volume fraction (%) measurement by picrosirius-red staining of 56 myocardial biopsies.*

Forty patients had determination of serum biomarkers of extracellular matrix (ECM) remodeling (see table 4 for demographic characterization). There were no differences between genders in serum CTGF, TGF β, MMP1 and MMP9, as well as TIMP 1 and TIMP2 levels (table 5). Female gender had a trend for higher serum levels of PIP (775.7±250.2 ng/ml vs 649.1±94.1 ng/ml, p=0.067), reflecting higher myocardial synthesis of collagen type I. Moreover, in women baseline indexed LVM positively correlated with TIMP1/MMP1 levels ($r_s$=0.681, p=0.003) and tended to correlate with PIP levels ($r_s$=0.465, p=0.052). The development of excessive LVM in women also tended to correlate with higher levels of TIMP1/MMP1 ($r_s$=0.582, p=0.060), favoring collagen deposition. An opposite trend was seen in men ($r_s$=-0.617, p=0.077). Importantly, female patients with higher baseline levels of TIMP2 and TIMP1/MMP1 experienced significantly less 6 months LVM regression, but this pattern was not seen in men (table 6).

*Table 4: Clinical characterization of aortic stenosis patients with serum biomarkers measurement, according to gender.*

| | | Female (n=26) | Male (n=14) | p | Age-adjusted p |
|---|---|---|---|---|---|
| **Age** | Mean (st. dev) | 69.6 (9.0) | 64 (11.9) | 0.066 | |
| **BMI** | Mean (st. dev) | 28.7 (4.8) | 28.4 (4.8) | 0.832 | 0.108 |
| **BSA** | Mean (st. dev) | 1.68 (0.16) | 1.91 (0.17) | **<0.001** | <0.001 |
| **Euroscore II** | Mean (st. dev) | 1.81 (1.18) | 1.08 (0.70) | 0.029 | 0.168 |
| **HT** | n (%) | 23 (60.5%) | 6 (40.0%) | 0.176 | |
| | Age adjusted % | 60.5% | 46.4% | | 0.176 |
| **DM** | n (%) | 11 (28.9%) | 2 (13.3%) | 0.305 | |
| | Age adjusted % | 28.8% | 16.2% | | 0.171 |
| **CKD** | n (%) | 15 (39.5%) | 2 (12.5%) | 0.051 | |
| | Age adjusted % | 38.0% | 15.5% | | 0.052 |
| **GFR** (ml/min) | Mean (st. dev) | 65.8 (19.4) | 71.8 (12.8) | 0.291 | 0.537 |
| **NYHA≥3** | n (%) | 8 (21.1%) | 3 (20.0%) | 0.932 | |
| | Age adjusted % | 19.4% | 26.4% | | 0.713 |
| **LVH** | n (%) | 34 (91.9%) | 13 (81.3%) | 0.351 | |
| | Age adjusted % | 92.4% | 81.1% | | 0.113 |
| **LV mass** (g) | Mean (st. dev) | 212.8 (46.7) | 260.1 (57.9) | **0.003** | 0.005 |
| **LV mass index** (g/m$^2$) | Mean (st. dev) | 126.6 (25.5) | 135.5 (27.0) | 0.256 | 0.231 |

BSA= body surface area; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate (MDRD formula); NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy. Results are presented as mean (standard deviation) unless otherwise noted.

*Table 5: Serum biomarkers levels in aortic stenosis patients according to gender.*

| | Female | Male | p |
|---|---|---|---|
| **MMP9** (ng/ml) | 157.8 (145.7) | 195.5 (189.5) | 0.669 |
| **MMP1** (ng/ml) | 2.98 (1.53) | 3.21 (2.02) | 0.633 |
| **TIMP2** (ng/ml) | 6.88 (13.46) | 5.59 (11.62) | 0.926 |
| **TIMP1** (ng/ml) | 124.4 (48.4) | 132.4 (77.8) | 0.925 |
| **TIMP1 / MMP1** | 60.20 (25.92) | 65.37 (36.93) | 0.920 |
| **TIMP1 / MMP9** | 1.87 (1.57) | 1.57 (1.54) | 0.715 |
| **TIMP2 / MMP1** | 11.84 (9.82) | 4.91 (4.34) | 0.355 |
| **TIMP2 / MMP9** | 0.29 (0.15) | 0.23 (0.22) | 0.669 |
| **PIP** (ng/ml) | 775.7 (250.2) | 649.1 (94.1) | 0.067 |

MMP= metalloproteinase; TIMP= tissue inhibitor of metalloproteinase; PIP= procollagen type I carboxy-terminal peptide. Results are presented as mean (standard deviation)

## *6 months follow-up*

At 6 months follow-up, overall there was a significant absolute decrease in LVM index of 20.22±30.83 g/m$^2$ and a relative decrease of 12.04±25.52%. Even so, 56.3% of patients still had residual LVH after AVR.

*Table 6: Correlation of ECM serum biomarkers with baseline left ventricular mass (LVM) and LVM regression in aortic stenosis patients according to gender*

| | LV mass index (g/m²) | | | | %observed/predicted LVM | | | | LVM regression | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | | Male | | Female | | Male | | female | | Male | |
| | rs | P | rs | P | rs | p | rs | p | rs | p | rs | p |
| **MMP9** | 0.083 | 0.729 | 0.175 | 0.587 | -0.044 | 0.887 | 0.503 | 0.138 | -0.004 | 0.990 | 0.262 | 0.531 |
| **MMP1** | -0.201 | 0.439 | 0.409 | 0.241 | 0.045 | 0.894 | 0.555 | 0.121 | 0.497 | 0.101 | -0.086 | 0.872 |
| **TIMP2** | 0.369 | 0.145 | -0.272 | 0.419 | -0.189 | 0.578 | 0.274 | 0.476 | -0.650 | **0.022** | 0.045 | 0.924 |
| **TIMP1** | 0.133 | 0.425 | 0.024 | 0.931 | 0.249 | 0.210 | -0.178 | 0.543 | -0.230 | 0.221 | -0.259 | 0.417 |
| **TIMP1 / MMP1** | 0.681 | **0.003** | -0.164 | 0.651 | 0.582 | 0.060 | -0.617 | 0.077 | -0.748 | **0.005** | -0.600 | 0.208 |
| **TIMP1 / MMP9** | 0.009 | 0.972 | -0.018 | 0.958 | 0.126 | 0.697 | -0.527 | 0.117 | -0.029 | 0.923 | -0.607 | 0.148 |
| **PIP** | 0.465 | 0.052 | 0.224 | 0.533 | 0.406 | 0.191 | 0.250 | 0.516 | -0.467 | 0.108 | -0.771 | 0.072 |

MMP= metalloproteinase; TIMP= tissue inhibitor of metalloproteinase; PIP= procollagen type I carboxy-terminal peptide

Women continued to have higher Zva, although they tended to have a greater relative increase in indexed effective orifice area (EOAi). There were no differences between genders in indexed LVM decrease (22.07±28.22 g/m$^2$ in women vs 27.35±33.04 g/m$^2$ in men, p=0.76), but at 6 months women continued to have a higher excess in LVM than men (% observed/predicted LVM: 170.15±47.15% vs 141.36±32.89%, age-adjusted p=0.019). Moreover, after surgery women more frequently had residual LVH (68.8% vs 39.6%, age-adjusted p=0.002) and regression of LVH was impaired when compared with men (LVH improvement: 33.3% vs 61.7%, p=0.003).

In a multivariate model for predicting residual LVH after surgery (table 7), including gender, age, arterial hypertension (HT), diabetes mellitus (DM), CKD, baseline LVM (continuous variable or divided in terciles) and % observed/predicted LVM (continuous variable or dichotomized >142%), only the female gender [OR: 4.83(1.58-14.77)], hypertension [OR: 3.39(1.23-9.36)] and baseline LVM [OR: 1.04(1.02-1.06) per 1 g/m$^2$ increase] were independent predictors of residual LVH after surgery. A higher baseline valvuloarterial impedance, although associated with persistent LVH, did not reach statistical significance. The combination of being female, a history of HT and the baseline LVM (continuous- model 1, or the second or third upper tercile- model 2) had a high predictive value for residual LVH [model 1 AUC 0.836 (95%CI:0.761-0.911), model 2 AUC 0.823 (95%CI:0.744-0.903), fig3].

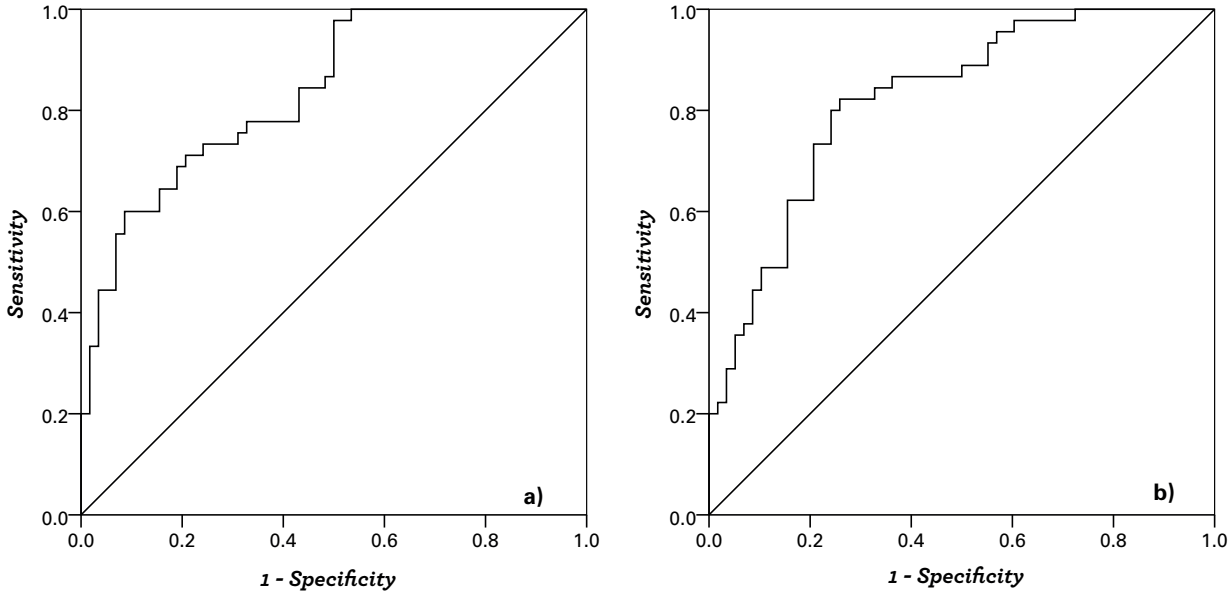


*Fig 3. ROC curve for the multivariate logistic model for residual LVH: a) Model 1 (arterial hypertension, female gender, basal indexed left ventricular mass) ; b) Model 2 (arterial hypertension, female gender, second or third upper terciles of basal indexed left ventricular mass).*

*Table 7: Univariate analysis and multivariate model for predicting residual LVH six months after surgery.*

| | p | Unadjusted OR (95%CI for OR) | p | Adjusted OR (95%CI for OR) Model 1 | p | Adjusted OR (95%CI for OR) Model 2 |
|---|---|---|---|---|---|---|
| **Age** (years) | **0.033** | 1.037 (1.003-1.072) | | | | |
| **HT** | **0.007** | 2.850 (1.327-6.119) | **0.018** | 3.390 (1.228-9.357) | **0.048** | 2.72 (1.009-7.334) |
| **DM** | 0.355 | 1.517 (0.627-3.669) | | | | |
| **CKD** | 0.322 | 1.452 (0.695-3.034) | | | | |
| **Sex** (female) | **0.002** | 3.293 (1.534-7.070) | **0.006** | 4.834 (1.582-14.769) | **0.013** | 3.685 (1.31-10.365) |
| **Basal valvuloarterial impedance** | **0.016** | 1.332 (1.055-1.682) | 0.057 | 1.311 (0.992-1.731) | 0.068 | 1.310 (0.980-1.753) |
| **Observed/predicted LVM** (%) | **<0.001** | 1.024 (1.012-1.036) | | | | |
| **Observed/predicted** LVM > 142% | **0.001** | 4.133 (1.808-9.447) | | | | |
| **Basal LV mass index** ($g/m^2$, per 1 $g/m^2$) | **<0.001** | 1.024 (1.011-1.038) | **<0.001** | 1.040 (1.020-1.061) | | |
| **Basal LV mass index** ($g/m^2$) <113 F or <118 M | **0.002** | 1 | | | <0.001 | 1 |
| **Basal LV mass index** ($g/m^2$) 113-139 F or 118-144 M | **0.011** | 3.580 (1.340-9.564) | | | 0.005 | 6.037 (1.713-21.275) |
| **Basal LV mass index** ($g/m^2$) >139 F or > M | **0.001** | 5.859 (2.153-15.943) | | | <0.001 | 14.022 (3.714-52.934) |
| | | | **AUC (95%CI): 0.836 (0.761-0.911)** | | **AUC (95%CI): 0.823 (0.744-0.903)** | |

HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; LV= left ventricle; F= female; M=male

## Discussion

In our study, women had a significantly higher excess in LVM than men, considering what would be expected according to height, load and gender. Likewise, six months after AVR, women had more residual LVH. Moreover, women had a trend for higher serum levels of PIP than men, reflecting higher synthesis of collagen type I, and only women had imbalance ofTIMP1/MMP1 favoring inhibition of collagen degradation, which positively correlates with baseline LVM values. At histological level women with AS presented more interstitial fibrosis than their men counterparts, confirming their plasmatic ECM biomarker profile.

### *Relevance of inappropriate left ventricular mass increase*

In this study we describe gender differences in hypertrophic response, with a higher excess of LVM in women. Some degree of hypertrophy is expected under chronic pressure overload to normalize wall stress and preserve systolic function. But in some patients this increase in LVM is maladaptive and produces an unfavorable concentric phenotype associated with diastolic dysfunction and worse prognosis (20). The appropriateness of LVM increase, measured by the ratio between the observed and the predicted LVM (considering workload, gender and body size), has been proposed as valuable tool to help to distinguish between compensatory from maladaptive (and often irreversible) left ventricular hypertrophy (4). Inappropriate LVM has been associated with worse cardiovascular outcomes both in hypertension (21) and asymptomatic severe AS (5). Moreover, its presence was associated with concentric LV geometry and LV systolic and diastolic abnormalities, even in the absence of LV hypertrophy (22), supporting the hypothesis that it can identify a more advanced stage of myocardial disease, probably beyond the compensatory phase. But the problem remains in its definition, since there is no consensus in its cutoff value and different cutoffs have been used in different populations (5,22,23). For this reason we used % observed/predicted LVM as a continuous variable in our analysis.

Considering the worse outcomes of patients with inappropriate LVM, known from previous studies, early surgery could be considered in AS patient with an excessive hypertrophic response, in particular in women. Its cutoff value is yet to be determined.

### *Gender differences in left ventricular remodeling in aortic stenosis*

Elderly women with AS respond to pressure overload with smaller, more hypertrophic and stiffer ventricles, and often have supranormal ejection fraction (8,12). Conversely, men have higher levels of wall stress and worse systolic function than women under similar load conditions (8). These distinctive LV remodeling responses to pressure overload can be partially explained by the effect of sex hormones. Estrogens seem to have antiproliferative effects on cardiac fibroblasts (15) and vascular smooth–muscle cells, while androgens have opposite effects (24). In animal models, estrogens down regulate proliferation of cardiac fibroblasts and gene expression of collagens type I and III in female, but have opposite

effect in male (14,25). Therefore, estrogens may prevent the up regulation of collagen in women with pressure overload until menopause. Given that older patients have relative hypogonadal hormone concentrations, with a decrease in estrogens and ovarian production of androgens in postmenopausal women, it is expected that this protective effect is lost with aging in women.

A faster early regression of LVM has been described in women, and their lower gene expression of collagen I and III and MMP2 (in a 10 patients subgroup) was considered as a possible explanation (14). In a comparable cohort of AS patients, we found that women had a similar degree of LVM regression but more residual LVH than men 6 months after AVR. This apparent contradiction may be due to the existence of coronary artery disease (CAD) and differences in the moment of evaluation of regression. In the study by Petrov et al (14) there was a trend for higher prevalence of CAD in men. The coexistence of CAD can influence regression and it was an exclusion criteria in our study. Moreover, results may be different if the evaluation is performed in the first days after AVR or at six months after surgery. One can speculate that women might regress faster than men before discharge after AVR, but there can be a slower but more significant regression overall in men.

Postoperative changes in myocardial structure are characterized by an initial decrease in muscle fiber diameter and a relative increase in interstitial fibrosis, whereas in a later phase, LVM regression continues more slowly, with no further change in muscle fiber diameter but an additional reduction in collagen volume fraction (26). Therefore, differences on gene expression of ECM components are unlikely to be responsible for early regression, but it is plausible that they can influence later remodeling. The presence of a profibrotic pattern of ECM biomarkers and evidence of more fibrosis in surgical biopsies of our elderly AS women can explain the existence of a more inappropriate increase in LVM and the persistence of LVH after AVR, since this is the myocardial component that takes longer to regress and some of these changes can even be irreversible.

## Limitations

This is a prospective observational study and we were unable to match for age and body surface area between genders. Still, age-adjustment was performed for clinical and echocardiographic parameters and differences were considered in the multivariate analysis. The small sample size and the fact that determinations of plasmatic biomarkers and fibrosis were done in only some patients, may have limited our ability to find differences. However, this is a frequent constraint in similar studies, and only multi-center registries or large scale randomized clinical trials can overcome this limitation.

## Conclusions

Among AS patients, women have a higher excess in hypertrophic response than men under similar workload conditions, and female gender is an independent predictor of residual hypertrophy after AVR. A gender-specific ECM remodeling, favoring interstitial fibrosis in women, might help to explain these differences. Identifying potential causes for gender differences in LV remodeling may raise hypothesis for distinct therapeutic interventions.

## Disclosures

Authors have no conflicts of interest do disclose.

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# RESULTS

## 5.3. Relevance of residual left ventricular hypertrophy after surgery for isolated aortic stenosis

## 5.3 Relevance of residual left ventricular hypertrophy after surgery for isolated aortic stenosis

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## Abstract

**Objectives:** To evaluate if residual left ventricular (LV) hypertrophy is associated with clinical outcomes after aortic valve replacement (AVR) for severe aortic stenosis (AS).

**Background:** Persistent LV hypertrophy (LVH) after surgery is frequent but its clinical relevance is controversial.

**Methods:** We analyzed clinical and echocardiographic parameters before and after AVR, in a prospective cohort of 132 severe AS patients. Mean follow-up was 6.0±1.5 years. Clinical endpoints were all-cause death and combined all-cause death and non-fatal cardiovascular hospitalization. At time of AVR, myocardial biopsies for collagen volume fraction (CVF) evaluation were done in 56 random patients.

**Results:** Residual LVH was present in 44% of patients after AVR. Patients with residual LVH were older, more frequently women and had hypertension (HT). Preoperatively, they had higher indexed LV mass (LVMI), higher E/e' and indexed left atrial volume, as well as lower peak systolic annular velocity (Sm). Female gender, HT, LVMI and E/e´ were independent predictors of persistent LVH. CVF at the time of surgery was higher in those with residual LVH (20.0±14.6% vs 13.2±11.5%, p=0.027). The risk of all-cause death and non-fatal cardiovascular hospitalization was higher in patients with residual LVH [OR 2.89 (95%CI:1.12-7.44); p=0.035], but there were no differences in all-cause mortality. Residual LVH was associated with a worse outcome in women but not in men.

**Conclusions:** Residual LVH after AVR is common and is associated with worse prognosis, particularly in women. In addition, HT, higher baseline LVM and worse diastolic dysfunction can help to identify patients at risk for incomplete mass normalization.

## Introduction:

In chronic pressure overload, left ventricular hypertrophy (LVH) is an adaptive mechanism that contributes for normalization of systolic wall stress [1, 2]. In aortic stenosis (AS) patients, severe LVH is related with worse left ventricular (LV) function and higher early and late mortality, even after successful aortic valve replacement (AVR) [3, 4]. Likewise, incomplete regression of LVH, commonly observed in these patients [5, 6], may be a marker of irreversible remodeling and, as so, of worse prognosis.
Our aim was to evaluate the prognostic impact of residual LVH late after surgery and identify baseline independent predictors of its occurrence.

## Methods:

### *Patient selection and follow-up*

Between January 2006 and December 2009 we included 141 consecutive patients over 18 years old with severe symptomatic AS (aortic valve area <1 $cm^2$ or mean transaortic gradient ≥40 mmHg) referred for AVR at the Cardiothoracic Surgery Department of Centro Hospitalar São João, Porto, Portugal. We excluded patients with aortic regurgitation >II/IV or other significant valve diseases (>mild), significant coronary artery disease (lesions >50% on coronary angiography) or previous cardiac surgery. All patients were in sinus rhythm at the time of inclusion. From the initial 141 patients, 132 were considered for this prospective analysis: one was refused for surgery, other died before surgery from non-cardiovascular reason (cholangitis with sepsis), and there was incomplete clinical data in seven of them. All patients had clinical follow-up up-to 8.2 years and echocardiographic follow up was achieved in 123 (93.2%) patients. There were 2 perioperative deaths (one fatal stroke and one due to sepsis) and 3 sudden deaths in the first 30 days after surgery. These patients had no echocardiographic evaluation after AVR. Four additional patients refused coming to our hospital for echocardiographic evaluation, 3 of them were alive at the end of follow-up but one had died 4 months after surgery from non-cardiovascular cause). Mean follow-up was 6.0±1.5 years for clinical outcomes and final echocardiographic evaluation was performed 5.0±2.2 years after surgery.

Clinical endpoints were defined as all-cause of death and a composite of all-cause of death or non-fatal cardiovascular hospitalization (heart failure, myocardial infarction, re-operation for prosthesis dysfunction, new-onset atrial fibrillation or advanced AV block requiring hospitalization).

### *Surgical technique*

All surgeries were performed using standard procedure for AVR. The patients were placed on cardiopulmonary bypass and cardiac arrest was induced and maintained with cold blood cardioplegia. The

majority of patients received a bioprosthesis (73.3%). Two patients also had ascending aorta aneurism and underwent aortic root replacement with valved composite grafts (Bentall technique). At the time of surgery, 56 random patients underwent myocardial biopsy from the LV interventricular septum.

## *Echocardiographic studies*

Echocardiographic examination was performed by a trained cardiologist and recorded on digital support. All recordings were examined by an experienced echocardiographer, in an accredited independent echocardiography laboratory (Hospital Clínico San Carlos in Madrid, Spain), blinded to patient details. Studies were performed using Phillips IE-33 equipment with a S5-1 transducer and M-mode, two dimensional, pulsed, continuous, color-flow and tissue Doppler capabilities. Correct orientation of imaging planes, cardiac chambers dimensions and function measurements were performed according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations [7].

LV mass was estimated according to the joint recommendations of the ASE and EAE using Devereux's formula for ASE measurements in diastole: LV mass=0.8 x (1.04 x ([LV internal dimension + posterior wall thickness + interventricular septal thickness] $^3$ – [LV internal dimension] $^3$) + 0.6 g . Left ventricular hypertrophy was defined by LV mass index greater than 115 g/m$^2$ in men and greater than 95 g/m$^2$ in women.

Relative wall thickness (RWT) was calculated for the assessment of LV geometry using the formula 2x posterior wall thickness/ LV diastolic diameter. Increased RWT was present when this ratio was greater than 0.42. Left atrium (LA) volume was measured at LV end-systole in the frame preceding mitral valve opening. The volume was measured using the biplane area length method and corrected for body surface area. Aortic valve area was estimated using quantitative Doppler by the continuity equation.

Mitral inflow was assessed in the apical 4-chamber view using pulsed wave Doppler with the sample volume placed at the tips of mitral leaflets during diastole. From the mitral inflow profile, the peak flow velocity of early filling (E wave), peak flow velocity of atrial contraction (A wave), the E/A ratio, and early filling deceleration time (DT) were measured. Doppler tissue imaging (DTI) of the mitral annulus was obtained from the apical 4-chamber using a sample volume placed in the septal mitral valve annulus. Peak systolic annular velocity (Sm) and early diastolic septal velocity (e') were determined, and the E/e' ratio was derived. As a measure of global LV load, we calculated the valvuloarterial impedance: Zva =SAP+ MG)/SVI, where SAP is the systolic arterial pressure and MG is the mean transvalvular pressure gradient and SVI is stroke volume index.

## *Histological determination of fibrosis*

Light microscopic quantification of fibrosis has previously been described and validated. Fibrosis analysis of myocardial biopsies was performed using picrosirius-red–stained, 4-µm-thick-sections of tissue (± 5 sections of each sample). Images of these sections were acquired with a projection microscope

(x50). Subsequent image analysis with Slidebook 4.0 software (3I, Denver, Colo) was performed to determine the extent of reactive interstitial fibrosis, which was expressed as collagen volume fraction (%). Areas of reparative and perivascular fibrosis were excluded. Myocardial fibrosis was calculated as the sum of all connective tissue areas divided by the sum of connective tissue and muscle areas averaged over 4 to 6 representative fields of the section of 56 random AS patients (18 male and 38 female). In our laboratory, normal values of fibrosis for LV myocardial biopsy material are 5.4±2.2% [8].

### *Statistical analysis*

Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation, unless otherwise specified. Continuous variables were compared between groups using an unpaired t-test (for normally distributed variables) or the Mann–Whitney U-test (for non-normally distributed variables). For comparison between baseline and follow-up a paired Student's t-test was applied or a Wilcoxon test (for non-normally distributed variables). Chi-square test (or Fisher exact test) was used to compare categorical variables. Spearman's rank correlation was used for the assessment of correlations between LVM index and its variation and clinical, echocardiographic and molecular continuous variables. Univariable and multivariable binary logistic regression models (Wald backward stepwise method, p=0.05 for covariate inclusion and p=0.20 for exclusion) were used for predicting residual LVH and the outcome of all-cause death and cardiovascular hospitalization. The Kaplan-Meier and Cox models were used to evaluate survival times after surgery for both all-cause death and for all-cause death and cardiovascular hospitalization, and the log-rank test was used to compare survival curves. All reported probability values are two-tailed, and P <0.05 was considered statistically significant. Analyses were performed with the IBM® SPSS® Statistics software package (version 21.0) (SPSS Inc, Chicago, IL, USA).

## Results

The study group included 132 patients with a mean age of 66±12 years, 58% were women and 81% had left ventricular hypertrophy (LVH) before surgery. Most patients had preserved ejection fraction (EF) with a mean value of 62±10%. Detailed demographic, clinical and echocardiographic (before and after AVR) characterization can be found in tables 1 and 2.

A bioprosthesis was implanted in 73% of patients and in all cases that a mechanical valve was chosen it was bileaflet. The valve size was >21 mm in 42% of cases (size 19 mm: 14; size 20 mm: 1; size 21 mm: 61; size 23 mm: 37; size 25 mm: 19). There were 2 perioperative deaths (1.5%), and the median time of hospitalization was 6 days. Non-fatal post-surgery complications were atrial fibrillation in 30 patients (22.7%), perioperative renal failure in 24 patients (defined as a fall in GFR >25% from baseline, 18.2%), pacemaker implantation due to AV block in 9 patients (6.8%), bleeding needing surgical reexploration in 2 patients (1.5%), stroke in 2 patients (1.5%), and respiratory infection in 2 patients (1.5%). No patient needed inotropic support beyond 24 hours.

A final echocardiographic evaluation was performed 5.0±2.2 years after surgery. After AVR, we observed significant improvement in transprosthetic gradients, in EOAI and a significant reduction in LVMI (table 2), but 54 (44 %) of patients still had LVH.

*Table 1: Clinical characterization of aortic stenosis (AS) patients.*

| | **AS patients (n=132)** |
|---|---|
| **Age** | 66.6 (±12.0) |
| **Female** | 77 (58%) |
| **BSA** | 1.8 (±0.2) |
| **Euroscore II** | 1.6 (±1.4) |
| **HT** [n (%)] | 74 (56.5%) |
| **DM** [n (%)] | 27 (20.6%) |
| **CKD** [n (%)] | 48 (36.4%) |
| **GFR** (ml/min) | 68.9 (±18.5) |
| **NYHA≥3** [n (%)] | 31 (23.7%) |
| **LVH baseline** [n (%)] | 104 (80.6%) |

BSA= body surface area; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate; NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy.

*Table 2: Baseline and follow-up echocardiographic characterization of aortic stenosis (AS) patients.*

| | **Baseline** | **Follow-up** | **p*** |
|---|---|---|---|
| **LV geometry** | | | |
| **Interventricular septum** (cm) | 1.46 (±0.25) | 1.33 (±0.2) | **<0.001** |
| **Posterior wall** (cm) | 1.09 (±0.18) | 0.99 (±0.16) | **<0.001** |
| **Relative wall thickness** | 0.47 (±0.1) | 0.43 (±0.08) | **<0.001** |
| **LV mass index** ($g/m^2$) | 130.95 (±32.47) | 107.8 (±31.23) | **<0.001** |
| **LV end-diastolic volume index** ($ml/m^2$) | 51.48 (±16.08) | 50.61 (±17.86) | 0.282 |
| **LV end-systolic volume index** ($ml/m^2$) | 20.75 (±11.85) | 20.08 (±11.83) | 0.530 |
| **Aortic/Prosthesis stenosis severity** | | | |
| **Maximal transaortic velocity** (cm/s) | 463.78 (±60.92) | 261.69 (±59.06) | **<0.001** |
| **Medium transaortic gradient** (mmHg) | 54.25 (±14.34) | 15.95 (±7.11) | **<0.001** |
| **Effective orifice area index** ($cm/m^2$) | 0.42 (±0.12) | 0.86 (±0.24) | **<0.001** |
| **Increase in effective orifice area** (%) | 121.79 (±85.36) | | |
| **Hemodynamic load** | | | |
| **Valvuloarterial impedance** (mm $Hg/ml/m^2$) | 6.43 (±2.29) | 5.45 (±1.92) | **0.003** |
| **Peak LV wall stress** ($dynes/cm^2$) | 226.17 (±78.61) | 175.58 (±44.5) | **<0.001** |
| **Systolic function** | | | |
| **Ejection fraction** (%) | 61.68 (±10.3) | 63.58 (±7.68) | 0.114 |
| **Peak systolic annular velocity** (cm/s) | 5.49 (±1.24) | 6.3 (±1.31) | **<0.001** |

Values are mean (±SD) unless otherwise indicated. **Bold values** indicate statistical significance. * Wilcoxon test

## *Residual left ventricular hypertrophy after AVR*

Patients with residual LVH after AVR were older (69.2±10.6 vs 64.5±12.5 years, p=0.036), more frequently women (72.2% vs 27.8%; p=0.002), hypertensive (71.7% vs 28.3%; p=0.006), had higher surgical risk and tended to have lower glomerular filtration rate (GFR) (table 3).

*Table 3: Patients Clinical and Echocardiographic characterization, by the presence of residual left ventricular hypertrophy after surgery.*

| | | Normal LVM | Residual LVH | p* |
|---|---|---|---|---|
| **Age** | mean (±SD) | 64.5 (±12.5) | 69.2 (±10.6) | **0.029** |
| **Women** | n (%) | 30 (44.1%) | 39 (72.2%) | **0.002** |
| **HT** | n (%) | 32 (47.1%) | 38 (71.7%) | **0.006** |
| **DM** | n (%) | 12 (17.6%) | 13 (24.5%) | 0.354 |
| **euroSCORE II** | mean (±SD) | 1.33 (±0.78) | 2.13 (±1.97) | **0.003** |
| **GFR** (ml/min) | mean (±SD) | 71.5 (±16.6) | 65.9 (±20.6) | 0.093 |
| **LVEDVI** (ml/ $m^2$) | mean (±SD) | 52.0 (±15.5) | 53.5 (±17.1) | 0.745 |
| **LVESVI** (ml/ $m^2$) | mean (±SD) | 20.0 (±9.4) | 23.5 (±14.6) | 0.347 |
| **RWT** | mean (±SD) | 0.48 (±0.1) | 0.47 (±0.11) | 0.730 |
| **LVMI** (g/$m^2$) | mean (±SD) | 121.2 (±27.3) | 143.5 (±34.7) | **<0.001** |
| **ΔLVMI** (g/ $m^2$) | mean (±SD) | -33.5 (±28.7) | -10.3 (±41.8) | **0.001** |
| **LAVI** (ml/ $m^2$) | mean (±SD) | 31.7 (±10.9) | 40.8 (±13.7) | **<0.001** |
| **Max Ao vel** (m/s) | mean (±SD) | 454.9 (±55.3) | 466.1 (±63.9) | 0.307 |
| **EOAI** ($cm^2/m^2$) | mean (±SD) | 0.42 (±0.13) | 0.41 (±0.11) | 0.511 |
| **ΔEOAI** (%) | mean (±SD) | 123.4 (±88.1) | 119.8 (±82.8) | 0.923 |
| **Zva** | mean (±SD) | 5.92 (±1.56) | 6.91 (±2.27) | **0.019** |
| **SVI** ($ml/m^2$) | mean (±SD) | 32.3 (±7.7) | 29.6 (±8.2) | 0.111 |
| **Sm** (cm/s) | mean (±SD) | 214.1 (±59.4) | 231.8 (±81.3) | **0.001** |
| **E/e'** | mean (±SD) | 14.5 (±5.2) | 19.0 (±7.4) | **0.002** |
| **EF** (%) | mean (±SD) | 63.1 (±8.5) | 58.6 (±12.3) | **0.030** |
| **PPM** | n (%) | 33 (50.8%) | 27 (52.9%) | 0.816 |

HT= hypertension; DM= diabetes mellitus; GFR= glomerular filtration rate (MDRD formula) ; LVEDVI = left ventricle end-diastolic volume index; LVESVI= left ventricle end-systolic volume index; RWT=relative wall thickness; LVMI= left ventricular mass index; Δ LVMI= baseline LVMI-final LVMI; LAVI= left atrial volume index; Max Ao vel= maximal aortic velocity; EOAI= effective orifice area index; Δ EOAI= (baseline EOAI-final EOAI)/baseline EOAI*100; Zva= valvuloarterial impedance; SVI= stoke volume index; Sm= peak systolic annular velocity; EF= ejection fraction; PPM= patient prosthesis mismatch; *Mann-Whitney test, except for age and EF where the t-Student test was used, n (%) compared using Chi-square test.

When analyzing baseline echocardiographic parameters, patients with residual LVH had higher baseline left ventricular mass index (LVMI) and valvuloarterial impedance (Zva), but no differences in mean gradient or indexed aortic valve area (AVAI). Moreover, these patients had worse diastolic function with higher values of E/e' and indexed LA volume, as well as worse LV systolic function, given the lower peak systolic annular velocity (Sm) and lower EF (table 1). Patients with residual LVH had less relative mass regression after surgery, with a median decrease of 8.3% (P25-75: 21.9%-6.5%) vs 25.7% (P25-75: 41.3%-13.9%) in those with normalization of LV mass (p<0.001). The frequency of PPM (defined as an indexed effective orifice area ≤0.85 $cm^2$) was not different in patients with and without residual LVH (52.9% vs 50.8%; p=0.82).

In a multivariate Cox regression model (table 4), including age, gender, hypertension (HT), Euroscore II value, baseline GFR, and baseline LVMI, Zva, Sm, EF and E/e' (table 2), the independent predictors of residual LVH were female gender, history of hypertension, higher baseline LVMI and higher LV filling pressures evaluated by E/e'. Lower values of peak systolic annular velocity also showed a trend to predict residual LVH.

*Table 4: Univariate and multivariate Cox regression analyses for prediction of residual left ventricular hypertrophy.*

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95%CI) | p | OR (95%CI) | p |
| **Gender** (Female) | 3.293 (1.534-7.070) | **0.002** | 3.797 (1.047-13.771) | **0.042** |
| **Age** (10 years increment) | 1.439 (1.031-2.010) | **0.033** | | |
| **HT** (Yes) | 2.850 (1.327-6.119) | **0.007** | 4.160 (1.255-13.792) | **0.020** |
| **Euroscore II** | 1.768 (1.179-2.650) | **0.006** | | |
| **GFR** (MDRD) | 0.984 (0.964-1.004) | 0.112 | | |
| **LVMI** (10 units increment) | 1.272 (1.113-1.454) | **<0.001** | 1.350 (1.067-1.708) | **0.010** |
| **Zva** | 1.332 (1.055-1.682) | **0.016** | | |
| **EF** | 0.958 (0.922-0.996) | **0.030** | | |
| **E/e'** (1 unit increment) | 1.123 (1.049-1.201) | **0.001** | 1.117 (1.024-1.219) | **0.013** |
| **Sm** (1 unit decrease) | 1.969 (1.311-2.957) | **0.001** | 1.653 (0.990-2.755) | 0.055 |
| **CVF** ≥15.4% | 5.2 (1.475-18.332) | **0.010** | 7.076 (1.406-35.604) | **0.018** |

## Fibrosis and residual hypertrophy after surgery

Fifty-six random patients underwent myocardial biopsy at the time of surgery for fibrosis determination. There were no clinical differences between these patients and the overall group (table 5).

*Table 5: Clinical and echocardiographic characterization of aortic stenosis patients with and without fibrosis determination.*

| | Without fibrosis determination | With fibrosis determination | p* |
|---|---|---|---|
| | n=76 (57.6%) | n=56 (42.4%) | |
| **Echo Follow-up time** (months) | 70.1±16.6 | 71.5±14.7 | 0.45 |
| **Age** | 66.9 ±12.4 | 66.3±11.5 | 0.80 |
| **Women** | 39 (51.3%) | 38 (67.9%) | 0.057 |
| **HT** | 47 (62.7%) | 27 (48.2%) | 0.099 |
| **DM** | 17 (22.7%) | 10 (17.9%) | 0.50 |
| **euroSCORE II** | 1.73±1.65 | 1.54±1.06 | 0.71 |
| **GFR** (ml/min) | 70.9±16.5 | 66.2±20.8 | 0.20 |
| **LVMI** (g/m$^2$) | 133.2±34.5 | 128.1±30.9 | 0.28 |
| **LAVI** (ml/ m$^2$) | 36.0±12.7 | 35.7±13.3 | 0.89 |
| **Max Ao vel** (m/s) | 457.1±52.8 | 463.2±65.6 | 0.77 |
| **EOAI** (cm$^2$/m$^2$) | 0.42±0.12 | 0.41±0.12 | 0.27 |
| **Zva** | 6.28±1.71 | 6.51±2.23 | 0.96 |
| **SVI** (ml/m$^2$) | 31.5±8.1 | 30.2±7.8 | 0.42 |
| **E/e'** | 16.5±7.2 | 16.8±6.1 | 0.500 |
| **EF** (%) | 59.1±12 | 63.6±7.6 | **0.013** |
| **PPM** | 36 (52.9%) | 25 (51%) | 0.84 |

HT= hypertension; DM= diabetes mellitus; GFR= glomerular filtration rate; LVMI= left ventricular mass index; LAVI= left atrial volume index; Max Ao vel= maximal aortic velocity; EOAI= effective orifice area index; Zva= valvuloarterial impedance; SVI= stroke volume index; EF= ejection fraction; PPM= patient prosthesis mismatch

From the patients who had a determination of collagen volume fraction (CVF) at the time of surgery, those with residual LVH had a significantly higher level of CVF (20.0±14.6% vs 13.2±11.5%, p=0.027) (Fig. 1).

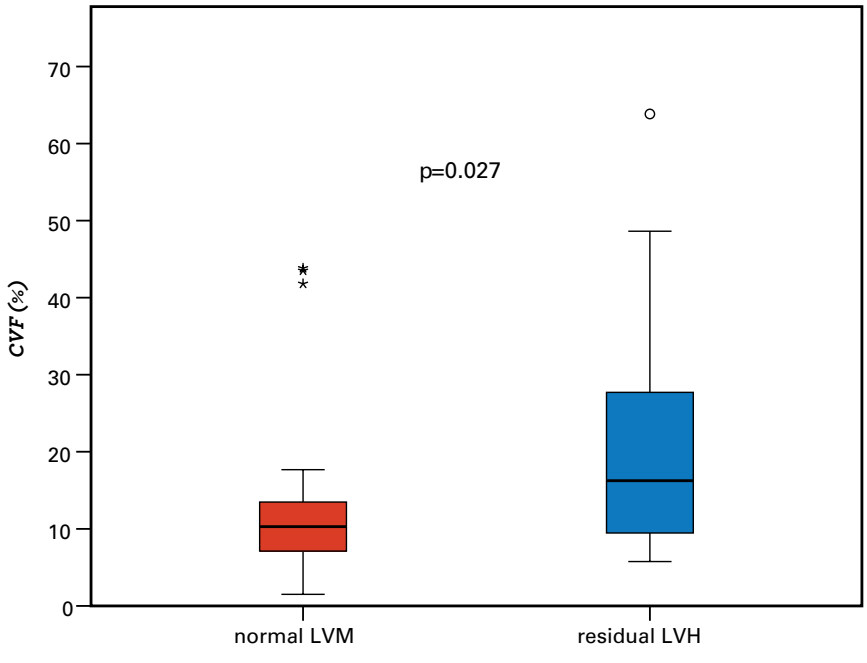



*Fig. 1 Levels of collagen volume fraction (CVF) according to the existence of residual LVH after surgery.*

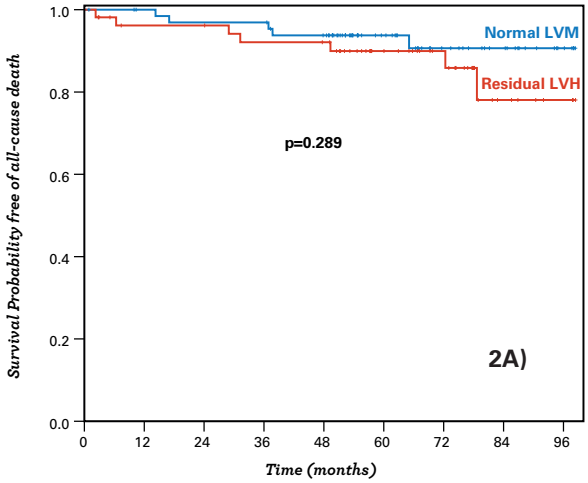
## Clinical outcomes

After 6.0±1.5 years of follow-up, 17 patients (12.9%) had died and 12 patients had a non-fatal cardiovascular hospitalization (5 for heart failure, 3 re-operations for prosthesis dysfunction, 2 for symptomatic new-onset atrial fibrillation, and 2 for advanced AV block requiring pacemaker implantation).

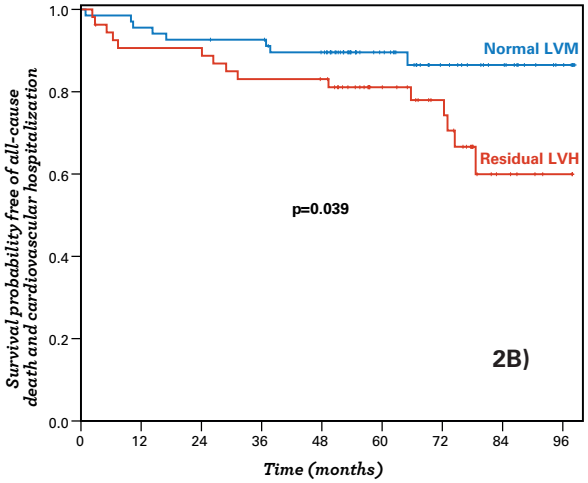
Patients with residual LVH after surgery had no differences in the risk of all-cause mortality (HR 1.88, 95% CI:0.56-6.28; p=0.366) , as well as no differences in the risk of cardiovascular death (HR 2.00, 95% CI:0.56-7.10; p=0.283), but had a significantly higher risk of non-fatal cardiovascular hospitalization (HR 3.82, 95%CI:1.03-14.13, p=0.045) and the combination of all-cause mortality and non-fatal cardiovascular hospitalization (HR 2.89, 95%CI:1.12-7.44; p=0.035), when compared with those with normal LVM.

Event-free survival curves for each group are displayed in Fig. 2 . Patients with residual LVH after surgery had worse results for survival free of non-fatal cardiovascular hospitalization (83.3% vs 95.6%, p=0.032) and the composite endpoint of all-cause mortality and non-fatal cardiovascular hospitalization, compared with those with normal LVM (60.0% vs 86.5%, p=0.039). There was no significant difference for all-cause mortality between the groups (78.1% vs 90.6%, p=0.289).

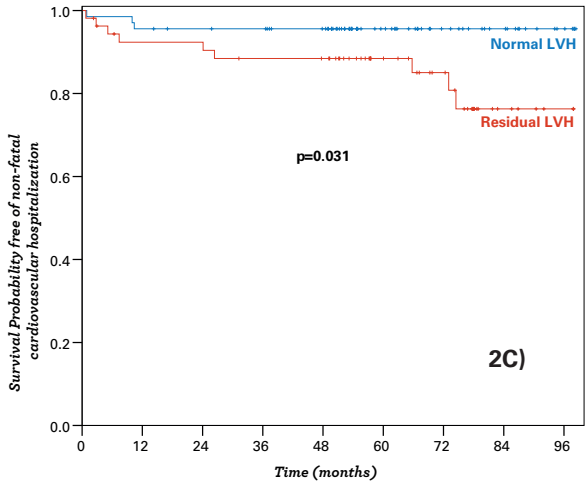
*Fig 2. Kaplan-Meier survival curves for all-cause death, for all-cause death and cardiovascular hospitalization and for non-fatal cardiovascular hospitalization, by the presence of residual left ventricular hypertrophy (LVH)*




| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 67 | 67 | 65 | 65 | 63 | 63 | 62 | 62 | 62 | 62 |
| Residual LVH | 53 | 51 | 51 | 49 | 49 | 48 | 48 | 46 | 46 | 46 |




| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 67 | 64 | 62 | 62 | 60 | 60 | 59 | 59 | 59 | 59 |
| Residual LVH | 53 | 48 | 48 | 44 | 44 | 43 | 42 | 38 | 38 | 38 |



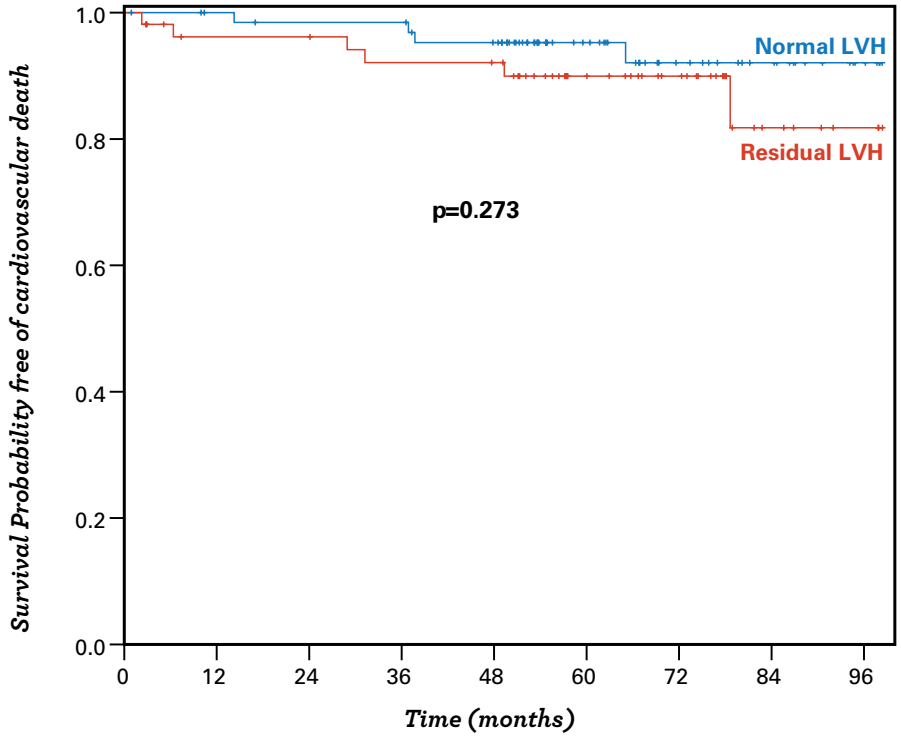

| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 67 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 |
| Residual LVH | 53 | 49 | 49 | 47 | 47 | 47 | 46 | 44 | 44 | 44 |

**2A)** Patients with normal LVM have similar survival free of all-cause death compared to the ones with residual LVH.

**2B)** Patients with normal LVM have significantly better survival free of all-cause death and cardiovascular hospitalization compared to the ones with residual LVH.

**2C)** Patients with normal LVM have significantly better survival free of non-fatal cardiovascular hospitalization compared to the ones with residual LVH.

*Fig 3. Kaplan-Meier survival curves for cardiovascular death according to the presence of residual left ventricular hypertrophy (LVH). Patients with normal LVM have similar survival free of cardiovascular death compared to the ones with residual LVH.*




| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| **number at risk** | | | | | | | | | | |
| normal LVM | 67 | 67 | 66 | 66 | 64 | 64 | 64 | 63 | 63 | 63 |
| Residual LVH | 53 | 51 | 51 | 49 | 49 | 48 | 48 | 48 | 47 | 47 |

There was a difference in event-free survival in those with residual LVH, according to gender (Fig. 4 and 5). Women with residual LVH have lower event-free survival for the combined endpoint (50.0% vs 93.2%, p=0.019) and a trend for lower survival free of all-cause death (67.8% vs 96.4%, p=0.059) and cardiovascular mortality (84.6% vs 96.7%, p=0.086), when compared with women with normal final LVM. This result was not seen in men, as there was no significant difference in event-free survival, between those with and without residual LVH.

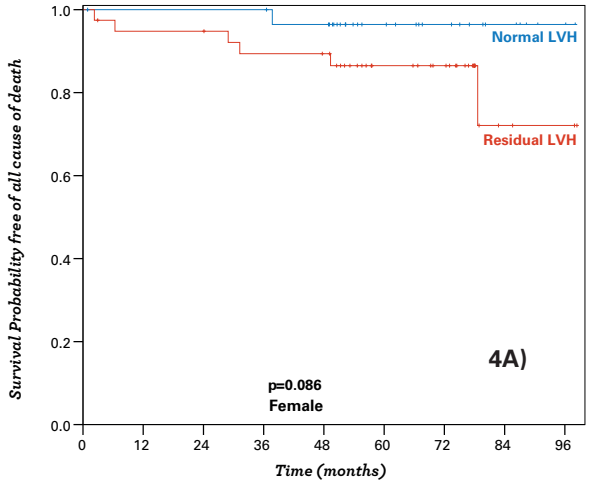
*Fig 4. Gender specific Kaplan-Meier survival curves for all-cause death or for all-cause death and cardiovascular hospitalization by the presence of residual left ventricular hypertrophy (LVH).*

**4A)** Female with normal LVM have similar survival compared with female with residual LVH.

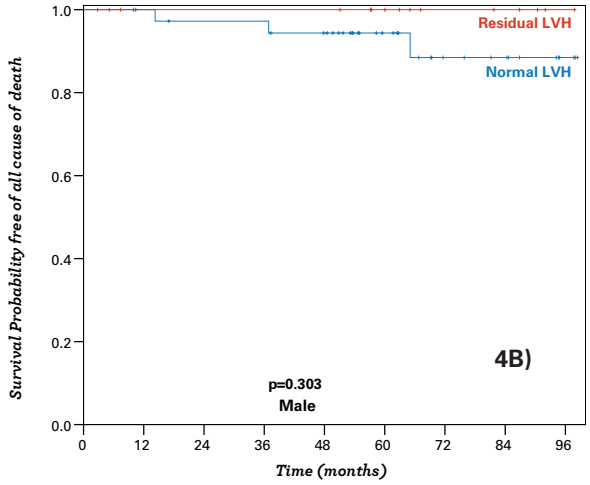
**4B)** Male with normal LVM have similar survival compared with male with residual LVH.

**4C)** Female with normal LVM have significantly better survival free of all-cause death and cardiovascular hospitalization compared with female with residual LVH.

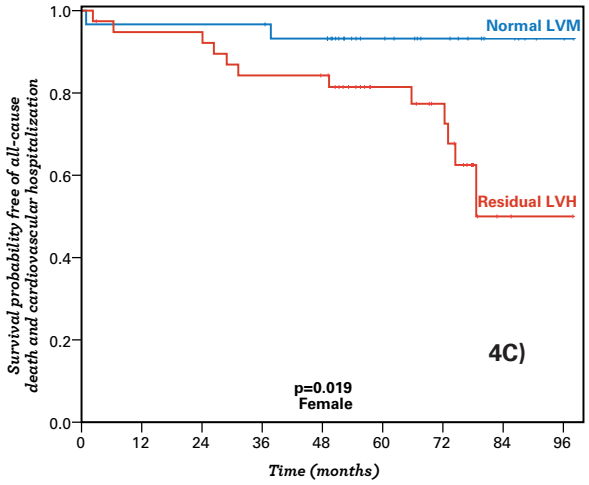
**4D)** Male with normal LVM have similar survival free of all-cause death and cardiovascular hospitalization compared with male with residual LVH.



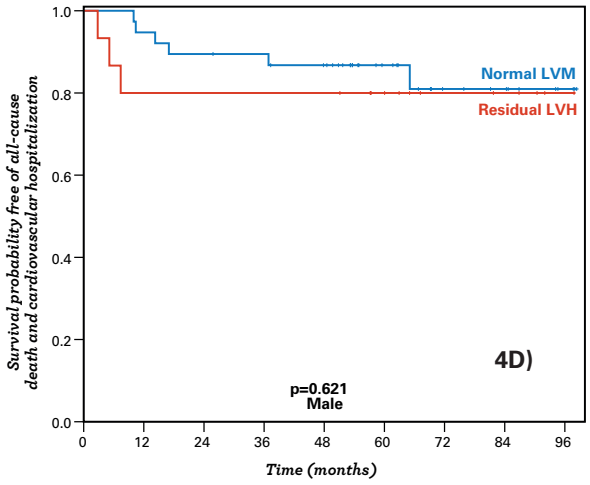

| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 29 | 29 | 29 | 29 | 28 | 28 | 28 | 28 | 28 | 28 |
| Residual LVH | 38 | 36 | 36 | 34 | 34 | 33 | 33 | 31 | 31 | 31 |




| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 37 | 37 | 35 | 34 | 34 | 34 | 33 | 33 | 33 | 33 |
| Residual LVH | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |



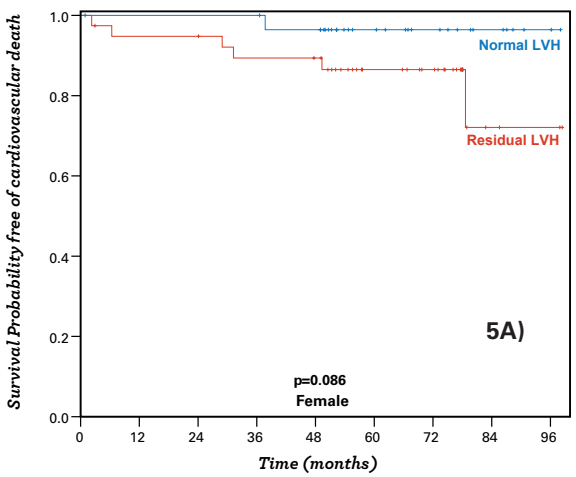

| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 29 | 29 | 29 | 29 | 28 | 28 | 28 | 28 | 28 | 28 |
| Residual LVH | 38 | 36 | 36 | 32 | 32 | 31 | 30 | 26 | 26 | 26 |

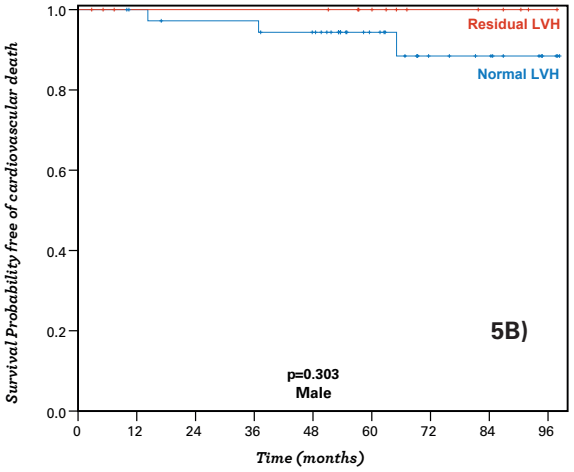



| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 37 | 35 | 33 | 33 | 32 | 32 | 31 | 31 | 31 | 31 |
| Residual LVH | 14 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |

*Fig 5. Gender specific Kapplan-Meier survival curves for cardiovascular death by the presence of residual left ventricular hypertrophy (LVH).*

Female patients showed a trend to higher cardiovascular mortality if they have residual LVH **(5A)** while cardiovascular mortality was not different in men with or without residual LVH **(5B)**.




| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 29 | 29 | 29 | 29 | 28 | 28 | 28 | 28 | 28 | 28 |
| Residual LVH | 38 | 36 | 36 | 34 | 34 | 33 | 33 | 33 | 32 | 32 |

| time | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | >96 |
|---|---|---|---|---|---|---|---|---|---|---|
| number at risk | | | | | | | | | | |
| normal LVM | 37 | 37 | 36 | 36 | 35 | 35 | 34 | 34 | 34 | 34 |
| Residual LVH | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |

## Discussion

We analyzed a prospective cohort of patients with isolated severe AS who underwent AVR, with echocardiographic follow-up at 5 years and clinical follow-up at 6.0±1.5 years. In our study residual LVH was present in 44% of patients late after AVR and was associated with a worse prognosis, with nearly a three-fold increase in the risk of death or non-fatal cardiovascular hospitalization. We also found that female gender, history of hypertension, higher baseline LVMI and higher baseline LV filling pressures were independent predictors of residual LVH. Moreover, in women the persistence of LVH late after AVR was associated with a worse outcome. This was not seen in men, suggesting that the prognostic impact of residual LVH is gender-specific.

The association of residual LVH in AS with worse prognosis is controversial. Others have described this association including patients with other types of valve lesions and coexisting coronary artery disease (CAD), which may have influenced results. Indeed, the coexistence of CAD has been considered as an independent predictor of clinical outcomes after AVR [6] and the presence of aortic insufficiency can elicit a different remodeling response[9]. In our study we excluded these patients.

The lack of normalization of LV mass after surgery occurs in nearly half of patients with AS, and it has been considered as a "natural" consequence of the replacement of a native valve for a somewhat obstructive valve substitute with a residual gradient [10]. Thus the focus has been on avoiding significant PPM and new prostheses have been developed with better hemodynamic profiles. In our study PPM was frequent and occurred in nearly half of the patients, but only in about 20% of them PPM was severe. However, in our study residual LVH was not associated with PPM. Beach et al, described that high postoperative transprosthesis gradients had only a minimal effect on residual left ventricular hypertrophy, in a study including a very large number of patients [5]. Therefore, hemodynamic factors, such as type of valve and residual gradients, are not the only determinants of incomplete regression. The worse long-term outcome of patients with residual LVH after AVR can be explained by the existence of more extensive preoperative disease and persistent diastolic and/or systolic dysfunction [11, 12].

One important finding in our study was the observed differences in the prognostic impact of residual LVH according to gender. Only in women the absence of normalization of LVM was associated with worse survival free from non-fatal cardiovascular hospitalization or and all-cause mortality. Recently, Petrov et al [13] described that women with preoperative maladaptive LVH had worse survival than those with adaptive LVH, a pattern that was not seen in men. These results are in accordance with ours, showing a gender-specific prognosis of LVH determined before or after AVR. Thus, it seems that, in women, the search for early predictors of negative remodeling after AVR could be particularly relevant.

### *Predictors of residual LVH*

Patients with a higher baseline LVMI and worse diastolic dysfunction (higher filling pressures evaluated by E/e´) had a higher probability of having residual LVH after surgery. Moreover, those with worse longitudinal systolic function (evaluated by Sm) were also less likely to have LV mass normalization late after surgery. Our results are in accordance with those of other authors, who also found that the existence of a more severe preoperative hypertrophy [5, 14-16] and the presence of early signs of myocardial dysfunction, even with preserved ejection fraction, may be a surrogate of a more advanced disease[17] and could help to explain the worse long-term outcome of patients with residual LVH.

Female gender and a history of HT were also independently associated with persistent LVH. In previous analyses, we have found that women had more interstitial fibrosis than men. They also had levels of biomarkers of extracellular matrix (ECM) favoring collagen deposition, and these correlated negatively with LV mass regression (unpublished data). Moreover, hypertension negatively impacts LV mass regression after surgery, and several authors have stressed the need for rigorous blood pressure control in these patients [18, 19]. Nevertheless, our group has shown that this impairment in reverse remodeling happens independently of load, and might be related to the neuro-hormonal milieu [20].

### *Myocardial fibrosis and residual LVH*

At the histological level, we found that a higher amount of fibrous tissue at the time of surgery is an independent predictor of residual LVH, altogether suggesting the presence of irreversible remodeling. Our results are in accordance with earlier landmark studies that established the relationship between myocardial fibrosis, systolic and diastolic function and incomplete LVM regression in aortic stenosis [21, 22]. More recently, the presence of severe fibrosis at the time of surgery has been associated with lesser functional improvement [23] and higher mortality after AVR [24], confirming its prognostic importance.

Based on results from previous studies [23, 25], the worse baseline longitudinal systolic function in our patients with residual LVH might reflect the existence of more advanced myocardial disease and higher levels of fibrosis, making its evaluation an important tool for risk-stratifying AS patients without class I recommendation for AVR.

## Limitations

This was a single center observational study and the limited size of our cohort, although similar to those reported on literature about this subject, limits our statistical power. For fibrosis determination, we were unable to achieve myocardial biopsies for all patients. Still these patients were randomly chosen and are believed to be representative of the overall study group.

## Conclusion

Residual LVH late after AVR is associated with a worse prognosis, in particular in women. The presence of more severe myocardial disease, as suggested by higher LVM and worse LV diastolic and systolic function, can help to explain the poorer clinical outcome of these patients. Interstitial fibrosis could be the missing link in the pathophysiology of residual LVH. Early intervention may be needed in women, those with HT and those with higher baseline LVM and worse diastolic dysfunction, independently of symptoms. Our study is hypothesis generating and brings light for the need of additional research to evaluate the impact of earlier surgery in specific subgroups of patients with a higher risk of residual LVH after surgery, such as women and those with HT.

## Acknowlegdments

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# RESULTS

## 5.4. Load independent impairment of reverse remodeling after valve replacement in hypertensive aortic stenosis patients

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# Load independent impairment of reverse remodeling after valve replacement in hypertensive aortic stenosis patients

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ABSTRACT

*Background:* We evaluated the impact of hypertension on the left ventricular mass regression in aortic stenosis after aortic valve replacement.
*Methods:* We prospectively studied 135 patients with severe aortic stenosis at baseline and 1 year after surgery. In 32 patients we analyzed myocardial gene expression of collagen types I and III, connective tissue growth factor, transforming growth factor-β1, metalloproteinase-2 and its tissue inhibitor and compared its levels vs controls.
*Results:* Seventy-six patients (56.3%) had a history of hypertension. Hypertensive patients were older, had higher Euroscore-II and NYHA class, with no differences in stenosis severity. At 1 year follow-up there was a median decrease of mass index of 14.2% (P25–75: −4.3%–30.4%; $p < 0.001$). Mass regression was significantly higher in patients without hypertension, with a median decrease of 25.9% (P25–75: 12.0%–38.7%) vs 5.4% (P25–75: −12.5%–20.1%; $p = 0.001$), despite similar increase in effective orifice area and no differences in valvuloarterial impedance. After 1 year, higher baseline left ventricular mass index ($p = 0.005$) and the absence of hypertension ($p = 0.002$) or diabetes ($p = 0.041$) were the only independent predictors of mass regression higher than the median. Comparing with controls, aortic stenosis patients had an increased expression of collagen types I and III, but only hypertensive patients had higher relative expression of collagen type I vs III. In hypertensive patients TIMP2 expression was up-regulated and correlated with higher baseline left ventricular mass index ($r = 0.61$; $p = 0.020$).
*Conclusions:* In aortic stenosis, hypertension impairs mass regression one year after valve replacement, independently of total afterload. Differences in the expression of extracellular matrix remodeling genes might contribute to this finding.



## 1. Introduction

Hypertension (HT) is a common comorbidity in patients with aortic valve stenosis (AS), with a previously reported prevalence of 33–72% [1–4].

In chronic pressure overload states, like systemic HT and AS, the left ventricle (LV) responds with hypertrophy and altered geometry as an adaptative mechanism that helps to maintain contractile performance despite abnormal loading conditions. LV hypertrophy (LVH) allows for normalization of systolic wall stress and has been considered as compensatory [5], but it is also associated with impaired coronary blood-flow reserve [6] and changes in cardiomyocytes and extracellular matrix (ECM) connective tissue, some of them irreversible [7]. Moreover, the presence of residual hypertrophy after aortic valve replacement (AVR) has been associated with incomplete recovery of left ventricular function and worse prognosis [8–10].

The coexistence of hypertension and valvular aortic stenosis (AS) is common, but few studies have assessed the impact of concomitant hypertension on LV structure and function in patients with AS. Moreover, although we have evidence of changes in the composition and structure of ECM in the progression to heart failure in AS [11] and HT [4], there is no published data comparing the expression of genes regulating ECM production in patients with both types of pressure overload.

Therefore our aim was to evaluate the importance of HT on LV remodeling and LV mass regression in AS patients one year after AVR.

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Additionally, we did a subgroup analysis on myocardial expression of genes involved in ECM remodeling in aortic stenosis patients with and without HT, and compared its results with those of a control group.

## 2. Methods

### 2.1. Patient selection and follow-up

Between January 2006 and December 2009 we included 141 consecutive patients over 18 years old with severe symptomatic AS (aortic valve area < 1 $cm^2$ or mean transaortic gradient ≥ 40 mm Hg) referred for aortic valve replacement (AVR) at the Cardiothoracic Surgery Department of Hospital São João, Porto, Portugal. This investigation conforms to the Declaration of Helsinki, had institutional ethical review board approval and each study participant signed an informed consent before enrolment. We excluded patients with aortic regurgitation >II/IV or other significant valve diseases (>mild), and significant coronary artery disease (lesions > 50% on coronary angiography). All patients were in sinus rhythm at the time of inclusion for a more accurate evaluation of diastolic function parameters. From the initial 141 patients, 135 were considered for this prospective analysis: one was refused for surgery, other died before surgery from cholangitis with sepsis, and there was incomplete clinical data in four of them. One year clinical and echocardiographic follow-up was achieved in 91 (67.4%) patients. The remaining patients were not lost to follow-up, except for two cases, but had echocardiographic evaluation at 6 months or beyond 1 year and those values were not considered. The diagnosis of hypertension was considered whenever it was registered in the clinical records of the assistant physician. Renal insufficiency was determined when estimated glomerular filtration rate (GFR) <60 ml/min/1.73 $m^2$ by the Cockcroft–Gault formula and perioperative renal failure if there was an increase in serum creatinine >25% the preoperative value. Medical therapy was at the discretion of assistant physician.

Given the shortage of human myocardial samples in normal adults, for the control group of the molecular substudy we recruited nine mitral stenosis (MS) patients without coronary artery disease or significant mitral regurgitation and/or aortic valve disease. These patients had no significant left ventricular overload and should have a local expression of ECM genes more similar to the normal left ventricles.

### 2.2. Surgical technique and biopsies

All surgeries were performed using standard procedure for aortic or mitral valve replacement. The patients were placed on cardiopulmonary bypass and cardiac arrest was induced and maintained with cold blood cardioplegia. The majority of patients received a bioprosthesis (73.3%). Two patients also had ascending aorta aneurism and underwent aortic root replacement with valved composite grafts (Bentall technique). In 32 patients with AS myocardial biopsies were procured at the time of surgery from the LV interventricular septum. In 9 mitral stenosis patients undergoing mitral valve replacement, excised papillary muscles were collected and used as control myocardial biopsies. In both cases, excised myocardium was immediately snap-frozen in liquid nitrogen and stored at −80 °C.

### 2.3. Echocardiographic studies

Echocardiographic examination was performed by a trained cardiologist and recorded on digital support. All recordings were examined by an experienced echocardiographer in an accredited independent echocardiography laboratory (Hospital Clínico San Carlos in Madrid, Spain) blinded to patient details. Studies were performed using Phillips IE-33 equipment with a S5-1 transducer and M-mode, two dimensional, pulsed, continuous, color-flow and tissue Doppler capabilities. Correct orientation of imaging planes, cardiac chamber dimension, function measurements, LV mass index and relative wall thickness were performed according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations [12]. LV mass index greater than 115 g/m2 in men and greater than 95 g/m2 in women was considered indicative of LV hypertrophy. LA volume was measured in LV end systole in the frame preceding mitral valve opening, using the biplane area length method and corrected for body surface area. To evaluate systolic function we used LV ejection fraction (EF), estimated using Simpson's biplane method, and longitudinal systolic function, assessed by peak systolic mitral annular motion.

Aortic valve area was estimated using quantitative Doppler by continuity equation. Mitral inflow by pulsed wave Doppler and septal e′ tissue Doppler velocity of the mitral annulus were obtained from the apical 4-chamber and according to ASE guidelines [13]. Patients with an E/e′ septal >15 were considered to have increased filling pressure, whereas patients with E/e′ septal <8 were considered to have normal filling pressure. In the remaining patients with an indeterminate E/e′, those with LAVi ≥34 ml/$m^2$ were considered to have increased filling pressure. The presence of increased filling pressures was considered indicative of diastolic dysfunction.

Peak wall stress (WS) was estimated using a previously validated formula: $WS = 0.8 \times [0.334 \times (SAP + MaxG) \times LVID] / [PWTd \times (1 + (PWTd / LVID)] - 2(\times 10^3\ dyn/cm^2)$, where SAP = systolic arterial pressure, MaxG = maximal transvalvular pressure gradient, LVID = LV internal diameter, and PWTd = posterior wall thickness in diastole [14]. As a measure of global LV load, we calculated the valvuloarterial impedance: Zva = (SAP + MG) / SVI, where SAP = systolic arterial pressure, MG = mean transvalvular pressure gradient and SVI = stroke volume index. Blood pressure was measured before echocardiography with patients in supine position, and a mean of 3 measurements was considered.

### 2.4. mRNA quantification

For gene expression evaluation, RNA was extracted with TriPure (Roche) according to the manufacturer's instructions. RT-PCR was performed with total RNA, followed by real time PCR analyses using the SYBR Green method, in a LightCycler 2.0 (Roche) as previously described [15]. Results are normalized for GAPDH and expressed in arbitrary unit. Specific PCR primer pairs for the studied genes are displayed in Supplementary material.

### 2.5. Statistical analysis

Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation or median and interquartile range, according to their distribution. Continuous variables were compared between groups using an unpaired *t*-test (for normally distributed variables) or the Mann–Whitney *U*-test (for non-normally distributed variables). For comparison between baseline and follow-up a paired Student's *t*-test was applied (normally distributed variables). Chi-square test was used to compare proportions. Spearman's rank correlation was used for the assessment of correlations between LVM index and its variation and clinical, echocardiographic and molecular continuous variables. Following univariate analysis, a stepwise binary logistic multivariate regression model (Wald backward stepwise method, $p = 0.05$ for covariate inclusion and 0.2 for exclusion) was performed (including potential confounders) for LVM index regression analysis 1 year after AVR (relative LVM index regression variable was dichotomized according to its median value: ≤14% (no LVM index regression or regression below median value) and >14% (LVM index regression higher than the median).

All reported probability values are two-tailed, and $p < 0.05$ was considered statistically significant. Analyses were performed with the IBM®SPSS® Statistics software package (version 19.0) (SPSS Inc, Chicago, IL, USA).

## 3. Results

Demographics and clinical parameters of the 135 patients with severe symptomatic AS are described in Table 1. Heart failure was the most prevalent presentation feature (81.5%), 72 (53.3%) patients had echocardiographic evidence of LV diastolic dysfunction and most patients had LVH (68.1%) with a mean LVM index of 129.6 ± 34.0 g/$m^2$ (Table S1, Supplementary data). Ninety nine cases (73.3%) had a bioprosthesis implanted (size 21 mm: 46.6%; 23 mm: 27.4%; 25 mm: 14.8%; 19 mm: 10.5%; and 17 mm: 0.7%). The median time of hospitalization was 6.0 days (P25–75: 6.0–8.0 days) and 2 hospital deaths occurred (1 from pneumonia and 1 due to stroke). At 1 year follow-up, there was an increase in the EOA index, decrease in valvuloarterial impedance and peak wall stress (Table S1), and a significant median decrease in LVM index of 20.6 g/$m^2$ (P25–75: −5.1 g/$m^2$–40.7 g/$m^2$) with a median relative decrease of 14.2% (P25–75: −4.3%–30.4%; $p < 0.001$).

Clinical and echocardiographic comparison between patients with (HT + AS) and without HT (ASwHT) are described in Tables 1 and 2. Hypertensive patients were older, had higher surgical risk and were in higher NYHA class. However there were no differences in AS severity.

One year after aortic valve replacement LVM regression was significantly higher in ASwHT, with a median decrease of 25.9% (P25–75: 12.0%–38.7%) vs 5.4% (P25–75: −12.5%–20.1%) in HT + AS ($p = 0.001$). In ASwHT only 25.6% had LVH at 1 year follow-up, but, in the presence of associated HT, 56.2% had persistent LVH ($p = 0.003$). At this time-point, patients with HT + AS had higher LVM index (118.9 ± 35.2 vs 101.0 ± 31.3 g/$m^2$; $p = 0.042$) when compared with ASwHT, despite similar increase in effective orifice area and similar prosthetic gradients (Table 2). LV reverse remodeling at 1 year was only significant in ASwHT (Fig. 1), with a decrease in LV end-diastolic (92.3 ± 33.0 vs 80.5 ± 29.8 ml, $p = 0.019$) and end-systolic (37.2 ± 23.0 vs 28.8 ± 12.3 ml, $p = 0.004$) volumes and relative wall thickness (0.48 ± 0.09 vs 0.45 ± 0.08, $p = 0.048$), with no change in indexed LA volume (Table 2). In HT + AS there was an increase in LA volume index, although there were no significant changes in estimated LV filling pressure (Table 2). As expected, there was a trend for higher systolic blood pressure in hypertensive patients (137.6 ± 18.8 vs 130.2 ± 17.1 mm Hg; $p = 0.069$), but there were no differences in valvuloarterial impedance (5.30 ± 1.60 vs

**Table 1**
Demographics and clinical parameters of studied groups (HT + AS = aortic stenosis with hypertension; ASwHT = aortic stenosis without hypertension; p value for HT + AS vs ASwHT).

| Clinical characteristics | Study group (n = 135) | HT + AS (n = 76) | ASwHT (n = 59) | P |
|---|---|---|---|---|
| *Baseline* | | | | |
| Age | 66.7 ± 11.9 | 69.4 ± 9.1 | 63.1 ± 14.2 | 0.004 |
| Women [n (%)] | 78 (57.8) | 48 (63.2) | 30 (50.8) | 0.151 |
| BSA ($m^2$) | 1.75 ± 0.19 | 1.76 ± 0.19 | 1.75 ± 0.18 | 0.861 |
| Diabetes [n (%)] | 27 (20) | 14 (18.4) | 13 (22) | 0.603 |
| Renal Insufficiency [n (%)] | 46 (34.1) | 25 (32.9) | 21 (35.6) | 0.743 |
| Glomerular filtration rate CKD-EPI (ml/min) | 71.6 ± 19.3 | 69.4 ± 19.2 | 74.5 ± 19.2 | 0.141 |
| Euroscore II (%) [Me (P25–P75)] | 1.25 (0.75–1.99) | 1.39 (0.99–2.19) | 0.94 (0.69–1.64) | 0.003 |
| Heart Failure [n (%)] | 110 (81.5) | 68 (89.5) | 42 (71.2) | 0.007 |
| NYHA class [n (%)] | | | | 0.016 |
| I | 23 (17) | 8 (10.5) | 15 (25.4) | |
| II | 81 (60) | 44 (57.9) | 37 (62.7) | |
| III | 30 (22.2) | 23 (30.3) | 7 (11.9) | |
| IV | 1 (0.7) | 1 (1.3) | – | |
| LVH [n (%)] | 93 (81.6) | 50 (79.4) | 43 (84.3) | 0.498 |
| Diastolic dysfunction [n (%)] | 72 (73.5) | 44 (75.9) | 28 (70) | 0.518 |
| *Surgery/perioperatory period* | | | | |
| Bioprosthesis (%) | 99 (73.3) | 59 (77.6) | 40 (67.8) | 0.202 |
| Atrial fibrilation (%) | 28 (22.4) | 19 (27.5) | 9 (16.1) | 0.126 |
| Definite pacemaker (%) | 7 (5.6) | 2 (2.9) | 5 (8.9) | 0.151 |
| Renal failure (%) | 25 (20.2) | 16 (23.5) | 9 (16.1) | 0.303 |
| Death (%) | 2 (1.6) | – | 2 (3.5) | 0.119 |
| Discharge (days) [Me (P25–P75)] | 6 (6–8) | 6 (6–8) | 6 (6–8) | 0.450 |
| | Study group (n = 91) | HT + AS (n = 48) | ASwHT (n = 43) | |
| *1 year* | | | | |
| NYHA class [n (%)] | | | | 0.019 |
| I | 72 (79.1) | 34 (70.8) | 38 (88.4) | |
| II–III | 19 (20.9) | 14 (29.2) | 5 (11.6) | |
| LVH [n (%)] | 38 (51.4) | 27 (67.5) | 11 (32.4) | 0.003 |
| Diastolic dysfunction [n (%)] | 48 (72.7) | 29 (82.9) | 19 (61.3) | 0.0496 |
| *Current therapy* | | | | |
| ACE/ARB [n (%)] | 48 (51.6) | 30 (60) | 18 (41.9) | 0.081 |
| Beta blockers [n (%)] | 63 (67.7) | 34 (68) | 29 (67.4) | 0.954 |
| Spironolactone [n (%)] | 3 (3.2) | 3 (6) | – | 0.103 |
| Diuretics [n (%)] | 35 (37.6) | 23 (46) | 12 (27.9) | 0.073 |
| Calcium channel blockers [n (%)] | 21 (23.1) | 19 (39.6) | 2 (4.7) | 0.001 |
| Statins [n (%)] | 50 (53.8) | 28 (56) | 22 (51.2) | 0.641 |

**Table 2**
Echocardiographic characterization of aortic stenosis patients according to HT status, before and after AVR (HT + AS = aortic stenosis with hypertension; ASwHT = aortic stenosis without hypertension; p value for baseline vs 1 year).

| | HT + AS | | | ASwTH | | |
|---|---|---|---|---|---|---|
| | Baseline | 1 year | p | baseline | 1 year | p |
| *Aortic/prosthesis stenosis severity* | | | | | | |
| Maximal transaortic velocity (m/s) | 4.63 ± 0.65 | 2.69 ± 0.62 | <0.001 | 4.68 ± 0.63 | 2.64 ± 0.65 | <0.001 |
| Medium transaortic gradient (mm Hg) | 54.67 ± 15.22 | 16.73 ± 10.61 | <0.001 | 56.62 ± 14.46 | 16.23 ± 8.43 | <0.001 |
| Effective orifice area index (EOAi, $cm^2/m^2$) | 0.39 ± 0.12 | 0.81 ± 0.21 | <0.001 | 0.39 ± 0.1 | 0.82 ± 0.23 | <0.001 |
| % increase EOAi [Me (P25–P75)] | 93.5 (71.3–150.3)[a] | | | 119.6 (57.9–179.1)[a] | | |
| *Hemodynamic load* | | | | | | |
| Systolic blood pressure (mm Hg) | 135.14 ± 21.69 | 137.14 ± 17.98 | 0.647 | 128.72 ± 20.01 | 130.67 ± 17.04 | 0.576 |
| Valvulo-arterial impedance (mm Hg/ml/$m^2$) | 6.9 ± 2.57 | 5.3 ± 1.61 | 0.006 | 6.34 ± 1.81 | 6.16 ± 2.03 | 0.692 |
| Peak LV wall stress ($10^3$ dyn/$cm^2$) | 248.89 ± 101.29 | 175.81 ± 42.97 | 0.002 | 211.49 ± 57.78 | 164.73 ± 37.28 | <0.001 |
| *Systolic function* | | | | | | |
| EF (%) | 59.12 ± 12.28 | 62.06 ± 10.19 | 0.099 | 61.61 ± 9.8 | 64.42 ± 5.82 | 0.126 |
| Stoke volume index (ml/$m^2$) | 29.13 ± 7.75 | 30.47 ± 7.03 | 0.412 | 31.1 ± 7.99 | 26.56 ± 6.6 | 0.031 |
| Systolic velocity mitral annulus (cm/s) | 5.51 ± 1.27 | 6.14 ± 2.11 | 0.088 | 5.44 ± 1.41 | 6 ± 1.31 | 0.082 |
| *Diastolic function* | | | | | | |
| E/A | 0.85 ± 0.43 | 0.97 ± 0.32 | 0.065 | 0.84 ± 0.3 | 1.07 ± 0.52 | 0.003 |
| E-wave deceleration time (ms) | 239 ± 79.83 | 257.85 ± 75.15 | 0.262 | 231.88 ± 68.08 | 246.06 ± 77.78 | 0.298 |
| e′ (cm/s) | 5.32 ± 2.22 | 6.24 ± 2.26 | 0.059 | 5.59 ± 2.36 | 6.37 ± 1.89 | 0.020 |
| E/e′ | 16.75 ± 6.72 | 15.71 ± 5.8 | 0.333 | 15.48 ± 6.19 | 15.23 ± 6.26 | 0.785 |
| Isovolumetric relaxation time (ms) | 104.05 ± 26.82 | 171.76 ± 196.28 | 0.042 | 97.59 ± 26.28 | 162.76 ± 188.81 | 0.074 |
| LA volume index (ml/$m^2$) | 37.59 ± 12.61 | 43.81 ± 13.96 | 0.051 | 32.66 ± 12.44 | 32.69 ± 7.18 | 0.987 |

[a] Mann–Whitney *U*-test, p = 0.692.

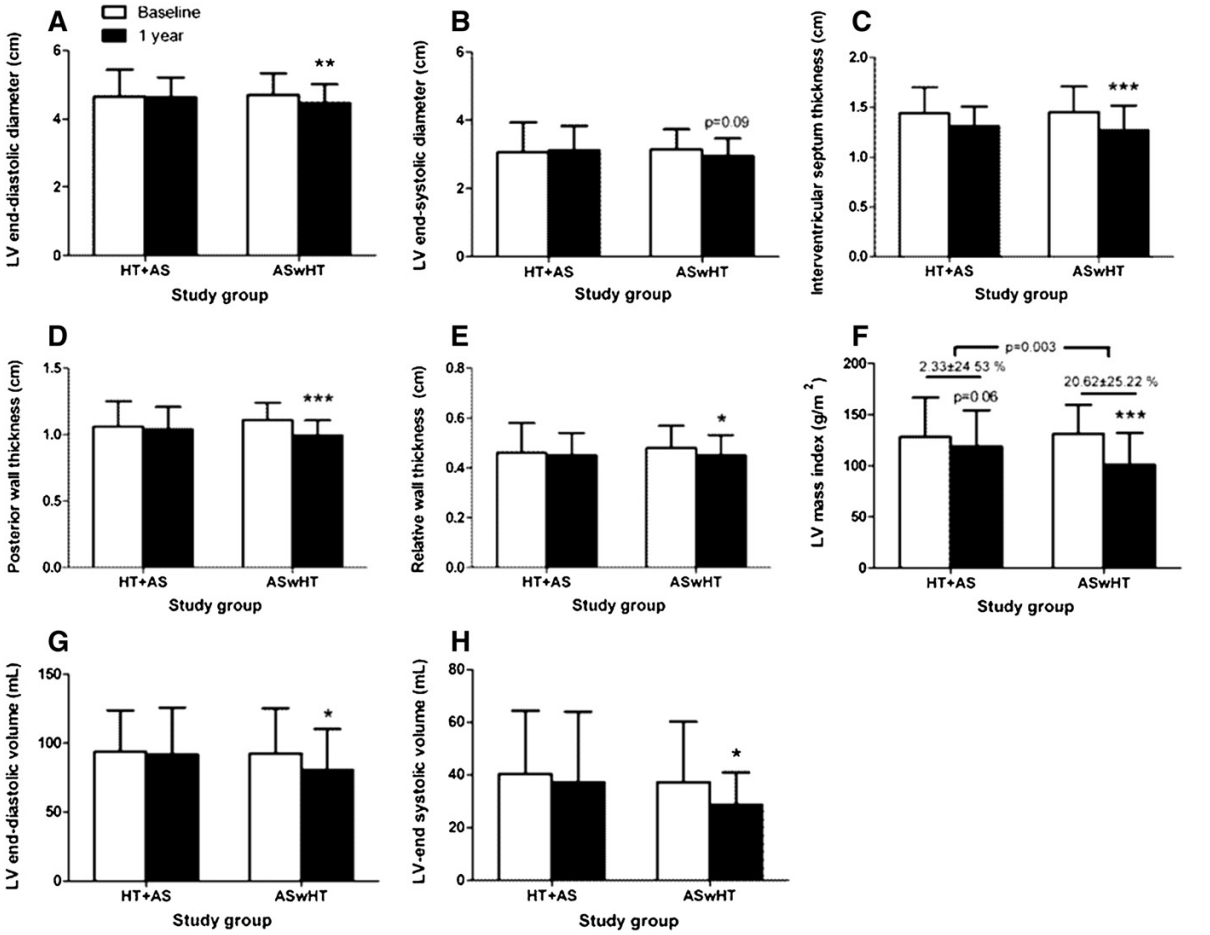


**Fig. 1.** Left ventricle remodeling before and after AVR (*p < 0.05, **p < 0.01, ***p < 0.001).

6.15 ± 2.02 mm Hg/ml/m$^2$; p = 0.104) or peak wall stress (175.8 ± 43.0 vs 164.1 ± 36.8 $10^3$ dyn/cm$^2$; p = 0.223). Patients with HT + AS had a trend for higher rates of prescription of angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) (62.5% vs 41.8%; p = 0.098), but this medical therapy had no correlation with one year LVM regression (median LVM regression with ACE/ARB 12.3% (P25–75: −5.8–25.3) vs 18.9% without (P25–75: −3.7–32.3); p = 0.538).

### 3.1. Predictors of LVM regression 1 year after AVR

The LVM regression outcome was considered to be relative LVM index decrease higher than the median (>14%). Baseline LVM index (p = 0.008) and the absence of HT (p = 0.002) were the only predictors of LVM regression at one year follow-up in univariate binary logistic analysis. After performing a multivariate logistic analysis, including potential confounders such as age, gender, diabetes mellitus, baseline LV ejection fraction, one year valvuloartial impedance, and the use of ACEI/ARB, the absence of HT (p = 0.002) and diabetes mellitus (p = 0.041) as well as higher baseline indexed LVM (p = 0.005) remained the only independent predictors of significant LVM regression (Table 3). Aortic stenosis patients without HT had a 6 fold higher probability of LV mass regression than patients without HT. ACEI/ARB prescription had no influence on LVM regression (Table 3).

## 4. Correlations between clinical data and myocardial expression of extracellular matrix remodeling components

We analyzed mRNA expression in the LV of 9 controls and 32 patients with severe AS, 19 of which also had hypertension (Figs. 2 & 3). Comparing with controls, patients with AS had significantly higher levels of collagen types I (AS 2.57 ± 0.34 vs CTRL 1.00 ± 0.14; p < 0.001) and III expression (AS 1.94 ± 0.25 vs CTRL 1.00 ± 0.13; p = 0.003), with no differences in the expression of CTGF, TGFβ1, MMP2 or TIMP2. Both HT + AS and ASwHT have an increase expression in collagens type I (HT + AS: 2.88 ± 1.68 vs 1.00 ± 0.14 AU, p = 0.002; ASwHT: 2.07 ± 1.30 vs 1.00 ± 0.14 AU, p = 0.06) and type III (HT + AS: 1.71 ± 0.84 vs 1.00 ± 0.13 AU, p = 0.03; ASwHT: 2.36 ± 1.24 vs 1.00 ± 0.13 AU, p = 0.04), but only the HT + AS have shown significant differences in collagen turnover with higher expression of TIMP2 (1.55 ± 0.63 vs 1.00 ± 0.18 AU; p = 0.047) and a trend for higher expression of MMP2 (2.28 ± 1.91 vs 1.00 ± 0.20 AU, p = 0.08). Moreover, the presence of hypertension was associated with a preponderance of collagen type I vs type III, which was not seen in ASwHT (collagen I/III in HT + AS: 1.51 ± 0.50 vs 0.99 ± 0.19, p = 0.03; ASwHT: 1.08 ± 0.67 vs 0.99 ± 0.19, p = 0.76, Fig. 3). When directly comparing HT + AS with ASwHT, there is an upregulation of TIMP2 mRNA expression in the former (1.55 ± 0.63 vs 0.73 ± 0.36 AU; p = 0.001), which correlates with higher baseline LV

**Table 3**
Risk factors for LVM index regression (outcome: LVM index regression >14%): uni- and multivariate logistic regression analysis.

| | Univariate | | Multivariate[a] (n = 70) | |
|---|---|---|---|---|
| Variable | p | OR [95%CI] | p | OR [95%CI] |
| Gender, male | 0.231 | 1.79 [0.69–4.65] | | |
| age, 1 year increase | 0.488 | 0.99 [0.95–1.03] | | |
| HT, without | 0.002 | 4.79 [1.70–13.19] | 0.002 | 6.19 [1.93–19.88] |
| DM, without | 0.152 | 2.4 [0.72–7.95] | 0.041 | 4.45 [1.06–18.66] |
| LV mass index, 1 g/m$^2$ increase | 0.008 | 1.02 [1.01–1.04] | 0.005 | 1.03 [1.01–1.06] |
| Valvulo-arterial impedance (1 year), 1 mm Hg/ml/m$^2$ increase | 0.152 | 1.27 [0.92–1.75] | | |
| EF baseline, 1% increase | 0.560 | 0.99 [0.94–1.03] | | |
| ACE/ARB, without | 0.260 | 1.77 [0.65–4.82] | | |
| | Area under the curve (ROC curve) | | 0.821 [0.721–0.922] | |
| | % of correctly predicted outcome | | 75.70% | |

[a] Variable(s) entered on step 1: gender (male), age, hypertension (without HT), diabetes (without DM), baseline left ventricular mass index, baseline ejection fraction, one year valvuloarterial impedance, one year use of ACEI (without).

mass index (r = 0.61, p = 0.020), with no differences in the expression of other studied genes.

## 5. Discussion

This is a prospective study of patients with isolated severe AS analyzing the impact of HT on LV mass regression and reverse remodeling. Overall, baseline LVM index and the absence of HT and DM were the only independent predictors of LVM regression at one year follow-up. In addition, we report two major findings. First, HT impairs LV mass regression and reverse remodeling after AVR, independently of total LV afterload. Secondly, the combination of HT with AS is associated with a different pattern of expression of genes related to ECM remodeling favoring collagen accumulation and higher relative levels of collagen type I, which could help to explain its negative impact on reverse remodeling.

Although it was not the objective of this study, we found that the presence of DM is also a predictor of impaired LVM regression. It is known that the presence of DM is associated with higher LV mass and worse systolic function in AS, independently of pressure overload and gender [16]. Moreover, our group has already described structural changes in AS diabetic patients with increased fibrosis, advance glycation end-product (AGE) deposition and raised cardiomyocyte passive force, which can explain their worse diastolic LV dysfunction [17]. What is less studied is if it also influences LV mass regression after valve replacement, and this should warrant further study.

### 5.1. HT impact on LV mass regression and reverse remodeling after AVR

After successful AVR, LV pressure and wall stress are significantly reduced (44) and LVH regression is expected. Even so, nearly half of patients with AS have residual LVH late after surgery [8,18]. This persistent increase in LVM is an independent predictor of cardiac-related morbidity [8] and mortality [9,19], making LVM regression a target for achieving a good outcome.

When investigating hemodynamic factors that influence LVM regression after AVR, most studies focused solely on pressure gradient and valve related parameters, but these can only explain a small part of the observed variability of LV load and LV mass regression [20,21]. In fact, the trigger stimulus for LVH is not pressure gradient itself but the elevated LV pressure, which also depends on systemic blood pressure [21]. This led some authors to propose combined indices of systolic load such as the valvuloarterial impedance, which has proven useful for stratification of prognosis in patients with asymptomatic AS [22].

Hypertension and AS frequently coexist. In our study we found a prevalence of HT of 56.3% and these patients had more severe heart

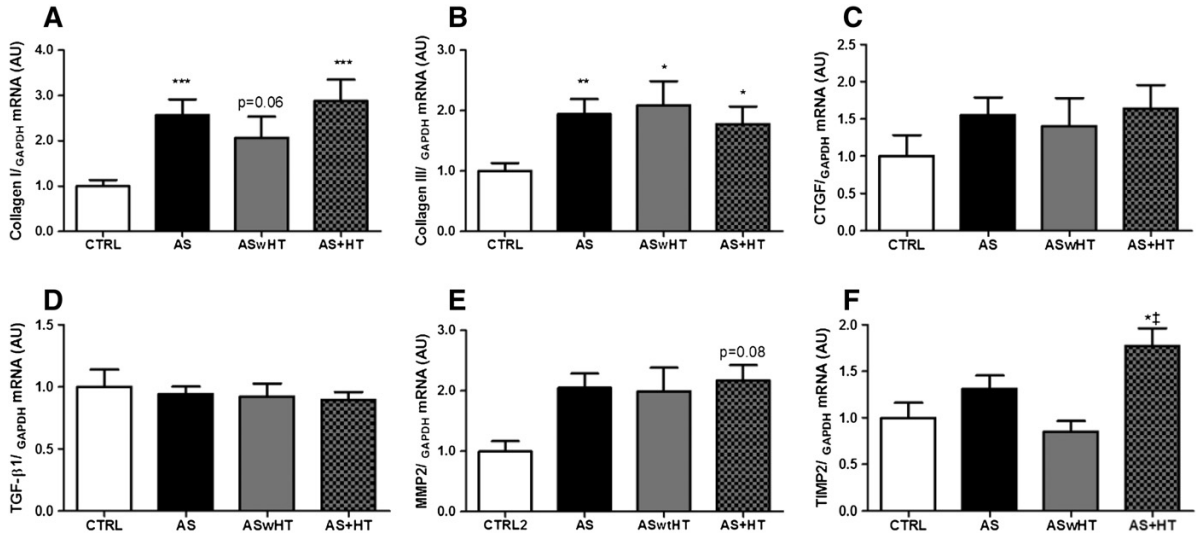


**Fig. 2.** Myocardial expression of collagen types I (A; ***p < 0.001 vs CTRL e p = 0.06 vs CTRL) and III (B; *p < 0.05, **p < 0.01 vs CTRL), connective tissue growth factor (CTGF, C), transforming growth factor-β1 (TGF-β1, D), matrix metaloprotease-2 (MMP2, E) and MMP2 specific tissue inhibitor (TIMP2, F). HT + AS = aortic stenosis with hypertension; ASwHT = aortic stenosis without hypertension.

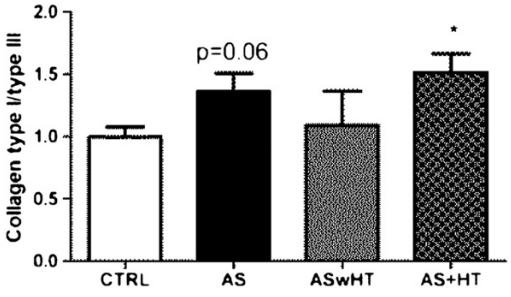


**Fig. 3.** Relation collagen type I/type III expression *p < 0.05 vs CTRL. HT + AS = aortic stenosis with hypertension; ASwHT = aortic stenosis without hypertension.

failure symptoms when compared to non-hypertensive AS patients with the same echocardiographic parameters of AS severity.

Data on systemic blood pressure before and after AVR is absent in most studies on incomplete LVH regression and only recently some authors have reported its relation to postoperative LVM [23–26]. In a retrospective observational study with 79 pure AS patients, the only independent predictors of postoperative LVM index were preoperative LVM and postoperative systolic blood pressure (defined as normal if <130 mm Hg) [23]. Uncontrolled hypertension was not only related to higher LVM after AVR but also with worse survival, with higher incidence of heart failure and bleeding as causes of death [26]. These observations resulted in a general recommendation for strict blood pressure control after AVR [23,26].

What is less understood is if the existence of HT *per se* can influence LVM regression even under similar load conditions. Our results suggest that HT blunts LVM normalization and reverse remodeling after AVR for isolated AS independently of load. As expected, there was a trend for higher systolic blood pressure in hypertensive patients, but the total LV afterload, evaluated by valvuloarterial impedance, that takes into account systolic blood pressure, prosthetic gradient and stoke volume, was not significantly different between patients with and without HT. One can speculate that this could be due to the systemic nature of hypertensive disease, with a generalized neurohumoral activation, with particular focus on the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system (SNS), which directly promote myocyte hypertrophy and matrix deposition independently of their effects on systemic arterial pressure [27]. These same factors, promote both hypertension and LVH and there is the possibility that increased blood pressure is the consequence, rather than the cause, of LVH and associated vascular structural changes. Data from the Framingham Heart Study demonstrated a direct and continuous relationship between LVM and the subsequent development of hypertension in previously normotensive subjects [28]. Also, in a study in young healthy subjects, plasma angiotensin II was an independent predictor of LVM and its effect was independent of systolic blood pressure and body size [29]. Moreover, the magnitude of LVH regression achieved by inhibiting the RAAS and SNS is greater than that produced by comparable BP reduction alone [30]. All this data supports the hypothesis that neuroendocrine mechanisms are important in the regression of LVH, independently of blood pressure.

### *5.2. HT and extracellular matrix remodeling in AS*

In chronic pressure overload, the development of LVH is simultaneous with remodeling of the ECM with progressive interstitial fibrosis, reduced ventricular compliance and diastolic dysfunction [7,31,32]. Altered levels of matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) are crucial in remodeling of the ECM during LV hypertrophy and in the failing heart [33,34].

In LVH associated with AS, there is an increased in collagen production and inhibition of collagen degradation [11,35,36]. When compared with controls, myocardial biopsies of our AS patients have higher collagen synthesis, in accordance with previous reports [11,35]. The levels of MMPs and their inhibitors (TIMPs), however, were not different in the total study group vs controls. In the literature results in the MMP and TIMP expression in AS are variable [11,36], but their balance is always favoring decreased ECM degradation.

In isolated hypertension, a growing number of studies have shown imbalanced MMP activity, confirming findings obtained with animal models [37]. Most of these studies have concentrated on circulating levels of MMPs and TIMPs and a considerable number of them have provided inconsistent results [38–43]. What is not well established is if the coexistence of HT and AS can influence MMP/TIMP balance.

To the best of our knowledge this is the first report on the myocardial expression of MMPs and their tissue inhibitors in combined HT and AS. In our study only HT + AS patients have an upregulation of TIMP 2 and an increase in collagen type I/type III ratio, suggesting a shift towards collagen accumulation and a stiffer form of collagen meshwork. When comparing patients with and without hypertension, it becomes clear that collagen degradation seems to be more impaired in HT + AS given the higher levels of TIMP2 expression and this finding is accompanied by higher levels of LVM index.

## 6. Study limitations

Although our study is hypothesis generating, it has some limitations: (1) Our evaluation was done one year after AVR because in the literature significant LVM regression is maximal after the first year, and has only a non-significant slight decrease after 18 months to 10 years [25,44,45]. To know if HT definitely impairs LV mass regression or if it only makes it slower a longer follow-up period is needed. (2) At follow-up blood pressure values were determined only at office visit. Given blood pressure values are labile, for a more accurate evaluation of the impact of blood pressure on the hypertrophic response, it would have been important to have more determinations at other time points. (3) Medical therapy, namely ACEI/ARB, was prescribed in a non-randomized manner and can be a cause of bias. (4) The small number of patients enrolled may have precluded the identification of other factors influencing LVM regression besides baseline LVM, HT and DM. (5) Moreover, the molecular substudy included only 32 AS patients and had no statistical power to find correlations between ECM gene expression and clinical and echocardiographic data.

## 7. Conclusions

Our results, showing that HT in AS is associated with ECM remodeling favoring collagen deposition and higher LVM, together with the negative impact of HT on LV mass regression and reverse remodeling after AVR, gives strength to the concept of modulation of the RAAS system in AS, particularly if HT is also present. Therefore, therapeutic intervention with antagonists of the RAAS is very appealing, especially considering the pro-hypertrophic and pro-fibrotic effects of angiotensin II. New and large scale randomized clinical trials, including morbidity and mortality endpoints, are needed to establish the role of RAAS blockade after AVR.

## Acknowldgements

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2013.11.006.

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# RESULTS

## 5.5. Prognostic implications of fibrosis in low risk aortic stenosis patients

## 5.5 Prognostic implications of fibrosis in low risk aortic stenosis patients

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## Abstract

**Background:** Among aortic stenosis (AS) patients, interstitial fibrosis has been associated with the progression to heart failure and is a marker of worse prognosis.

**Objectives:** We aimed to evaluate the impact of myocardial fibrosis on clinical events after aortic valve replacement (AVR) in low risk severe AS.

**Methods:** We prospectively followed 56 severe AS patients with ejection fraction (EF) >40%, who underwent AVR with simultaneous myocardial biopsies and collagen volume fraction (CVF) determination. Mean follow-up was 5±2 years. Outcomes were all-cause death and the combined endpoint of all-cause death or non-fatal cardiovascular hospitalization after 8 years of follow-up.

**Results:** Patients' mean age was 66±12 years, 67.9% women, mostly mildly symptomatic (NYHA class II:76.8%), They had low risk of operative mortality (Euroscore II:1.5±1.0%), and EF was normal or mildly compromised (EF:63.7±7.6%). At follow-up, there was a significant decrease in transaortic gradients and wall stress, as well as regression in indexed LV mass (LVMI). Mean value of CVF was 16.9±13.5%. There were 7 deaths (12.5%) and 4 non-fatal cardiovascular hospitalizations (7.1%). Baseline clinical characteristics, aortic stenosis severity, LVMI and EF, were similar between patients with or without an event. Patients who suffered a fatal event or the combined endpoint had higher degree of fibrosis (27.1±20.7% vs 15.4±11.8%, p=0.035; 24.0±18.2% vs 15.3±12.0%, p=0.038, respectively). Patients with CVF≥15.4% had lower survival (37.5% vs 97.0%, p=0.001) and survival free of the combined endpoint (0 vs 91.2%, p<0.001). On Cox regression analysis, CVF was the only independent predictor of all-cause death (HR1.88; 95%CI:1.08-3.29 for 10% increase; p=0.026) and all-cause death or cardiovascular hospitalization (HR1.73; 95%CI:1.03-2.911 for 10% increase; p=0.038).

**Conclusions:** In low risk AS patients, higher levels of fibrosis are independent predictors of all-cause death and the composite of all-cause death and non-fatal cardiovascular hospitalization. Further advances on anti-fibrotic therapies in the setting of AS are needed.

## Introduction:

In chronic pressure overload states, like systemic hypertension (HT) and AS, the left ventricle (LV) responds with hypertrophy and altered geometry as an adaptive mechanism that helps to maintain contractile performance despite abnormal loading conditions. Concomitantly, there are changes in cardiomyocytes and extracellular matrix connective tissue, some of them irreversible, which may help to explain the deterioration of diastolic and systolic function that take place after longstanding overload [1].

Aortic valve replacement increases long-term survival, which becomes similar to age-matched population, reduces symptoms and improves quality of life in patients with aortic valve stenosis [2, 3]. Late outcome after AVR depends mainly on the stage of heart disease before surgery, prosthetic related complications, and co-morbidities.

At histopathological level, fibrosis has been implicated in the progression from the compensate phase to heart failure [4] and those with higher grades of fibrosis have more severe myocardial disease and lower long-term survival rates [5, 6].

Our aim was to determine if fibrosis levels, in patients with severe aortic stenosis but milder forms of remodeling and EF >40%, had any impact in long-term clinical outcomes.

## Methods:

### *Patient selection and follow-up*

Between January 2006 and December 2009 we included consecutive patients over 18 years old with severe symptomatic AS (aortic valve area <1 $cm^2$ or mean transaortic gradient ≥40 mmHg) referred for aortic valve replacement (AVR) at the Cardiothoracic Surgery Department of Centro Hospitalar São João, Porto, Portugal. We excluded patients with aortic regurgitation > II/IV or other significant valve diseases (> mild), significant coronary artery disease (lesions > 50% on coronary angiography), ejection fraction ≤ 40%, or previous cardiac surgery. All patients had to be in sinus rhythm at the time of inclusion. From the initial 141 patients included in a prospective cohort, at time of AVR, 56 random patients were submitted to myocardial biopsies for collagen volume fraction evaluation and were considered for this prospective analysis. Clinical and echocardiographic follow up was achieved in all patients. Mean clinical follow-up was 5.0±2.2 years and mean final echocardiographic follow-up was 4.0±1.8 years.

The diagnosis of hypertension was performed by the clinical records of the assistant physician. Renal insufficiency was determined when creatinine clearance <60 ml/kg by the Cockcroft-Gaul formula. Clinical endpoints were defined as all-cause death and a composite of all-cause death or non-fatal cardiovascular hospitalization (for heart failure, myocardial infarction, stroke, new-onset atrial fibrillation or advanced AV block requiring hospitalization).

## *Surgical technique*

All surgeries were performed using standard procedure for AVR. The patients were placed on cardiopulmonary bypass and cardiac arrest was induced and maintained with cold blood cardioplegia. The majority of patients received a bioprosthesis (64.3%) and prosthesis sizes used were <21 mm in 12.5%, 21 mm in 39.3%, 23 mm in 32.1% and 25 mm in 16.1%. At the time of surgery, patients underwent myocardial biopsies from the LV interventricular septum.

## *Echocardiographic studies*

Echocardiographic examination was performed by a trained cardiologist and recorded on digital support. All recordings were examined by an experienced echocardiographer in an accredited independent echocardiography laboratory (Hospital Clínico San Carlos in Madrid, Spain) blinded to patient details. Studies were performed using Phillips IE-33 equipment with a S5-1 transducer and M-mode, two dimensional, pulsed, continuous, color-flow and tissue Doppler capabilities. Correct orientation of imaging planes, cardiac chambers dimensions and function measurements were performed according to the European Association of Echocardiography (EAE)/American Society of Echocardiography (ASE) recommendations [7].

LV mass was estimated according to the joint recommendations of the ASE and EAE using Devereux's formula for ASE measurements in diastole: LV mass=0.8 x (1.04 x [LV internal dimension + posterior wall thickness + interventricular septal thickness]$^3$ – [LV internal dimension]$^3$) + 0.6 g [7]. Left ventricular hypertrophy was defined by LV mass index greater than 115 g/m$^2$ in men and greater than 95 g/m$^2$ in women.

Relative wall thickness (RWT) was calculated for the assessment of LV geometry using the formula 2x posterior wall thickness/ LV diastolic diameter. Increased RWT was present when this ratio was greater than 0.42[7]. LA volume was measured in LV end systole in the frame preceding mitral valve opening. The volume was measured using the biplane area length method and corrected for body surface area. Aortic valve area was estimated using quantitative Doppler by continuity equation.

Mitral inflow was assessed in the apical 4-chamber view using pulsed wave Doppler with the sample volume placed at the tips of mitral leaflets during diastole. From the mitral inflow profile, the peak flow velocity of early filling (E wave), peak flow velocity of atrial contraction (A wave), the E/A ratio, and early filling deceleration time (DT) were measured. Doppler tissue imaging (DTI) of the mitral annulus was obtained from the apical 4-chamber using a sample volume placed in the septal mitral valve annulus. The peak systolic annular velocity (Sm) and early diastolic septal velocity (e') was determined, and the E/e' ratio was derived.

As a measure of global LV load, we calculated the valvuloarterial impedance: Zva =(SAP+ MG)/SVI, where SAP is the systolic arterial pressure and MG is the mean transvalvular pressure gradient and SVI is stroke volume index [8].

### *Histological determination of fibrosis*

Light microscopic quantification of fibrosis has previously been described and validated [9]. Fibrosis analysis of myocardial biopsies was performed using picrosirius-red–stained, 4-µm-thick-sections of tissue (± 5 sections of each sample). Images of these sections were acquired with a projection microscope (x50). Subsequent image analysis with Slidebook 4.0 software (3I, Denver, Colo) was performed to determine the extent of reactive interstitial fibrosis, which was expressed as collagen volume fraction (%). Areas of reparative and perivascular fibrosis were excluded. Myocardial fibrosis was calculated as the sum of all connective tissue areas divided by the sum of connective tissue and muscle areas averaged over 4 to 6 representative fields of the section in 18 male and 38 female AS patients. In our laboratory, normal values of fibrosis for LV myocardial biopsy material is 5.4±2.2%.

### *Statistical analysis*

Categorical variables were expressed as percentages and continuous variables as mean±standard deviation unless otherwise specified. Continuous variables were compared between groups using an unpaired t-test (for normally distributed variables) or the Mann–Whitney U-test (for non-normally distributed variables). For comparison between baseline and follow-up a paired Student's t-test was applied or a Wilcoxon test (for non-normally distributed variables). Chi-square test (or Fisher exact test) was used to compare categorical variables. Univariable binary logistic regression models (Wald method, p=0.05/0.20 for covariate inclusion/exclusion) were used in conjunction with the area under the curve (ROC) to assess the best cutoff-point (highest sensibility and specificity) of a continuous variable to predict a particular outcome of all-cause death and all-cause death and cardiovascular hospitalization. The Kaplan-Meier and Cox models were used to evaluate survival times after surgery for all-cause death, for non-fatal cardiovascular hospitalization, and for all-cause death and cardiovascular hospitalization, and the log-rank test was used to compare survival curves. All reported probability values are two-tailed, and $P<0.05$ was considered statistically significant. Analyses were performed with the IBM® SPSS® Statistics software package (version 22.0) (SPSS Inc, Chicago, IL, USA).

## Results

Patients were mainly women (67.9%) and mean age was 66.3±11.5 years (table 1). They had severe aortic stenosis (aortic valve area of 0.41±0.13 cm2), the majority were mildly symptomatic (NYHA class II in 76.8%), had low risk of operative mortality (mean Euroscore II of 1.5±1.0%), and global systolic function was normal or mildly compromised, with a mean LV ejection fraction (EF) of 63.7±7.6% (3 patients had EF<50 % and the minimum was 42.7%).

*Table 1: Clinical characterization of aortic stenosis patients.*

| | **n=56** |
|---|---|
| **Sex (Female)** [n (%)] | 38 (67.9%) |
| **Age** | 66.3±11.5 |
| **BSA** | 1.7±0.2 |
| **Euroscore II** | 1.5±1.0 |
| **HT** [n (%)] | 27 (48.2%) |
| **DM** [n (%)] | 10 (17.9%) |
| **CKD** [n (%)] | 22 (39.3%) |
| **GFR** (ml/min) | 66.2±20.8 |
| **NYHA≥3** [n (%)] | 13 (23.2%) |
| **LVH basal** [n (%)] | 47 (83.9%) |
| **LVH final** [n (%)] | 24 (47.1%) |

BSA= body surface area; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate; NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy. Results are presented as mean± standard deviation unless otherwise noted.

In final echocardiographic evaluation, performed 4.0±1.8 years after surgery, patients experienced a significant decrease in transaortic gradients and wall stress, as well as regression in indexed LV mass (LVMI) (table 2). The median absolute and relative decrease in LVMI was 20.9 g (P25-75: 1.0-39.9 g) and 17.2% (P25-75: 1.0-26.9%), respectively.

Mean value of collagen volume fraction (CVF), evaluated at the time of surgery, was 16.9±13.5% (median 12.8%, P25-75: 7.7-18.8%). There was no correlation between CVF and preoperative LVMI, LV diameters or volumes, relative wall thickness or EF. Although patients in NYHA class ≥ III before surgery had higher levels of myocardial fibrosis, this difference was not statistically significant (CVF 21.2±15.8% vs 15.6±12.7%, p=0.327). When comparing final with baseline NYHA class, patients with functional improvement after AVR had lower values of CVF at the time of surgery (11.5±9.3% vs 17.3±8.4%, p=0.036) (Fig.1).

Collagen volume fraction (CVF) was higher in patients with persistence of left ventricular hypertrophy (LVH) late after aortic valve replacement (20.0±14.6% vs 13.2±11.5%, p=0.027).

*Table 2: Baseline and final echocardiographic characterization of aortic stenosis patients.*

| | Basal | Final | p |
|---|---|---|---|
| **LV geometry** | | | |
| **Interventricular septum** (cm) | 1.45 ±0.26 | 1.27 ±0.23 | <0.001 |
| **Posterior wall** (cm) | 1.08 ±0.18 | 1.02 ±0.17 | 0.033 |
| **Relative wall thickness** | 0.47 ±0.1 | 0.45 ±0.09 | 0.126 |
| **LV mass index** (g/m²) | 129.5 ±31.9 | 109.8 ±30.8 | 0.000 |
| **LV end-systolic volume index** (ml/m²) | 17.8 ±7 | 18.5 ±8.8 | 0.779 |
| **LV end-diastolic volume index** (ml/m²) | 48.2 ±12.5 | 49.4 ±17.2 | 0.819 |
| **Aortic/Prosthesis stenosis severity** | | | |
| **Maximal transaortic velocity** (cm/s) | 472.4 ±69.8 | 263 ±73.6 | <0.001 |
| **Maximal transaortic gradient** (mmHg) | 89.6 ±26.2 | 29.4 ±17.6 | <0.001 |
| **Medium transaortic gradient** (mmHg) | 54.7 ±16.5 | 16.1 ±8.7 | <0.001 |
| **Effective orifice area index** (cm/m²) | 0.41 ±0.13 | 0.86 ±0.27 | <0.001 |
| **Increase effective orifice area** (%) | 124.5 ±83.3 | | |
| **Hemodynamic load** | | | |
| **Valvuloarterial impedance** (mm Hg/ml/m²) | 6.6 ±2.6 | 5.8 ±2.3 | 0.072 |
| **Peak LV wall stress** (dynes/cm²) | 227.1 ±89.6 | 168.3 ±41.6 | 0.005 |
| **Systolic function** | | | |
| **Ejection fraction** (%) | 63.7 ±7.6 | 64.3 ±6.9 | 0.525 |
| **Systolic annular velocity** (cm/s) | 5.53 ±1.11 | 6.01 ±1.10 | 0.159 |

Values are mean±SD unless otherwise indicated.

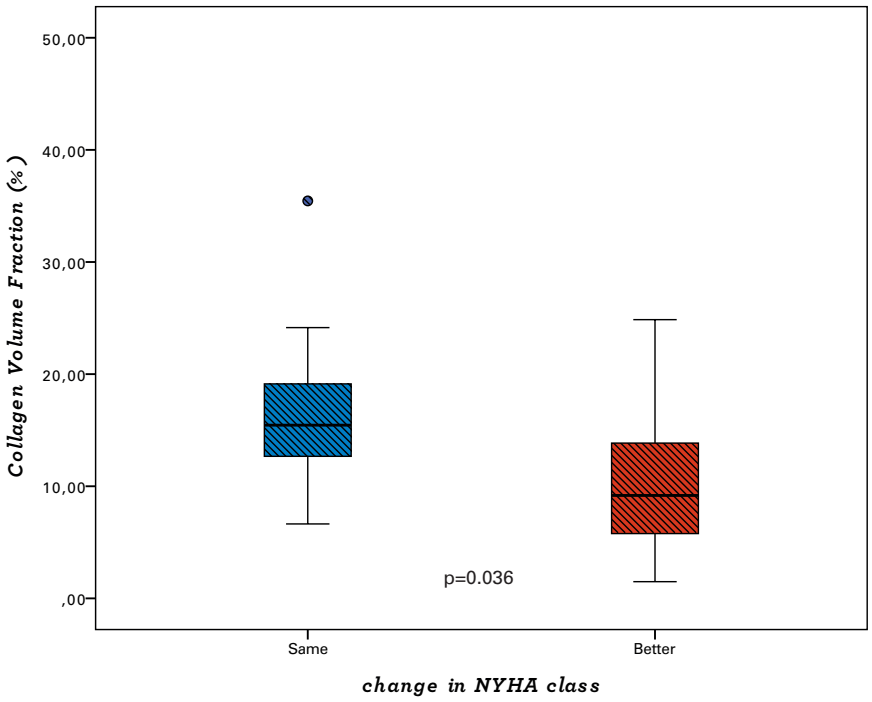



*Fig. 1 Levels of collagen volume fraction (CVF) in patients according to improvement in NYHA class after AVR.*

## *Clinical outcomes*

At the end of 5.0±2.2 years of follow-up, there were 7 deaths (12.5%) and 4 non-fatal cardiovascular hospitalizations (7.1%, 2 patients for heart failure, 1 for de novo atrial fibrillation and 1 for biologic prosthesis dysfunction).

*Table 3: Baseline characterization according to the occurrence of all-cause death or non-fatal cardiovascular hospitalization.*

| | **All-cause death or non-fatal cardiovascular hospitalization** | | |
|---|---|---|---|
| | **No (n=46)** | **Yes (n=10)** | **p** |
| **Sex (Female)** [n (%)] | 30 (65.2%) | 8 (80.0%) | 0.474 |
| **Age** | 64.7±11.6 | 73.8±8.0 | 0.260 |
| **Euroscore II** | 1.58±0.74 | 1.15±0.18 | 0.414 |
| **HT** [n (%)] | 21 (45.7%) | 6 (60.0%) | 0.497 |
| **DM** [n (%)] | 8 (17.4%) | 2 (20.0%) | 1.000 |
| **CKD** [n (%)] | 17 (37.0%) | 5 (50.0%) | 0.490 |
| **GFR** (ml/min) | 59.0±16.8 | 79.4±30.3 | 0.293 |
| **NYHA≥3** [n (%)] | 10 (21.7%) | 3 (30.0%) | 0.682 |
| **LVH basal** [n (%)] | 38 (82.6%) | 9 (90.0%) | 1.000 |
| **LVH final** [n (%)] | 17 (37.0%) | 7 (70.0%) | 0.042 |
| **LV geometry** | | | |
| **LV end-diastolic diameter index** ($cm/m^2$) | 2.66±0.38 | 2.65±0.29 | 0.563 |
| **LV end-systolic diameter index** ($cm/m^2$) | 1.76±0.28 | 1.63±0.12 | 0.078 |
| **Relative wall thickness** | 0.46±0.10 | 0.43±0.01 | 0.422 |
| **LV mass index** ($g/m^2$) | 120.6±34.5 | 119.1±8.5 | 0.684 |
| **LV end-diastolic volume index** ($ml/m^2$) | 48.7±12.2 | 26.6±5.2 | 0.147 |
| **LV end-systolic volume index** ($ml/m^2$) | 19.2±6.7 | 8.4±1.0 | 0.095 |
| **Aortic/Prosthesis stenosis severity** | | | |
| **Maximal transaortic velocity** (cm/s) | 456.4±58.9 | 500.1±85.8 | 0.283 |
| **Effective orifice area index** ($cm/m^2$) | 0.38±0.11 | 0.41±0.05 | 0.974 |
| **Hemodynamic load** | | | |
| **Valvuloarterial impedance** (mm Hg/$ml/m^2$) | 6.7±1.6 | 10.1±3.5 | 0.404 |
| **Peak LV wall stress** ($dynes/cm^2$) | 227.3±51.7 | 241.1±52.4 | 0.699 |
| **Diastolic function** | | | |
| **E/A** | 0.77±0.26 | 1.03±0.64 | 0.181 |
| **e'** | 5.2±1.7 | 5.1±1.9 | 0.836 |
| **E/e'** | 17.6±6.3 | 21.8±0.35 | 0.240 |
| **Systolic function** | | | |
| **Ejection fraction** (%) | 61.0±7.9 | 68.3±2.3 | 0.330 |
| **Systolic annular velocity** (cm/s) | 5.37±1.13 | 4.38±0.56 | 0.031 |
| **Collagen volume fraction** (%) | 24.0±18.2 | 15.3±12.0 | 0.038 |

HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; GFR= glomerular filtration rate; NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy. Results are presented as mean± standard deviation unless otherwise noted.

Patients with an event (all-cause death or cardiovascular hospitalization) had no significant differences in baseline clinical characteristics (table 3) comparing with those without events. In preoperative echocardiogram, these patients had worse longitudinal function, with a lower value of peak systolic annular velocity (Sm) (table 3), and no differences in aortic stenosis severity, LV mass or ejection fraction.

There was a positive association between the level of fibrosis, evaluated by CVF, and clinical outcomes (Fig.2). Patients who died had significantly higher degree of fibrosis at the time of surgery (27.1±20.7% vs 15.4±11.8%, p=0.035), and the same trend was observed for non-fatal cardiovascular hospitalization (24.8±17.7% vs 6.7±3.2%, p=0.114). Myocardial fibrosis levels were also higher in those with the composite outcome of all-cause death or non-fatal cardiovascular hospitalization (24.0±18.2% vs 15.3±12.0%, p=0.038).

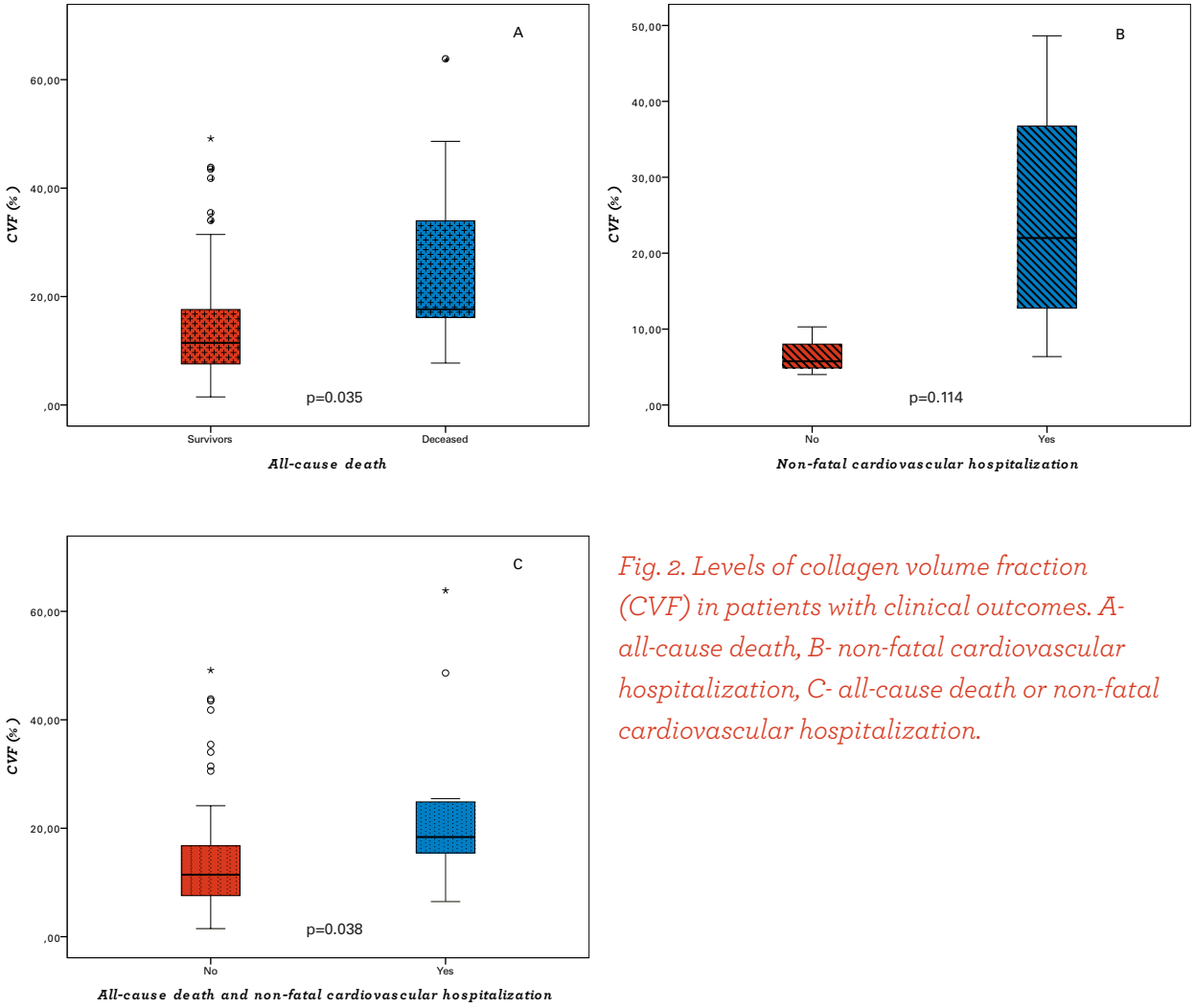



*Fig. 2. Levels of collagen volume fraction (CVF) in patients with clinical outcomes. A- all-cause death, B- non-fatal cardiovascular hospitalization, C- all-cause death or non-fatal cardiovascular hospitalization.*

After multivariate Cox regression analysis, CVF was the only independent predictor of all-cause death (HR 1.88; 95%CI:1.08-3.29, for each 10% increase; p=0.026) and all-cause death or cardiovascular hospitalization (HR 1.73; 95%CI:1.03-2.911, for each 10% increase; p=0.038) (table 4).

*Table 4: Univariate and multivariate associations with all-cause death and the outcome all-cause death and non-fatal cardiovascular hospitalization.*

| | All-cause death | | | | | All-cause death and non-fatal cardiovascular hospitalization | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variable** | | **Univariable analysis** | | **Multivarible analysis** | | **Univariable analysis** | | **Multivariable analysis** | |
| | **p** | **Unadjusted HR (95%CI)** | **p** | **Adjusted HR (95%CI)** | **p** | **Unadjusted HR (95%CI)** | **p** | **Adjusted HR (95%CI)** | |
| **Sex** | 0.370 | 2.035 (0.431-9.614) | | | 0.306 | 3.025 (0.363-25.179) | | | |
| **Age** (10y increment) | 0.022 | 2.509 (1.142-5.515) | 0.171 | 1.684 (0.798-3.552) | 0.035 | 2.802 (1.077-7.288) | | | |
| **HT** | 0.307 | 1.938 (0.545-6.893) | | | 0.525 | 1.627 (0.363-7.284) | | | |
| **DM** | 0.754 | 1.283 (0.271-6.066) | | | 0.380 | 2.09 (0.403-10.844) | | | |
| **Euroscore II** | 0.013 | 1.553 (1.099-2.195) | | | 0.012 | 1.626 (1.112-2.378) | 0.059 | 1.519 (0.984-2.345) | |
| **GFR** | 0.138 | 0.975 (0.944-1.008) | | | 0.198 | 0.973 (0.934-1.014) | | | |
| **NYHA class** ≥ III | 0.639 | 1.383 (0.357-5.354) | | | 0.710 | 1.365 (0.265-7.046) | | | |
| **LVMI** | 0.992 | 1.000 (0.980-1.020) | | | 0.948 | 1.001 (0.977-1.025) | | | |
| **EOAI** | 0.682 | 0.312 (0.001-81.386) | | | 0.845 | 0.532 (0.001-294.369) | | | |
| **SVI** | 0.641 | 0.979 (0.896-1.070) | | | 0.583 | 0.972 (0.877-1.077) | | | |
| **Zva** | 0.600 | 1.065 (0.841-1.350) | | | 0.938 | 1.013 (0.732-1.401) | | | |
| **EF** | 0.274 | 1.060 (0.955-1.176) | | | 0.641 | 1.028 (0.915-1.156) | | | |
| **Residual LVH** | 0.058 | 4.591 (0.952-22.138) | 0.097 | 3.825 (0.783-18.695) | 0.284 | 2.528 (0.463-13.815) | | | |
| **CVF** (10% increments) | 0.014 | 1.681 (1.113-2.539) | 0.038 | 1.733 (1.032-2.911) | 0.013 | 1.811 (1.135-2.889) | 0.026 | 1.882 (1.078-3.289) | |

Variables included in multivariable analysis: age, Euroscore II, glomerular filtration rate, residual LVH, collagen volume fraction
HT= arterial hypertension, DM= diabetes mellitus, GFR= glomerular filtration rate (MDRD formula), LVMI= left ventricular mass index, EOAI= effective orifice area index, SVI= stroke volume index, Zva= valvuloarterial impedance, EF= ejection fraction, LVH= left ventricular hypertrophy at follow-up, CVF= collagen volume fraction

### *Kaplan-Meier survival analysis*

A cut-off of 15.4% for CVF was used to calculate survival probability free of events. This value was chosen after ROC curve analysis to assess the best cutoff-point of CVF to predict the outcome of all-cause death (AUC 0.75, p=0.036) and all-cause death and cardiovascular hospitalization (AUC 0.92, p= 0.038). Comparative characteristics of patients with CVF ≥15.4% vs CVF <15.4% can be seen in tables 5 and 6. Patients with CVF ≥15.4% had lower probability of survival free of all-cause death (37.5% vs 97.0%, p=0.001), non-fatal cardiovascular hospitalization (84.4% vs 94.1%, p=0.018) and the composite of all-cause death or non-fatal cardiovascular hospitalization (0% vs 91.2%, p<0.001) (Fig. 3).

*Table 5: Clinical characterization according to the value of collagen volume fraction (CVF).*

| | | CVF ≥15.4% | CVF <15.4% | p |
|---|---|---|---|---|
| **Sex** (Female) | n (%) | 16 (76.2%) | 22 (62.9%) | 0.301 |
| **Age** (year) | | 68.4±11.3 | 65.1±11.8 | 0.297 |
| **BSA** | | 1.7±0.2 | 1.8±0.2 | 0.799 |
| **Euroscore II (%)** | | 1.9±1.4 | 1.4±0.7 | 0.182 |
| **HTA** | n (%) | 11 (52.4%) | 16 (45.7%) | 0.629 |
| **DM** | n (%) | 6 (28.6%) | 4 (11.4%) | 0.152 |
| **CKD** | n (%) | 10 (47.6%) | 12 (34.3%) | 0.323 |
| **GFR** (ml/min) | | 61.2±20.7 | 69.3±20.6 | 0.130 |
| **NYHA≥3** | n (%) | 7 (33.3%) | 6 (17.1%) | 0.201 |
| **LVH basal** | n (%) | 19 (90.5%) | 28 (80%) | 0.459 |
| **LVH final** | n (%) | 13 (72.2%) | 11 (33.3%) | 0.008 |

BSA= body surface area; HT= hypertension; DM= diabetes mellitus; CKD= chronic kidney disease; Cr clear= creatinine clearance; GFR= glomerular filtration rate; NYHA= functional class of New York Heart Association; LVH= left ventricle hypertrophy. Results are presented as mean± standard deviation unless otherwise

Patients with residual LVH after surgery had a lower survival free of a combined event (all-cause death or non-fatal cardiovascular hospitalization), when compared with those with normal LVM (70.8% vs 92.6%, p=0.037) (Fig. 4). Moreover, patients with Sm of <4.9 cm/s had significantly worse survival (81.6% vs 100.0%, p=0.021) and worse survival free from death or non-fatal cardiovascular hospitalization (55.8% vs 96.3%, p=0.017; AUC 0.77, p=0.027) (Fig. 5).

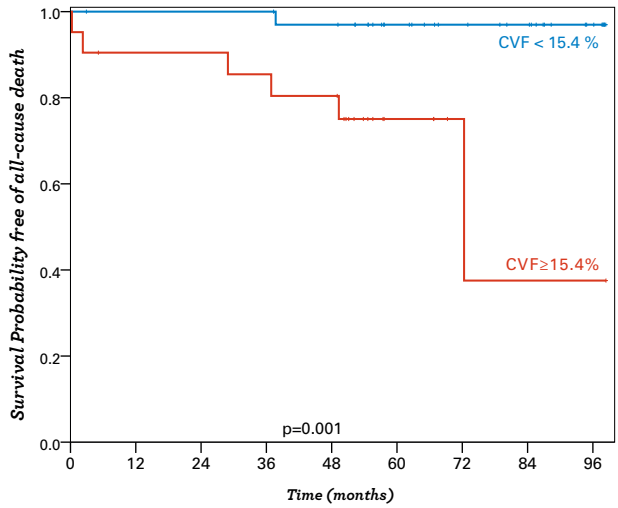
## Discussion

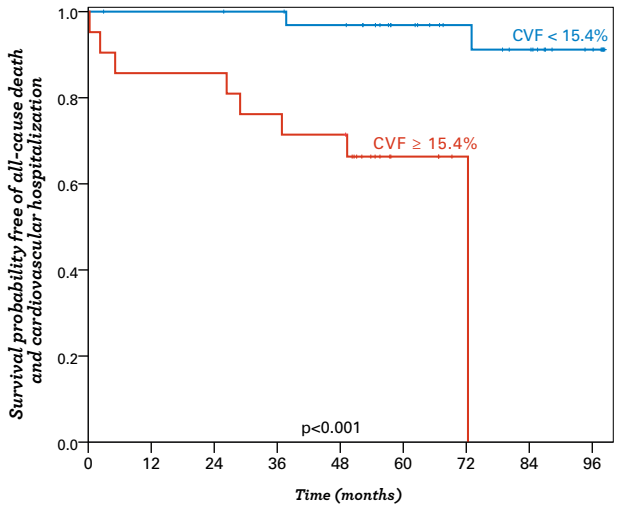
We found that, in our low risk cohort of patients with severe AS, higher levels of fibrosis have a negative prognostic impact with lower survival free of all-cause death or the composite of all-cause death or non-fatal cardiovascular hospitalization. Moreover, it was a predictor of events, independent of other well established prognostic factors such as EF, age, baseline LVMI or NYHA class. For our patients, a cut-off value of 15% of CVF had a good performance as a predictor of clinical events.

*Table 6: Echocardiographic characterization according to the according to the value of collagen volume fraction (CVF) .*

| | CVF ≥15.4% | | p | CVF <15.4% | | p |
|---|---|---|---|---|---|---|
| | **Preoperative** | **Postoperative** | | **Preoperative** | **Postoperative** | |
| **Relative wall thickness** | 0.46±0.9 | 0.48±0.11 | 0.525 | 0.48±0.11 | 0.43±0.1 | 0.015 |
| **LV mass index** (g/m²) | 133.3±35.3 | 114.1±20.4 | 0.023 | 127.4±30.3 | 107.5±35.3 | 0.002 |
| **LV end-diastolic volume index** (ml/m²) | 45.8±9.7 | 46.5±16.3 | 0.893 | 49.6±13.8 | 51.2±17.6 | 0.674 |
| **LV end-systolic volume index** (ml/m²) | 40.2±13.0 | 41.8±9.6 | 0.652 | 31.3±13.3 | 39.7±11.0 | 0.680 |
| **Maximal transaortic velocity** (m/s) | 4.83±0.82 | 2.63±0.69 | <0.001 | 4.68±0.67 | 2.63±0.76 | <0.001 |
| **Effective orifice area index** (cm/m²) | 0.39±0.11 | 0.95±0.34 | <0.001 | 0.42±0.14 | 0.81±0.21 | <0.001 |
| **Peak systolic anular velocity** (cm/s) | 5.7±0.6 | 5.5±1.3 | 0.484 | 5.5±1.2 | 6.2±1.0 | 0.065 |
| **E'** (cm/s) | 4.8±1.3 | 5.7±2.1 | 0.145 | 5.4±2.1 | 5.7±1.8 | 0.441 |
| **E/e'** | 18.9±5.8 | 19.2±7.3 | 0.882 | 15.5±5.3 | 16.6±5.5 | 0.310 |
| **Ejection fraction** (%) | 64.1±7.7 | 62.7±7.2 | 0.515 | 63.5±7.7 | 65.2±6.6 | 0.223 |
| **Stroke volume índex** (ml/m²) | 29.6±8.2 | 30.2±10.3 | 0.876 | 31.4±3.3 | 33.0±9.9 | 0.527 |

Results are presented as mean± standard deviation.



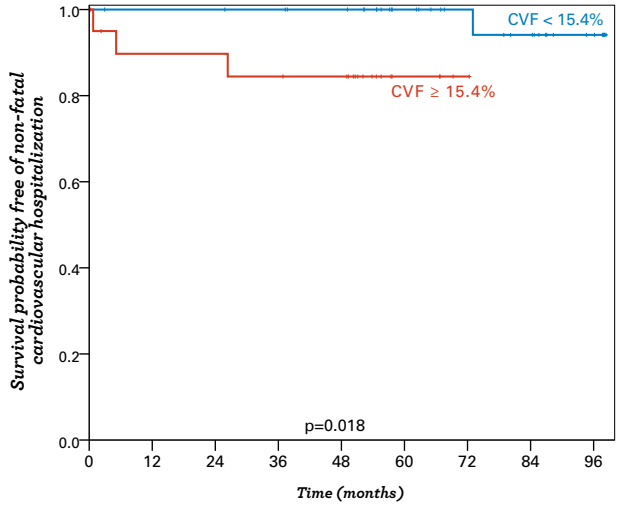




*Fig. 3 Amount of collagen volume fraction (CVF) and long-term survival free of events after aortic valve replacement.*

Others have already described the prognostic importance of myocardial fibrosis (MF) in aortic stenosis, either using histological assessment or noninvasive evaluation by cardiovascular magnetic resonance (CMR) with late gadolinium enhancement (LGE) [5, 6,10-12]. However, most of these studies have analyzed patients with worse preoperative NYHA class and more advanced forms of myocardial disease when compared with our cohort. Patients usually had lower values of EF, higher LV volumes and dimensions and higher levels of histological interstitial fibrosis, suggesting more extensive remodeling [5, 6, 10]. Likewise, the inclusion of aortic regurgitation patients [5] or the coexistence of other cardiovascular comorbidities, such as atrial fibrillation and coronary artery disease [10, 12], which were excluded in our study, may have influenced outcomes.

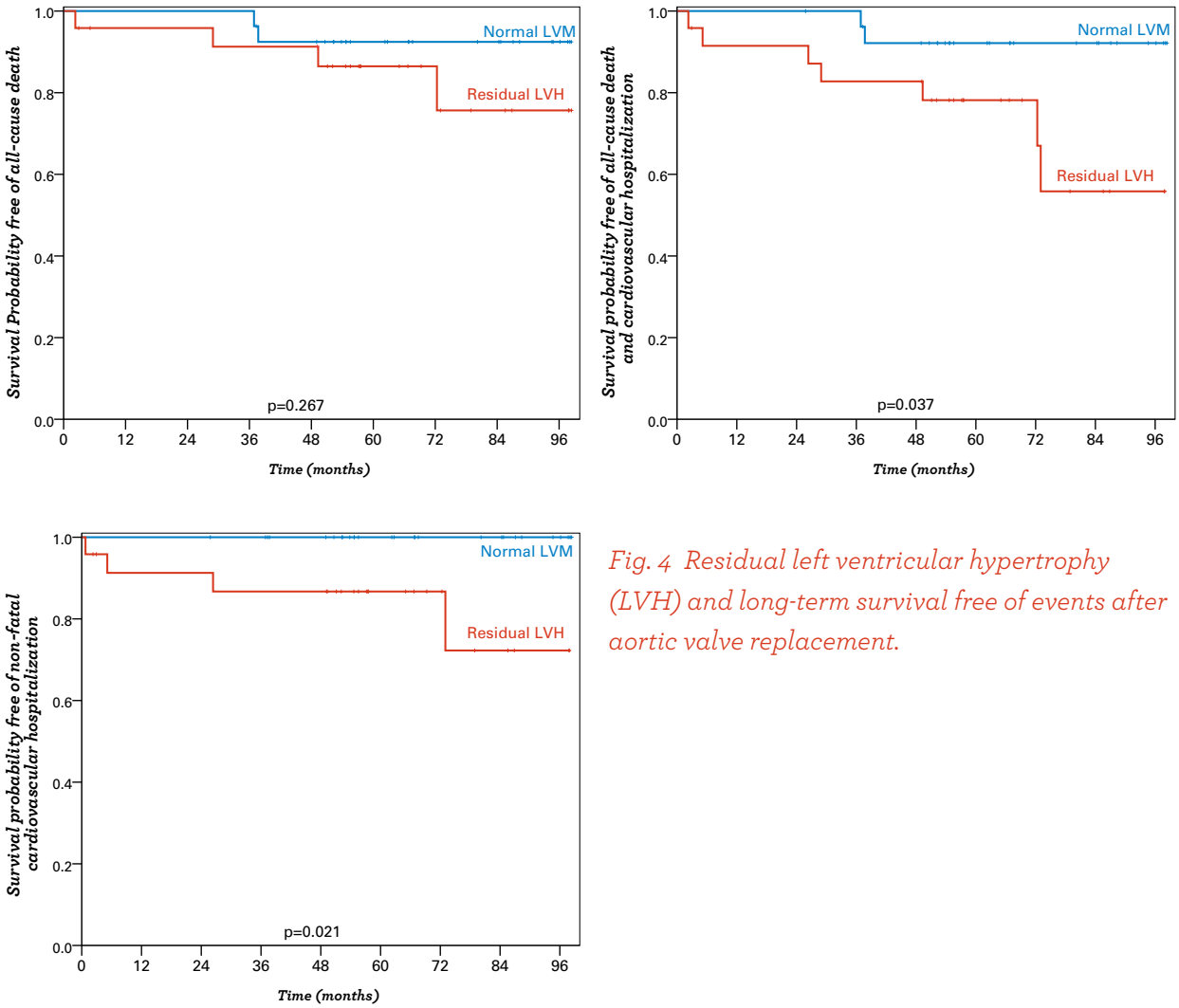



*Fig. 4 Residual left ventricular hypertrophy (LVH) and long-term survival free of events after aortic valve replacement.*

Milano and coworkers[6] have performed a very similar study in a group of 99 patients with AS in which fibrosis was calculated from myocardial biopsies obtained during surgery. In their retrospective analysis, 10 years survival rate was lower in patients with severe fibrosis (defined as fibrous index > 50%) and no significant improvement in NYHA class was seen in this the group. We also found worse long-term survival and lesser NYHA class improvement in those with more severe fibrosis, but ours was a prospective study and our cut-off was much lower. According to Milano and coworkers' criteria (no or mild fibrosis if < 20% and moderate fibrosis if 20-50%), most of our patients would have been included in the group with mild fibrosis. This can help to explain why our patients with higher level of fibrosis have less ventricular remodeling and dysfunction (only 9.5% of those with CVF ≥15.4% have EF<50%) comparing with their group with moderate or severe fibrosis. Moreover, not surprisingly, we didn´t find a significant correlation between fibrosis level and LV diameters, RWT or EF, which was described in the aforementioned work. It is expected for these correlations to be stronger in more severe grades of fibrosis. Even with lesser severe form of myocardial disease, we could still find an increase in events in our AS patients with increasing levels of fibrosis.

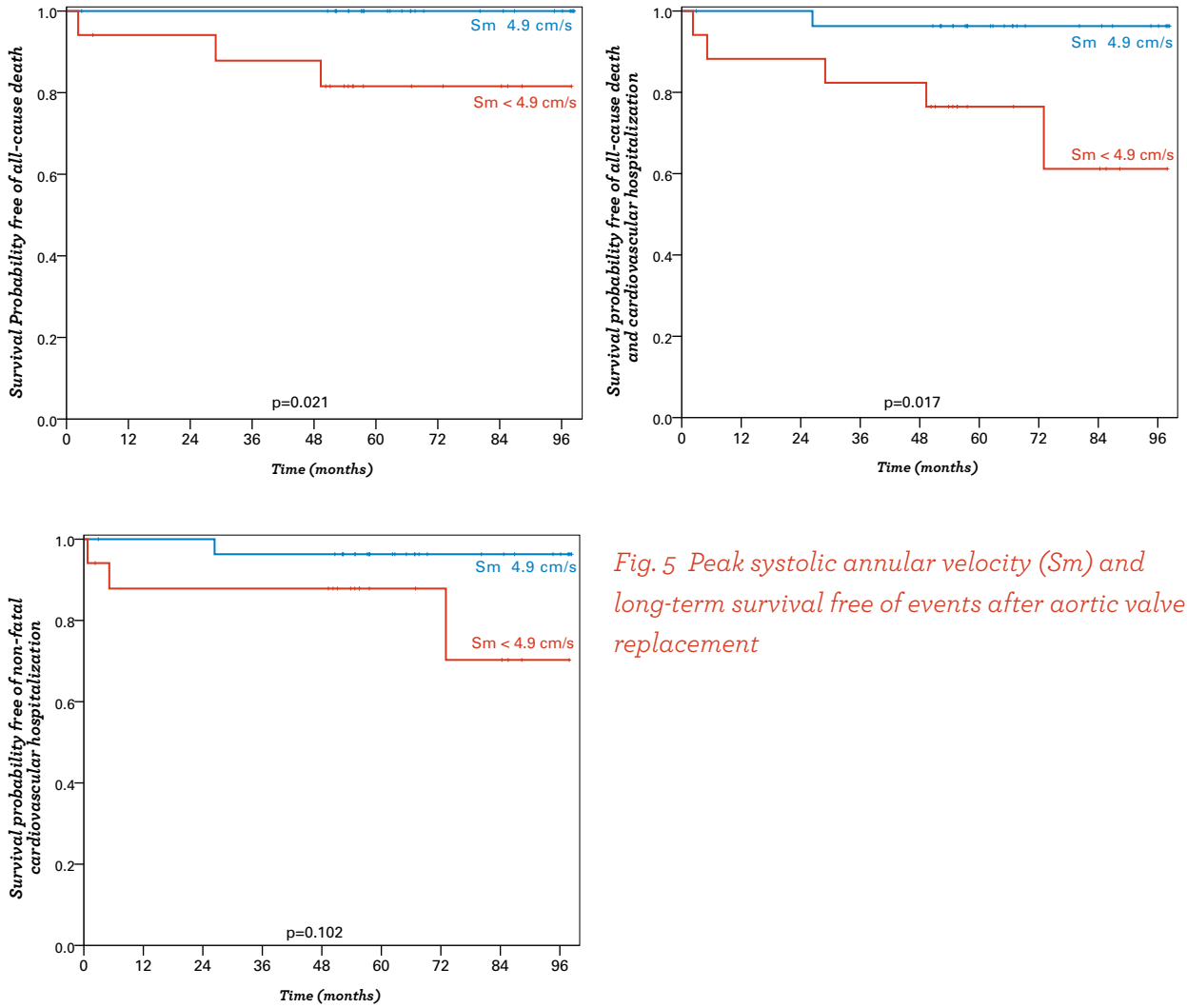



*Fig. 5 Peak systolic annular velocity (Sm) and long-term survival free of events after aortic valve replacement*

## *Myocardial fibrosis in aortic stenosis*

Fibrosis is an early morphological alteration in patients with AS and has been pointed as one of the reasons for impaired LVH regression after AVR [13]. Once established, fibrosis is a major determinant of diastolic and systolic dysfunction and it is one of the structural substrates for arrhythmogenicity, thus playing a major role for sudden death and the progression of HF [4, 14]. While myocyte hypertrophy is dependent on load, fibrosis seems also to be regulated by non-hemodynamic factors such as neuro-hormones [15].

In LVH associated with aortic stenosis, there is an increased production of collagen and a shift towards inhibition of collagen degradation [16-18]. When compared with controls, myocardial biopsies of aortic stenosis patients have higher expression of collagens and an up-regulation of TIMP 1 and 2 mRNA, favoring inhibition of collagen degradation, which significantly correlates with the degree of fibrosis [18]. Experimental studies have described total regression of MMP and TIMP gene expression as well as an association between changes in LVMI and MMP/TIMP gene expression after corrective surgical

therapy and LV hypertrophy regression [19]. The renin-angiotensin-aldosterone (RAA) system seems to be a key factor in this process. Mechanical stretch induces local production of angiotensin II, which in turn stimulates the release of multiple growth factors and cytokines from cardiac fibroblasts that act in an autocrine and paracrine fashion, affecting the progression of hypertrophy and remodeling [20-22].

## *Non invasive assessment of myocardial fibrosis*

The noninvasive evaluation of the level of myocardial fibrosis has been object of several studies, using surrogate echocardiographic parameters or cardiac resonance LGE technic.

CMR imaging using LGE allows for noninvasive evaluation of replacement fibrosis and it has been shown that, in moderate and severe AS, specific patterns like midwall fibrosis are associated with a more advanced hypertrophic response and increase mortality [12]. Late gadolinium enhancement is usually associated with more advanced forms of myocardial disease, worse systolic function and higher LV end-diastolic volumes [23, 24], and, therefore, worse prognosis [5]. But LGE has the limitation of only identifying regional differences in replacement myocardial fibrosis, and can miss diffuse interstitial fibrosis, which is an earlier event in disease progression. However, the more recent CMR contrast-enhanced T1 mapping enables detection of this kind of fibrosis and its prognostic value is being tested in a prospective observational study in patients with AS (The Role of Myocardial Fibrosis in Patients with Aortic Stenosis, NCT01755936).

In severe AS, the degree of myocardial fibrosis is crucial in the transition from compensated hypertrophy to heart failure [4]. With worsening of fibrosis, LV filling pressures increase and, later on, EF decreases. However, EF is reduced only in end-stage disease since it is related with global radial function [25] and fibrotic changes in AS hypertrophic hearts are initially subendocardial and affect basal segments (where regional wall stress is highest). This will impact mainly longitudinal function, which is not well represented by ejection fraction [11]. Mitral ring displacement and strain imaging seem to have overcome EF limitations in evaluation of early changes in systolic function. In a recent study, in patients with symptomatic AS and severe fibrosis, radial function was relatively preserved, while mitral ring displacement, a surrogate of overall longitudinal function of the septum, was reduced and predicted functional improvement[11]. Furthermore, using strain imaging, evidence of subclinical systolic LV dysfunction with decreased LV strain and strain-rate (radial, circumferential, and longitudinal) can be seen in patients with severe AS and preserved LVEF [26].

In our study we used peak systolic annular velocity (Sm) to evaluate longitudinal function and found that patients with Sm <4.9 cm/s had worse survival and survival free of death or non-fatal cardiovascular hospitalization. Our data support the prognostic importance of evaluating early parameters of systolic function in AS.

## Limitations

This was a single center study and the small size of our cohort can have precluded the identification of other predictors of clinical events. Moreover, the limited number of events in our study can result in overfitted multivariate model. These limitations have been described by other groups with published results on this topic and who have included similar sample sizes. Obtaining biologic samples for histological analysis and conducting prospective studies with long-term follow-up can be challenging and only overcome by multicentric collaboration.

## Conclusions

We have confirmed the prognostic relevance of myocardial fibrosis in severe AS, and extended this evidence to low risk patients with a less severe form of myocardial remodeling. Fibrosis is an ominous sign in AS in the continuous of myocardial structural changes and should be actively sought for risk stratification, not only in asymptomatic patients with preserved ejection fraction, but also in symptomatic patients undergoing AVR. After AVR, the use of additional medical therapy modulating the RAA system in patients with non-invasive evidence of fibrosis should be tested in large-scale randomized trials.

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# RESULTS

## 5.6. Determinants of clinical improvement after surgical replacement or transcatheter aortic valve implantation for isolated aortic stenosis

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CARDIOVASCULAR ULTRASOUND

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# Determinants of clinical improvement after surgical replacement or transcatheter aortic valve implantation for isolated aortic stenosis

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## Abstract

**Background:** Transcatheter aortic valve implantation (TAVI) is an alternative to surgical aortic valve replacement (SAVR) in patients with aortic stenosis (AS) and high surgical risk. Hemodynamic performance after TAVI is superior, but the impact of reverse remodeling on clinical improvement is controversial. We aim to address the differences in hemodynamic changes between SAVR and TAVI, and its correlation with LV remodeling and clinical improvement at 6 months follow-up.

**Methods:** Forty-two patients treated by TAVI were compared with 45 SAVR patients with a stented bioprosthesis. Clinical, 2D and 3D echocardiographic data were prospectively obtained before and six months after intervention.

**Results:** Patients had similar distribution for sex, body surface area and AS severity. TAVI patients were older, more symptomatic and had more comorbidities. They also had higher LV filling pressures, larger 3D indexed left atrium volume, but similar 3D indexed LV mass. At 6 months, TAVI patients had greater clinical improvement and higher effective orifice area index (EAOI), but only SAVR patients already had a significant decrease in 3D indexed LV mass and diastolic volume. In univariate analysis older age, NYHA class ≥ III, increase in EAOI and TAVI were related with functional class improvement. After multivariate analysis only NYHA class ≥ III (OR 8.81, CI:2.13-36.52; p = 0.003) and an increase in EAOI ≥ 105% (OR 3.87, CI:1.02-14.70; p = 0.04) were predictors of clinical improvement.

**Conclusions:** At 6 months, functional class improvement was greater after TAVI. Higher initial NYHA class and an increase in EAOI ≥ 105% were independently associated with functional enhancement. It is debatable if left ventricular remodeling is determinant for functional class improvement.

**Keywords:** Aortic stenosis, Transcatheter aortic valve replacement, Surgical aortic valve replacement, Left ventricular mass, Reverse remodeling, Clinical improvement

## Background

Aortic stenosis (AS) is the most prevalent of all valvular diseases in developed countries and its increase has a direct relation with population aging [1]. In elderly patients cardiac surgery can be challenging by the increased number of comorbidities, making transcatheter aortic valve implantation (TAVI) an atractive alternative treatment modality [2].

Surgical aortic valve replacement (SAVR) nearly normalizes long-term survival and improves quality of life in AS patients [3,4] but late outcomes depend mainly on the stage of heart disease before surgery, prosthetic related complications, and comorbidities. Although there is a significant reduction of wall stress and left ventricular (LV) pressure after SAVR, nearly half of patients have residual LV hypertrophy (LVH) late after surgery [5,6]. This persistent increase in LV mass is an independent predictor of cardiac-related morbidity [6] and mortality [7] making reverse remodeling an important outcome after surgery.

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Similarly, TAVI has shown good mid-term results, not inferior to SAVR in high-risk patients [2] and superior to medical therapy [8]. TAVI patients have higher effective orifice areas (EOA) and lower transprosthetic gradients but, in spite of remarkable clinical improvement, LV mass regression and reverse remodeling are less consistent in comparison with SAVR [9-11].

In this study we aim to address the differences in hemodynamic changes between SAVR and TAVI patients, and its correlation with LV remodeling and clinical improvement at 6 months follow-up.

## Methods

### Patient selection and follow-up

The present study is a comparison of two prospective cohorts of patients with symptomatic degenerative severe AS (defined as aortic valve area ≤1 $cm^2$) and LV ejection fraction (EF) ≥ 40%, who underwent SAVR with a stented bioprosthesis or TAVI. This was a collaborative work from two distinct institutions since one of them didn't have a TAVI program at the time of patient inclusion.

Patients with aortic regurgitation > II/IV, moderate to severe mitral or tricuspid regurgitation, significant coronary artery disease (lesions >50% on coronary angiography) or previous cardiac surgery were excluded. All patients had a clinical and echocardiographic evaluation before and at 6 months after the procedure, if alive.

This investigation conforms to the Declaration of Helsinki, had institutional ethical review board approval and each study participant signed an informed consent before enrolment.

### TAVI group

Forty-two consecutive patients with severe aortic stenosis and preserved LV systolic function, submitted to successful TAVI in one tertiary center, Hospital Clinico San Carlos, from April 2009 to April 2010, were included. These patients were obtained from a series of 97 consecutive patients who underwent TAVI, after excluding those with significant LV systolic dysfunction, concomitant moderate to severe mitral valve disease or aortic regurgitation, and those with significant coronary artery disease.

Patients were referred for TAVI due to an excessive risk for SAVR, which was estimated using the logistic EuroSCORE and/or clinical judgment.

Vascular access was obtained either by percutaneous approach through the common femoral artery (30 patients) or transapical approach (12 patients).

The procedure was performed under fluoroscopy and transesophageal echocardiography (TEE) guidance using the techniques described in detail in previous reports [12]. Among all, 31 (73.8%) patients were implanted with an Edwards SAPIEN (Edwards Lifesciences, Irvine, CA, USA). The CoreValve (Medtronic CoreValve Percutaneous System, Medtronic CV) was implanted in 11 (26.2%) patients exclusively by retrograde transfemoral approach. Two valve sizes were available, 23- and 26-mm expanded diameter for Edwards SAPIEN valve and 26- and 29-mm for CoreValve. The prosthesis size was decided according to annulus diameter, measured by TEE. The deployment was performed under the agreement of the interventionist and the echocardiographer. Device success was defined as stable device placement and function as assessed by angiography and echocardiography. All patients with developing new grade III atrioventricular block were implanted with a permanent pacemaker within 3 days after valve implantation.

### SAVR group

Between January 2009 and December 2009, among 141 consecutive patients with isolated symptomatic AS referred for SAVR at the Cardiothoracic Surgery Department of Centro Hospitalar São João, Porto, Portugal we included 45 patients with 3D echocardiographic evaluation. All surgeries were performed using standard procedure for aortic valve replacement. Patients were placed on cardiopulmonary bypass and cardiac arrest was induced and maintained with cold blood cardioplegia. The prosthetic substitutes used in this study were Carpentier-Edwards Perimount pericardial valve (Edwards Lifesciences, Irvine, CA) and the St Jude Medical Epic porcine valve (St Jude Medical, Inc, St Paul, Minn). Valve sizes were 19 mm (n = 4;8.9%), 21 mm (n = 25;55.6%), 23 mm (n = 11;24.4%) and 25 mm (n = 5;11.1%).

### Echocardiographic studies

Echocardiographic examination was performed by a trained cardiologist and recorded on digital support. All recordings were examined by an experienced echocardiographer in an accredited independent echocardiography laboratory (Hospital Clínico San Carlos in Madrid, Spain), blinded to patient details. Studies were performed using Phillips iE-33 equipment with a S5-1 transducer with M-mode, two dimensional, pulsed, continuous, color-flow and tissue Doppler capabilities, and an X3-1 transducer for 3D imaging. Imaging analysis was performed with Xcelera and QLab software. All measurements were performed in accordance with the recommendations of the American Society of Echocardiography [13]. Peak transvalvular gradient was estimated using the simplified Bernoulli equation. Aortic valve area (or effective orifice area, EOA) was estimated using quantitative Doppler by the continuity equation. The EOA values were then indexed to body surface area (EAOI). Patient prosthesis mismatch was considered present if EAOI ≤0.85 cm2/m2 and severe when EAOI ≤ 0.65 cm2/m2.

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The presence, degree, and type (paravalvular vs transvalvular) of aortic regurgitation (AR) were classified as trivial, mild, moderate, or severe according to The Valve Academic Research Consortium (VARC) II [14].

Mitral inflow was assessed in the apical 4-chamber view using pulsed wave Doppler with the sample volume placed at the tips of mitral leaflets during diastole. From the mitral inflow profile, the peak flow velocity of early filling (E wave), peak flow velocity of atrial contraction (A wave), the E/A ratio, and early filling deceleration time (DT) were measured. Doppler tissue imaging of the mitral annulus was obtained from the apical 4-chamber using a sample volume placed in the septal mitral valve annulus. The septal e' velocity value was determined, and the E/e' ratio was derived.

Left atrium (LA) volume, LV systolic and diastolic volumes, and resulting ejection fraction were calculated with direct 3D volumetric analysis.

All indexed values were obtained by dividing by body surface area according to the formula of Mosteler.

### Statistical analysis

Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation or median and interquartile range, according to their distribution. Continuous variables were compared between groups using an unpaired *t*-test (for normally distributed variables) or the Mann–Whitney *U*-test (for non-normally distributed variables). For comparison between baseline and follow-up a paired Student's *t*-test was applied (normally distributed variables) and a Wilcoxon test (for non-normal distributed variables). Chi-square test was used to compare proportions. Following univariate analysis, a stepwise binary logistic multivariate regression model (Wald backward stepwise method, $p = 0.05$ for covariate inclusion and 0.1 for exclusion) was performed, including potential confounders for NYHA improvement regression analysis 6 months after AVR. NYHA improvement was analyzed as worse or equal vs better. Of note, patients in baseline NYHA class I but symptomatic (angina or syncope) were considered improved if these additional symptoms disappeared.

All reported probability values are two-tailed, and $p < 0.05$ was considered statistically significant. Analyses were performed with the IBM®SPSS® Statistics software package (version 21.0) (SPSS Inc, Chicago, IL, USA).

## Results

Patient's baseline clinical characteristics are described in Table 1. TAVI patients were older, had more comorbidities and higher logistic Euroscore. Patients who underwent TAVI had worse functional class at baseline, although they had similar baseline severity of aortic stenosis (Table 2). Before intervention, when comparing to SAVR patients, TAVI patients had similar 3D LV mass index but, after normalizing LV mass to LV end-diastolic volume (LVMI/LVDVI), they had more concentric geometry (3.0 (P25-75:2.1-4.4) vs 2.4 (P25-75:1.8-3.0) g/ml; $p = 0.044$) due to smaller LV end-diastolic index volumes. TAVI patients also had worse diastolic dysfunction, with higher LV filling pressures and larger indexed LA volume (Table 3).

**Table 1 Baseline clinical characteristics of SAVR vs TAVI patients**

| | SAVR (n = 45) | TAVI (n = 42) | p-value |
|---|---|---|---|
| **Age (years)** [Me (P25-P75)] | 73 (68 - 78) | 83.5 (79 - 87) | <0.001 |
| **Female gender** [n(%)] | 28 (62.2) | 26 (61.9) | 0.976 |
| **BSA ($m^2$)** [Me (P25-P75)] | 1.76 (1.57 - 1.86) | 1.75 (1.6 - 1.8) | 0.639 |
| **Hypertension** [n(%)] | 28 (62.2) | 35 (83.3) | 0.028 |
| **Diabetes mellitus** [n(%)] | 13 (28.9) | 11 (26.2) | 0.778 |
| **Dislipidemia** [n(%)] | 28 (62.2) | 26 (61.9) | 0.976 |
| **COPD** [n(%)] | 12 (26.7) | 19 (45.2) | 0.071 |
| **PAD** [n(%)] | 3 (6.7) | 12 (28.6) | 0.007 |
| **Atrial Fibrilation** [n(%)] | 0 (0) | 7 (16.7) | 0.004 |
| **Logistic Euroscore** | 6.18 ± 2.71 | 17.86 ± 9.55 | <0.001 |
| **NYHA class III-IV** [n(%)] | 12 (26.7) | 37 (88.1) | <0.001 |

BSA = body surface area; COPD = chronic obstructive pulmonary disease; PAD = peripheral artery disease; NYHA = New York Heart Association; values are mean ± SD or median (P25-P75) according to distribution, or n (%).

At 6 months follow-up, 5 TAVI patients had died, 3 during hospitalization for TAVI, and 2 after hospital discharge from non-cardiovascular causes. There were no deaths in the SAVR group.

### Changes in LV remodeling and functional class after aortic valve replacement

At 6 months (Table 2), TAVI patients had a higher effective orifice area index (EAOI) and lower transprosthetic maximal velocity and mean gradient, as well as a greater absolute increase in EAOI. Patient-prosthesis mismatch (PPM) was more frequent in the SAVR patients and there were no severe PPM cases in the TAVI group. There was a significant increase in ejection fraction (EF) in both groups and, when considering LV remodeling (Table 3), although there was a decrease in LV mass index (LVMI) and LV diastolic volume index (LVDVI) in both groups, only in SAVR patients this decrease was significant when compared with baseline values (Figure 1).

The presence of patient-prosthesis mismatch had no correlation with changes in LVMI, LVDVI, LV end-systolic volume index (LVSVI), or LA volume index (Additional file 1: Table S1). Moreover, EAOI increase was not related to final LVMI ($r = 0.082$, $p = 0.512$), LVDVI ($r = 0.015$, $p = 0.925$), LVSVI ($r = 0.154$, $p = 0.331$), or LAVI ($r = 0.187$, $p = 0.143$).

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**Table 2 Baseline and 6 months 2D echocardiographic data on SAVR vs TAVI patients**

| | SAVR (n = 45) | | TAVI (n = 42) | | SAVR vs TAVI p-value |
|---|---|---|---|---|---|
| | mean ± SD | Me (P25-P75) | mean ± SD | Me (P25-P75) | |
| **AV annulus** (cm) | | | | | |
| Baseline | 21.34 ± 2.18 | 21 (20 - 22.75) | 20.73 ± 2.39 | 21 (19 - 22) | 0.337[b] |
| **AV peak velocity** (cm/sec) | | | | | |
| Baseline | 4.81 ± 0.60 | 4.76 (4.36 - 5.13) | 4.76 ± 0.61 | 4.81 (4.36 - 5.16) | 0.909[b] |
| 6 months | 2.69 ± 0.74 | 2.51 (2.21 - 3.00) | 2.07 ± 0.51 | 2.11 (1.72 - 2.31) | <0.001[a] |
| p-value* | <0.001 | | <0.001 | | |
| **AV mean gradient** (mmHg) | | | | | |
| Baseline | 57.89 ± 13.91 | 54.9 (47.13 - 66.45) | 54.67 ± 15.77 | 52.5 (44.0 - 60.3) | 0.317[a] |
| 6 months | 17.21 ± 12.05 | 13.9 (11 - 21) | 8.86 ± 4.72 | 8.1 (5.7 - 11.4) | <0.001[a] |
| p-value** | <0.001 | | <0.001 | | |
| **AVA** (EOA, $cm^2$) | | | | | |
| Baseline | 0.69 ± 0.2 | 0.7 (0.52 - 0.83) | 0.62 ± 0.15 | 0.6 (0.51 - 0.7) | 0.070[a] |
| 6 months | 1.5 ± 0.42 | 1.4 (1.2 - 1.75) | 1.95 ± 0.54 | 1.8 (1.5 - 2.2) | <0.001[a] |
| p-value** | <0.001 | | <0.001 | | |
| **AVA index** (EAOI $cm^2/m^2$) | | | | | |
| Baseline | 0.40 ± 0.11 | 0.38 (0.32 - 0.47) | 0.37 ± 0.1 | 0.37 (0.31 - 0.42) | 0.229[b] |
| 6 months | 0.87 ± 0.24 | 0.82 (0.68 - 1.00) | 1.16 ± 0.39 | 1.05 (0.88 - 1.36) | <0.001[a] |
| p-value* | <0.001 | | <0.001 | | |
| **Δ EAOI** ($cm^2/m^2$) | 0.47 ± 0.28 | 0.46 (0.3 - 0.59) | 0.79 ± 0.37 | 0.65 (0.55 - 1.03) | <0.001[a] |
| **PPM** [n (%)] | 24 (58.6) | | 9 (23.1) | | <0.001 |
| **Severe PPM** [n (%)] | 7 (17.1) | | 0 | | 0.012 |
| **Paravalvular AR** 6m [n (%)] | 3 (6.7) | | 23 (59) | | <0.001 |
| **E/A ratio** | | | | | |
| Baseline | 0.79 ± 0.34 | 0.72 (0.61 - 0.89) | 1.38 ± 0.87 | 1.07 (0.74 - 1.74) | <0.001[b] |
| 6 months | 0.85 ± 0.25 | 0.81 (0.69 - 0.93) | 1.34 ± 1.22 | 0.73 (0.57 - 1.74) | 0.703[b] |
| p-value* | 0.044 | | 0.212 | | |
| **E-wave deceleration time** (ms) | | | | | |
| Baseline | 235.11 ± 73.97 | 240 (180 - 280) | 207.69 ± 79.97 | 198 (148 - 238.5) | 0.039[b] |
| 6 months | 264.51 ± 72.84 | 250 (218.5 - 300) | 251.82 ± 66.02 | 260 (190 - 295) | 0.755[b] |
| p-value* | <0.001 | | <0.001 | | |
| **IVRT** (ms) | | | | | |
| Baseline | 99.09 ± 26.85 | 100 (80 - 120) | 73.4 ± 33.91 | 70 (50 - 100) | 0.001[a] |
| 6 months | 116.43 ± 20.41 | 110 (100 - 130) | 102.19 ± 28.93 | 100 (82.5 - 127.5) | 0.034[a] |
| p-value** | 0.049 | | <0.001 | | |
| **e'** (cm/s) | | | | | |
| Baseline | 4.77 ± 1.8 | 4.4 (3.6 - 5.8) | 5.56 ± 2.55 | 5 (4.1 - 6.1) | 0.157[b] |
| 6 months | 5.59 ± 1.52 | 5.4 (4.6 - 6.73) | 7 ± 3.07 | 6.1 (4.78 - 8.7) | 0.033[a] |
| p-value* | 0.005 | | 0.495 | | |
| **E/e'** | | | | | |
| Baseline | 18.62 ± 7.11 | 16.9 (13.27 - 23.68) | 23.55 ± 10.88 | 25.36 (15.99 - 30.78) | 0.024[b] |
| 6 months | 16.73 ± 5.96 | 15.75 (13.02 - 19.86) | 16.36 ± 10.77 | 11.72 (8.33 - 23.06) | 0.153[b] |
| p-value* | 0.096 | | 0.177 | | |

AV = aortic valve; AVA = aortic valve area; EAOI = effective orifice area index; Δ EAOI = absolute increase in EAOI; PPM = patient-prosthesis mismatch; IVRT = Isovolumetric relaxation time; 6m = six months; values are mean ± SD or median (P25-P75) or n (%). [a,b] – different letters stand for significant differences in mean or median values according to *t*-test (a) or the Mann–Whitney *U* test (b).*Wilcoxon test; **Paired-sample *t*-test.

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**Table 3 Baseline and 6 months 3D echocardiographic data on SAVR vs TAVI patients**

| | SAVR (n = 45) | | TAVI (n = 42) | | SAVR vs TAVI p-value* |
|---|---|---|---|---|---|
| | mean ± SD | Me (P25-P75) | mean ± SD | Me (P25-P75) | |
| **LVEDVi (ml/m$^2$)** | | | | | |
| Baseline | 61.77 ± 21.81 | 59 (43.49 - 75.58) | 46.29 ± 13.47 | 46.59 (34.44 - 55.74) | 0.007 |
| 6 months | 51.28 ± 16.63 | 47.64 (38.69 - 59.1) | 42.07 ± 12.87 | 38.89 (33.58 - 48.67) | 0.030 |
| p-value** | 0.001 | | 0.173 | | |
| **LVESVi (ml/m$^2$)** | | | | | |
| Baseline | 27.32 ± 14.68 | 21.7 (16.56 - 34.86) | 20.91 ± 9.81 | 18.54 (13.98 - 28.44) | 0.115 |
| 6 months | 21.17 ± 11.46 | 19.54 (13.55 - 24.99) | 16.29 ± 7.99 | 13.56 (10.68 - 23.01) | 0.074 |
| p-value** | 0.002 | | 0.004 | | |
| **EF (%)** | | | | | |
| Baseline | 57.63 ± 9.38 | 60.1 (54.8 - 63.5) | 55.73 ± 10.15 | 55.45 (49.45 - 59.83) | 0.079 |
| 6 months | 61.28 ± 8.98 | 61.7 (58.55 - 66.95) | 61.27 ± 11.35 | 61 (53.5 - 70.2) | 0.841 |
| p-value** | 0.037 | | 0.005 | | |
| **LVMI (g/m$^2$)** | | | | | |
| Baseline | 135.3 ± 37.5 | 120.1 (108.4 - 160.2) | 137.46 ± 47.76 | 123.13 (97.5 - 173.89) | 0.877 |
| 6 months | 119.5 ± 36.8 | 110.7 (96.5 - 128.0) | 124.44 ± 44.55 | 112.53 (98.75 - 155) | 0.588 |
| p-value** | 0.002 | | 0.537 | | |
| **LVMI/LVDVI** | | | | | |
| Baseline | 2.49 ± 0.81 | 2.35 (1.84 - 2.96) | 3.48 ± 1.7 | 3 (2.05 - 4.4) | 0.038 |
| 6 months | 2.46 ± 0.68 | 2.56 (1.9 - 2.92) | 3.01 ± 1.53 | 2.82 (1.81 - 3.65) | 0.334 |
| p-value** | 0.557 | | 0.424 | | |
| **LAVI (ml/m$^2$)** | | | | | |
| Baseline | 39.94 ± 14.27 | 37.27 (31.15 - 47.99) | 48.42 ± 14.81 | 47.5 (35.36 - 57.14) | 0.008 |
| 6 months | 38.16 ± 11.9 | 36.17 (30.19 - 45.24) | 40.99 ± 12.7 | 42.63 (29.44 - 50) | 0.425 |
| p-value** | 0.465 | | 0.006 | | |

LVDVI = left ventricular end-diastolic volume index; LVSVI = left ventricular end-systolic volume index; EF = ejection fraction; LVMI = left ventricular mass index; LAVI = left atrial volume index; *Mann–Whitney test; **Wilcoxon test.

After intervention, NYHA class was better in 30 (71.4%) TAVI patients compared with 22 (48.8%) SAVR patients (p = 0.001, Figure 2). Patients exhibiting a better NYHA class were more likely to have no PPM (81.6% vs 60.0%, p = 0.049) and a greater relative increase in EAOI (163.2% (P25-75: 118.9-234.8) vs 103.0% (P25-75: 52.1-170.2); p = 0.030). In univariate analysis, older age, baseline NYHA class ≥ III, a higher increase in EAOI and TAVI procedure were related with functional class improvement (Table 4). Moreover, we found no correlation between functional class improvement and parameters of LV reverse remodeling like the decrease in LV volumes or mass, or improvement in diastolic function suggested by the decrease in left atrial volume and E/e' (Table 4). After a stepwise logistic multivariate regression analysis, the only independent predictors of NYHA class improvement were initial NYHA class ≥ III (OR 8.81, CI: 2.13-36.52; p = 0.003) and relative increase in EAOI ≥ 105% (OR 3.87, CI: 1.02-14.70; p = 0.04).

Paravalvular regurgitation was more frequent after TAVI (59% vs 6.7%, p < 0.001), mostly of mild degree. Only 5 TAVI patients (5.8%) had moderate aortic regurgitation at six months. Paravalvular regurgitation had no correlation with the variation of indexed LVM, LVDV or LVSV (Additional file 2: Table S2). NYHA class improvement was similar in patients with and without paravalvular regurgitation (83.3% vs 66.7%, p = 0.241).

## Discussion

In this study we found that, at 6 months, TAVI patients had a better hemodynamic result and greater clinical improvement than those submitted to SAVR, but LV reverse remodeling was of a less significant degree than in SAVR patients.

### Reverse remodeling after aortic valve replacement

At 6 months follow-up, TAVI patients had a more favorable hemodynamic performance but the decrease in LVMI

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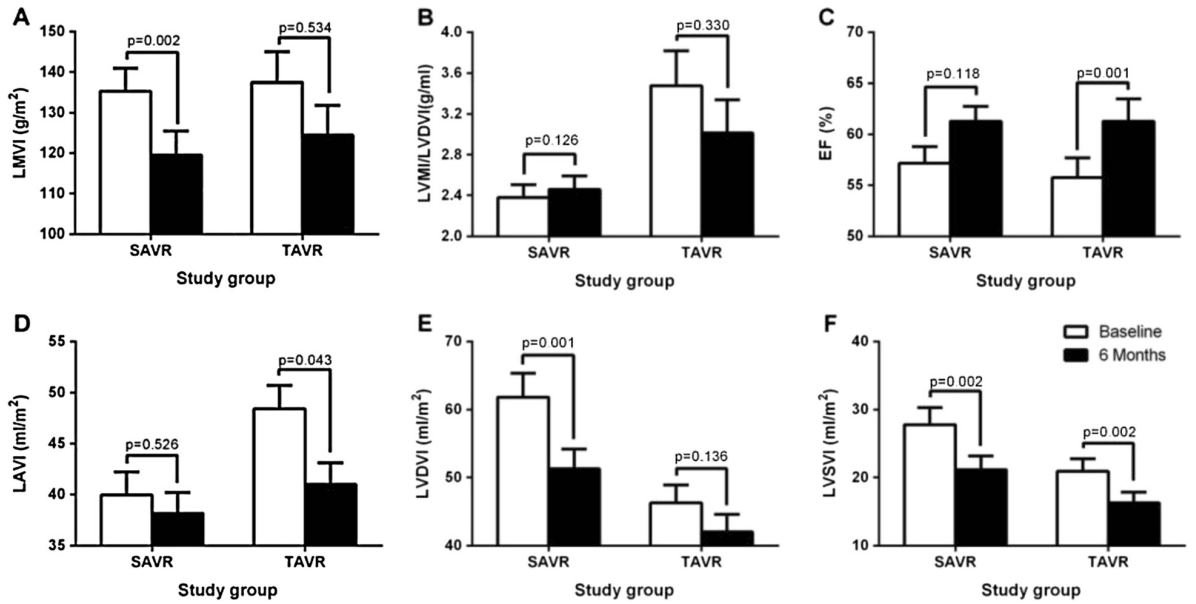


**Figure 1 Six months changes in parameters of remodeling in TAVI and SAVR groups. A**- change in left ventricular mass index (LVMI); **B**- change in ratio LVMI/left ventricular end-diastolic volume index (LVDVI); **C**- change in ejection fraction (EF); **D**- change in left atrial volume index (LAVI); **E**- change in LVDVI; **F**- change in left ventricular end-systolic volume index (LVSVI).

and LV volumes, although showing the same trend as SAVR patients, was less extensive.

A greater decrease in transvalvular gradients and increase in effective orifice area was seen after TAVI. It would be expected that patients undergoing this procedure had faster remodeling if load was the most important determinant of mass regression, but in our study EAOI increase was not associated with changes in LV mass and volumes after AVR. We can speculate that the baseline differences we have found, with older age and more comorbidities in TAVI patients, could have contributed to this result. Moreover, TAVI patients were "sicker", with worse diastolic dysfunction and worse functional class, despite similar severity of AS and EF, possibly due to

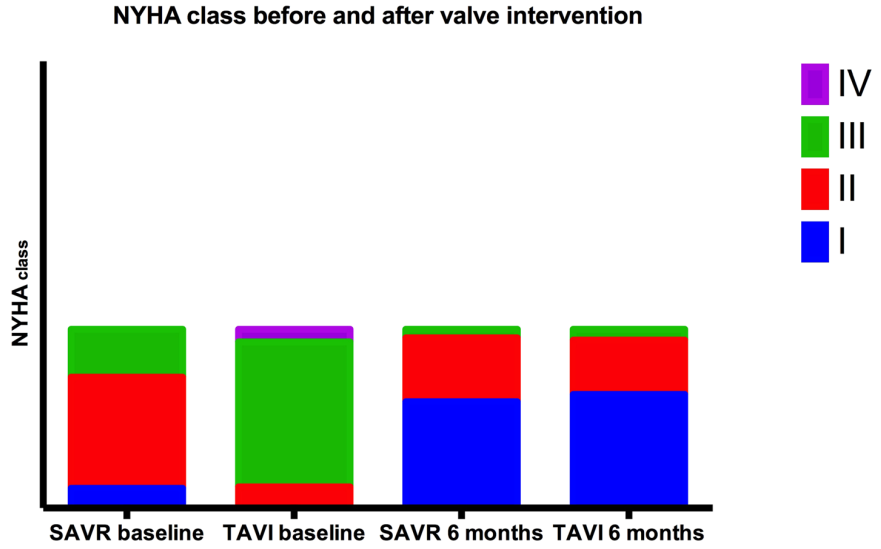


**Figure 2** Six months changes in NYHA class in TAVI and SAVR groups.

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**Table 4 Univariate and multivariate analysis of clinical and echocardiographic determinants of NYHA class improvement**

| NYHA improvement | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | p | | p |
| **Female gender [n(%)]** | 0.92 (0.31-2.67) | 0.87 | | |
| **Age (years)** | 1.09 (1.01-1.17) | 0.02 | | |
| **ΔEF (%)** | 0.99 (0.94-1.04) | 0.62 | | |
| **ΔLVMI (g/m$^2$)** | 0.99 (0.97-1.01) | 0.49 | | |
| **ΔLAVI (ml/m$^2$)** | 1.01 (0.99-1.02) | 0.38 | | |
| **ΔLVDVI (ml/m$^2$)** | 0.98 (0.94-1.02) | 0.28 | | |
| **ΔLVSVI (ml/m$^2$)** | 0.99 (0.93-1.05) | 0.64 | | |
| **ΔEAOI (%)** | 8.81 (0.90-86.10) | 0.06 | 3.87 (1.02-14.70) | 0.004 |
| **ΔE/e'** | 1.01 (0.94-1.08) | 0.85 | | |
| **Hypertension [n(%)]** | 1.70 (0.60-5.15) | 0.35 | | |
| **Diabetes Mellitus [n(%)]** | 0.48 (0.15-1.48) | 0.20 | | |
| **COPD [n(%)]** | 2.55 (0.75-8.66) | 0.14 | | |
| **PAD [n(%)]** | 0.80 (0.22-2.96) | 0.74 | | |
| **NYHA class ≥ III [n(%)]** | 11.33 (2.93-43.78) | <0.001 | 8.81 (2.13-36.52) | 0.003 |
| **TAVI [n(%)]** | 7.08 (1.86-27.04) | 0.004 | | |
| **Aortic regurgitation 6m [n(%)]** | 2.35 (0.69-8.03) | 0.17 | | |

LVDVI = left ventricular end-diastolic volume index; LVSVI = left ventricular end-systolic volume index;; LVMI = left ventricular mass index; LAVI = left atrial volume index; Δ EF = baseline- 6 months ejection fraction; Δ LVDVI = baseline- 6 months LVDVI; Δ LVSVI = baseline- 6 months LVSVI; Δ LVMI = baseline- 6 months LVMI; Δ LAVI = baseline- 6 months LAVI; Δ EAOI = relative increase in EAOI at 6 months; Δ E/e' = baseline- 6 months E/e'; COPD = chronic obstructive pulmonary disease; PAD = peripheral artery disease; TAVI = transcatheter aortic valve implantation.

longer time of LV overload exposure. This could explain a restricted ability of the myocardium to recover from pre-intervention changes.

Various groups have focused on the hemodynamic and structural effects of TAVI [10,15-18] with consistent results in afterload reduction and symptomatic improvement, but with conflicting results on reverse remodeling. Previous reports comparing the impact of TAVI and SAVR on LV remodeling addressed heterogeneous populations, including patients with coronary artery disease, different levels of EF, and several types of aortic prosthesis, including mechanical, stented and stentless bioprosthesis [9,11,19].

Clavel et al. [20] compared hemodynamic performances of TAVI or SAVR with stentless and stented bioprosthesis. At 6 to 12 months the increase in EAOI and reduction in transvalvular gradients in TAVI patients was similar to that obtained with stentless bioprosthesis and there was a clear advantage of TAVI in preventing PPM in patients with small annulus (≤20 mm). However, data on clinical improvement or reverse remodeling is absent.

Fairbairn et al. [19], using cardiac magnetic resonance imaging, showed a decrease in LVMI and indexed LV systolic volume at 6 months in both groups, but only SAVR patients had a decrease in indexed LV diastolic volume. The authors considered that the smaller reduction in LV end-diastolic volume post-TAVI could be related to a greater burden of coronary artery disease in these patients. In our study we found that, at 6 months, only SAVR patients had a significant decrease in LV mass and LV end-diastolic volume. Since we excluded patients with coronary artery disease, the absence of significant remodeling post-TAVI at this time point could indicate the existence of irreversible disease. Constantino et al. [18] compared reverse remodeling 2 months after TAVI and SAVR, and concluded that there was a more significant reduction in LVMI and relative wall thickness (RWT) in TAVI patients. These results are conflicting with ours, but were obtained in an earlier time point and the lack of adjustment for differences in baseline LVMI could have influenced results. Moreover, the authors found that these structural changes were paralleled by reduction in estimated filling pressures after TAVI. We also found a reduction in E/e´ after TAVI, but this occurred in absence of favorable remodeling and had no correlation with clinical improvement. Its association with prognosis is yet to be seen.

In the Cohort A of the PARTNER trial, LV diastolic volume was higher in TAVI patients in the first year of follow-up, but these differences were no longer present at two years. LV mass regression was faster in the SAVR group, although there were no significant differences after 6 months [11]. These results are similar to those reported in our study, suggesting that reverse remodeling can also occur in TAVI patients, but the process is slower than after SAVR, even after matching for age and major comorbidities.

Aortic regurgitation after TAVI, mostly paravalvular, is a common event [2,21] and has come to our attention because of its impact on mortality. Post-procedural moderate to severe AR increased in-hospital mortality in comparison with no or only mild AR [21] and, in the randomized PARTNER trial, there was a positive correlation between AR severity and long-term mortality [22]. The pathophysiology underlying this increase is mortality is unclear. It has been speculated that significant paravalvular aortic regurgitation can overload the LV and impair reverse remodeling [23], therefore worsening prognosis. In our study, the presence of paravalvular aortic regurgitation had no correlation with the variation in LV volumes or mass. Once only 5 patients had moderate regurgitation, the lack of association with ventricular remodeling could be due to the small sample size. Longer

follow-up and larger numbers are needed to take any definite conclusion on its impact in remodeling.

### Predictors of clinical improvement

In our sample, the increase in EAOI to more than the double was a strong predictor for clinical improvement, independent of changes in parameters of reverse remodeling. Conversely, the presence of patient-prosthesis mismatch (PPM) was correlated with impaired improvement in NYHA class.

Several studies reported that PPM is an independent predictor of cardiac events after AVR [24,25] while others failed to demonstrate a significant impact on outcomes [26,27].

Some authors found that persistent PPM results in less regression [28,29] but even patients with PPM or small prosthesis can have significant reduction in LVM [30,31]. The extent of regression is largely dependent on the extent of EOA increase after AVR [32]. Given the curvilinear relation of indexed EOA and transprosthetic gradients the degree of regression seems to be dependent on the original and final positions of an individual patient on the indexed EOA-gradient curve [33]. Although we found no correlation between PPM and impaired LVMI regression, we did find that the increase in valve area to more than twice the initial value was crucial for clinical improvement. This can be particularly important in elderly patients whose main concern is the achievement of a better quality of life.

Finally, as expected, patients who were in worse NYHA class before intervention more frequently experienced a clinical improvement. Using NYHA class to evaluate clinical improvement, although extensively used, has limitations and it is easier to demonstrate an improvement when a patient is class III/IV than NYHA class II/I. This fact can also help to explain the better improvement in the clinical status of patients undergoing TAVI, as they were in worse NYHA class than SAVR patients, before the intervention.

### Limitations

Since this was an observational study, we were not able to match patients for age, comorbidities or prosthesis size. These factors were considered in the multivariate model used for prediction of clinical improvement, but the authors recognize that, although logistic multivariate analysis is commonly used, it can't correct for all possible confounders.

In addition, we selected our population by excluding patients with concomitant coronary artery disease and significant associated valvular disease to reduce introduction of further bias. The limited number of patients included in this analysis has limited statistical power to detect small differences between groups.

The evaluation of functional improvement using NYHA class is subjective and a more objective method, like six-minute walk test, could have allowed a quantitative assessment. Nevertheless, in clinical practice, NYHA class is the most widely use classification of function status and has been proven useful over the years.

## Conclusions

At six months after aortic valve intervention, better hemodynamic result was seen after TAVI, but LV reverse remodeling was of a less significant degree than after SAVR. Older age, comorbidities and the existence of a more extensive myocardial disease, as suggested by worse diastolic dysfunction and worse functional class in TAVI patients, despite similar severity of AS and EF, could explain a restricted ability of the myocardium to recover even after load relief. Moreover, six months may be too early to draw definitive conclusions, namely regarding the consequences of paravalvular aortic regurgitation.

Mid-term clinical improvement was strongly related to the increase in EAOI and had no association with LV remodeling parameters. Thus, doubling the initial aortic valve area seems to be a key point to achieve clinical improvement after valve replacement, a particularly important endpoint in the elderly.

This study raises some important new questions but longer follow-up and large-scale randomized trials are needed to confirm these results.

## Additional files

**Additional file 1: Table S1.** Correlation of the presence of patient prosthesis mismatch (EAOI ≤ 0.85 $cm^2$) and changes in indexed 3D volumes and left ventricular mass.

**Additional file 2: Table S2.** Correlation of the presence of aortic regurgitation at 6 months and changes in indexed 3D volumes and left ventricular mass.

#### Abbreviations

SAVR: Surgical aortic valve replacement; TAVI: transcatheter aortic valve implantation; BSA: body surface area; COPD: chronic obstructive pulmonary disease; PAD: peripheral artery disease; DM: diabetes mellitus; HT: arterial hypertension; NYHA: New York Heart Association; AV: aortic valve; AVA: aortic valve area; EAOI: effective orifice area index; PPM: patient-prosthesis mismatch; IVRT: Isovolumetric relaxation time; LVDVI: left ventricular end-diastolic volume index; LVSVI: left ventricular end-systolic volume index; LVMI: left ventricular mass index; LAVI: left atrial volume index.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contribution

CG, AG, CA, RH, ALM, FRG take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. All authors read and approved the final manuscript.

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# 6

# DISCUSSION

# 6. DISCUSSION

We have followed for 8.2 years a cohort of 132 patients with severe symptomatic AS referred for AVR. Most patients had sex-specific criteria for LVH before surgery and, at echocardiographic evaluation, 5 years after surgery, only 66% achieved LVM normalization, despite a significant reduction in transvalvular gradients and total hemodynamic load. This raises the question if some of these patients may have a maladaptive response to chronic overload, dependent of non-hemodynamic factors, which impairs LVM regression and, presumably, impacts prognosis.

The event rate was low, with 17 deaths (1.6%/year) and 12 non-fatal cardiovascular hospitalizations (1.1%/year) at 8 years follow-up, which is in accordance with the low risk profile of our patients. Even so, we could find that an excess in LVM, above the predicted value for load, body size and gender, was an independent predictor of all-cause death and non-fatal cardiovascular hospitalization, after adjustment for important prognostic factors such as age, GFR, baseline LVM or EF. Moreover, higher baseline LV filling pressure, estimated by the ratio E/e', was also a predictor of the composite endpoint of death or non-fatal cardiovascular hospitalization.

The existence of inappropriate LVM, defined as an observed value above 78% of the predicted, was associated with worse event-free survival. Moreover, these patients presented more severe myocardial disease, probably irreversible, which can justify their worse prognosis.

### *Prognostic relevance of excessive LVM*

During childhood and adolescence, there is a close relationship between body size and LV mass, with no differences between genders, since body growth is the main determinant of cardiac development [8]. With advancing age, the discrepancy between the value of observed LVM and that predicted for body size increases. This variability is due to hemodynamic load, which becomes the fundamental stimulus for LV mass in adulthood [2]. At this time in life, the ratio of stroke work (SW), as a measure of hemodynamic load, to LVM is lower in men and gender differences exist in measured LVM.

In a normal-weight and normotensive cohort, de Simone et al have found that, 82% of the variance in LVM can be explained by SW (stroke volume times systolic pressure), height [2.7] and gender [2]. A multiple linear equation was then derived to calculate the predicted LVM. Still, about 18% of variance has to be attributed to non-hemodynamic factors such as genetics and other environmental influences.

The excess in LVM is associated with a specific phenotype of concentric LV geometry, depressed midwall systolic shortening and prolonged LV relaxation, in situations of chronic pressure overload such as hypertension and aortic stenosis [9-12], even in the absence of echocardiographic criteria of LV

hypertrophy [11]. Moreover, patients with inappropriate LVM have worse prognosis both in hypertension [13, 14] and in aortic stenosis [3], suggesting that this patients have a "maladaptive" response to increased LV load. Indeed, it has been advocated that this excess in LVM can identify a more advanced stage of myocardial disease, probably beyond the compensatory phase, and could be a sign of impending heart failure [15, 16].

Although the concept of inappropriate LVM has been studied mainly in the context of hypertension, where the cutoff was set at the 95$^{th}$ percentile of normal distribution, corresponding to an excess of 28% of the predicted value, some evidence exists suggesting its applicability in aortic stenosis [3, 12, 17] and, more recently in type 2 DM [18] and CKD [19].

In aortic stenosis, it has been used to help to predict the timing of surgery in asymptomatic patients, where those with an excess of LVM 10% above the predicted had a higher rate of the combined event of death from all causes, aortic valve replacement or hospital admission for non-fatal myocardial infarction and/or congestive heart failure [3]. Furthermore, in a sub-analysis of the SEAS (Simvastatin Ezetimibe in Aortic Stenosis) study, in patients with asymptomatic mild-moderate aortic stenosis, inappropriate LVM (defined an excess of 28% above the predicted LVM) was present in 16.6% and was associated with combined concentric geometry and reduced left-ventricular myocardial contractility, again suggesting that it is a marker of more advanced myocardial disease [17].

We have extended the evidence of excess of LVM usefulness to symptomatic severe aortic stenosis patients, describing a continuous gradient of risk with an adjusted HR of 1.02 (95%CI:1.01-1.04) for all-cause mortality, 1.02 (95%CI:1.00-1.04) for non-fatal cardiovascular hospitalization, and 1.01 (95%CI: 1.00-1.02) for the combined endpoint, for each 1% increase in excess LVM. Using the cutoff of 78% excess in LVM, we could identify a subgroup of patients with worse diastolic and systolic function and lower survival free of events, probably due to more advanced and irreversible myocardial disease. Inconsequence earlier intervention eventually might be beneficial in patients with an excessive LVM response to pressure overload to improve long-term outcomes after AVR.

### *E/e' and outcomes after AVR*

In our study, non-invasive evaluation of left ventricular filling pressures was based on the ratio between early diastolic mitral inflow and mitral annular velocity (E/e') since, in aortic stenosis, it has a good correlation with invasive determination of filling pressures [20]. Increased values of E/e´ are associated with the existence of diastolic dysfunction, worse functional class and pulmonary hypertension[21]. In our study, E/e´ was an independent predictor of the combined event all-cause mortality or non-fatal cardiovascular hospitalization, suggesting that patients with more severe diastolic dysfunction have worse long-term prognosis. This is in accordance with previous results reporting that increased E/´e levels are associated with more in-hospital cardiovascular complications and worse mid-term cardiovascular event-free survival [22].

### *Gender differences in hypertrophic response to aortic stenosis*

In our study, non-invasive evaluation of left ventricular filling pressures was based on the ratio between early diastolic mitral inflow and mitral annular velocity (E/e'), which is well correlated with invasive determination of filling pressures [20]. Increased values of E/e´ are associated with the existence of diastolic dysfunction, worse functional class and pulmonary hypertension [21]. In our study, E/e´ was an independent predictor of the combined event all-cause mortality or non-fatal cardiovascular hospitalization, suggesting that patients with more severe diastolic dysfunction have worse long-term prognosis. This is in accordance with previous results reporting that increased E/´e levels are associated with more in-hospital cardiovascular complications and worse mid-term cardiovascular event-free survival [22].

Elderly women with AS respond to pressure overload with smaller, more hypertrophic and stiffer ventricles, and often have supranormal ejection fraction [23, 24]. Conversely, men have higher levels of wall stress and worse systolic function than women under similar load conditions [23]. These distinctive LV remodeling responses to pressure overload can be partially explained by the effect of sex hormones. Estrogens seem to have antiproliferative effects on cardiac fibroblasts [25] and vascular smooth–muscle cells, while androgens have opposite effects [26]. In animal models, estrogens down regulate proliferation of cardiac fibroblasts and gene expression of collagens type I and III in female, but have opposite effect in male [27, 28]. Therefore, estrogens may prevent the up regulation of collagen in women with pressure overload until menopause. Given that older patients have relative hypogonadal hormone concentrations, with a decrease in estrogens and ovarian production of androgens in postmenopausal women, it is expected that this protective effect is lost with aging in women.

The presence of a profibrotic pattern of ECM biomarkers and evidence of more fibrosis in surgical biopsies of our elderly AS women can explain the existence of a more inappropriate increase in LVM and the persistence of LVH after AVR, since this is the myocardial component that takes longer to regress and some of these changes can even be irreversible. It is possible that women may benefit from earlier surgery before irreversible myocardial disease develops.

### *Relevance of residual left ventricular hypertrophy after surgery for isolated aortic stenosis*

In our study residual LVH was present in 44% of patients late after AVR and was associated with a worse prognosis, with nearly a three-fold increase in the risk of death or non-fatal cardiovascular hospitalization. We also found that women, history of hypertension, higher baseline LVMI and higher baseline LV filling pressures were independent predictors of residual LVH. Moreover, in women the persistence of LVH late after AVR was associated with a worse outcome. This was not seen in men, suggesting that the prognostic impact of residual LVH is gender-specific.

The lack of normalization of LV mass after surgery occurs in nearly half of patients with AS, and it has been considered as a "natural" consequence of the substitution of a native valve for a somewhat obstructive valve substitute with a residual gradient [29]. Thus the focus has been on avoiding significant

PPM and new prostheses have been developed with better hemodynamic profiles. Nevertheless, in our study residual LVH was not associated with PPM. Beach et al, described that high postoperative transprosthesis gradients had only a minimal effect on residual left ventricular hypertrophy, in a study including a very large number of patients [30]. Therefore, hemodynamic factors are not the only determinants of incomplete regression. We found that patients with a higher baseline LVMI and worse diastolic dysfunction had a higher probability of having residual LVH after surgery. Moreover, those with worse longitudinal systolic function were also less likely to have LV mass normalization late after surgery. Our results are in accordance with that of other authors, who also found that the existence of a more severe preoperative hypertrophy [30-33] and the presence of early signs of myocardial dysfunction, even with preserved ejection fraction, might be a surrogate of a more advanced disease [34]. At the histological level, our finding that a higher amount of fibrosis at the time of surgery is an independent predictor of residual LVH, reinforces the hypothesis that a more severe, and probably irreversible, form of myocardial disease is behind the lack of LVM normalization.

Female gender and a history of HT were also independently associated with persistent LVH. In the previous analysis, we had found that women had more interstitial fibrosis than men, had a profile of extracellular matrix (ECM) biomarkers favoring collagen deposition, and that these correlated negatively with LV mass regression, giving a plausible theory for the existence of more residual LVH in women. Moreover, hypertension negatively impacts LV mass regression after surgery, and several authors have stressed the need for rigorous blood pressure control in these patients [35, 36]. Nevertheless, our group has shown that this impairment in reverse remodeling happens independently of load, and might be related to the neuro-hormonal milieu[37].

The association of residual LVH in AS with worse prognosis is controversial. Others have described this association including patients with other types of valve lesions and coexisting coronary artery disease (CAD), which may have influenced results. Indeed, the coexistence of CAD has been considered as an independent predictor of clinical outcomes after AVR [38] and the presence of aortic insufficiency can elicit a different remodeling response [39]. In our study we excluded these patients.

The worse long-term outcome of patients with residual LVH after AVR can be explained by the existence of more extensive preoperative disease and persistent diastolic and/or systolic dysfunction [40, 41]. Based on results from previous studies [42, 43], the worse baseline longitudinal systolic function in our patients with residual LVH might reflect the existence of more advanced myocardial disease and higher levels of fibrosis.

One important finding in our study was the differences in the prognostic impact of residual LVH according to gender. Only in women the absence of normalization of LVM was associated with worse survival free of hospitalization or all-cause mortality. Recently, Petrov et al 44 described that women with preoperative maladaptive LVH had worse survival than those with adaptive LVH, a pattern that was not seen in men. These results are in accordance with ours, showing a gender-specific prognosis of LVH determined before or after AVR. Thus, it seems that, in women, the search for early predictors of negative remodeling after AVR could be particularly relevant.

### *Load independent impairment of reverse remodeling after valve replacement in hypertensive aortic stenosis patients*

Hypertension and AS frequently coexist. In our study we found a prevalence of HT of 56.3% and these patients had more severe heart failure symptoms when compared to non-hypertensive AS patients with the same echocardiographic parameters of AS severity. Moreover, in our cohort HT impaired LV mass regression and reverse remodeling after AVR, independently of total LV afterload. The combination of HT with AS was associated with a different pattern of expression of genes related to ECM remodeling favoring collagen accumulation and higher relative levels of collagen type I, which could help to explain its negative impact on reverse remodeling.

Data on systemic blood pressure before and after AVR is absent in most studies on incomplete LVH regression and only recently some authors have reported its relation to postoperative LVM [35, 36, 45, 46]. In a retrospective observational study with 79 pure AS patients, the only independent predictors of postoperative LVM index were preoperative LVM and postoperative systolic blood pressure (defined as normal if <130 mmHg) [36]. Uncontrolled hypertension was not only related to higher LVM after AVR but also with worse survival, with higher incidence of heart failure and bleeding as causes of death [35]. These observations resulted in a general recommendation for strict blood pressure control after AVR [35, 36].

It is not as well understood if the existence of HT *per se* can influence LVM regression even under similar load conditions. Our results suggest that HT blunts LVM normalization and reverse remodeling after AVR for isolated AS independently of load. As expected, there was a trend for higher systolic blood pressure in hypertensive patients, but the total LV afterload, evaluated by valvuloarterial impedance, that takes into account systolic blood pressure, prosthetic gradient and stoke volume, was not significantly different between patients with and without HT. One can speculate if this could be due to the systemic nature of hypertensive disease, with a generalized neurohumoral activation, with particular focus on the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS), which directly promote myocyte hypertrophy and matrix deposition independently of their effects on systemic arterial pressure [47]. These same factors, promote both hypertension and LVH and there is the possibility that increased blood pressure is the consequence, rather than the cause, of LVH and associated vascular structural changes. Data from the Framingham Heart Study demonstrated a direct and continuous relationship between LVM and the subsequent development of hypertension in previously normotensive subjects [48]. Also, in a study in young healthy subjects, plasma angiotensin II was an independent predictor of LVM and its effect was independent of systolic blood pressure and body size [49]. Moreover, the magnitude of LVH regression achieved by inhibiting the RAAS and SNS is greater than that produced by comparable BP reduction alone [50]. All this data supports the hypothesis that neuroendocrine mechanisms are important in the regression of LVH, independently of blood pressure

To the best of our knowledge we were the first to report myocardial expression of MMPs and their tissue inhibitors in combined HT and AS. In our study only HT+AS patients had an up regulation ofTIMP

2 and an increase in collagen type I/type III ratio, suggesting a shift towards collagen accumulation and a stiffer form of collagen meshwork. When comparing patients with and without hypertension, it becomes clear that collagen degradation seems to be more impaired in HT+AS given the higher levels of TIMP2 expression and their correlation with LVMI. This different pattern of ECM remodeling can help to explain the differences in LV remodeling in the presence of HT.

### *Prognostic implications of fibrosis in low risk aortic stenosis patients*

We found that, in our low risk cohort of patients with severe AS, higher levels of fibrosis had a negative prognostic impact with lower survival free of all-cause death or the composite of all-cause death or non-fatal cardiovascular hospitalization. Moreover, it was a predictor of events, independent of other well established prognostic factors such as EF, age, baseline LVMI or NYHA class. For our patients, a cut-off value of 15% of CVF had a good performance as a predictor of clinical events.

Fibrosis is an early morphological alteration in patients with AS and has been pointed as one of the reasons for impaired LVH regression after AVR [51]. Once established, fibrosis is a major determinant of diastolic and systolic dysfunction and it is one of the structural substrates for arrhythmogenicity, thus playing a major role for sudden death and the progression of HF [52, 53]. While myocyte hypertrophy is dependent on load, fibrosis seems also to be regulated by non-hemodynamic factors such as neurohormones [54].

In LVH associated with aortic stenosis, there is an increased production of collagen and a shift towards inhibition of collagen degradation [55-57]. When compared with controls, myocardial biopsies of aortic stenosis patients have higher expression of collagens and an up-regulation of TIMP 1 and 2 mRNA, favoring inhibition of collagen degradation, which significantly correlates with the degree of fibrosis[57]. Experimental studies have described total regression of MMP and TIMP gene expression as well as an association between changes in LVMI and MMP/TIMP gene expression after corrective surgical therapy and LV hypertrophy regression [58]. The renin-angiotensin-aldosterone (RAA) system seems to be a key factor in this process. Mechanical stretch induces local production of angiotensin II, which in turn stimulates the release of multiple growth factors and cytokines from cardiac fibroblasts that act in an autocrine and paracrine fashion, affecting the progression of hypertrophy and remodeling [59-61].

Others have described the prognostic importance of myocardial fibrosis (MF) in aortic stenosis, either using histological assessment or noninvasive evaluation by cardiovascular magnetic resonance (CMR) with late gadolinium enhancement (LGE) [42, 62-65]. However, most of these studies have analyzed patients with worse preoperative NYHA class and more advanced forms of myocardial disease when compared with our cohort. Patients usually had lower values of EF, higher LV volumes and dimensions and higher levels of histological interstitial fibrosis, suggesting more extensive remodeling [62-64]. Likewise, the inclusion of aortic regurgitation patients [62] or the coexistence of other cardiovascular comorbidities, such as atrial fibrillation and coronary artery disease [64, 65], which were excluded in our study, may have influenced outcomes.

Milano and coworkers [63] have performed a very similar study in a group of 99 patients with AS in which fibrosis was calculated from myocardial biopsies obtained during surgery. In their retrospective analysis, 10 years survival rate was lower in patients with severe fibrosis (defined as fibrous index >50%) and no significant improvement in NYHA class was seen in this the group. We also found worse long-term survival and lesser NYHA class improvement in those with more severe fibrosis, but ours was a prospective study and our cut-off was much lower. . According to Milano et al. criteria (no or mild fibrosis if <20% and moderate fibrosis if 20-50%), most of the patients in our study would have been included in the group with mild fibrosis. This can help to explain why in our study, patients with higher level of fibrosis have less ventricular remodeling and dysfunction (only 9.5% of those with CVF ≥15.4% have EF<50%) comparing with their group with moderate or severe fibrosis. Moreover, not surprisingly, we did not find a significant correlation between fibrosis level and LV diameters, RWT or EF, which was described in the aforementioned work. It is expected for these correlations to be stronger in more severe grades of fibrosis. Even with lesser severe form of myocardial disease, we could still find an increase in events in our AS patients with increasing levels of fibrosis.

Fibrosis is an ominous sign in AS in the continuous of myocardial structural changes associated with aortic stenosis and should be actively sought for risk stratification, not only in asymptomatic patients with preserved ejection fraction, but also in symptomatic patients undergoing AVR. After AVR, the use of additional medical therapy modulating the RAA system in patients with non-invasive evidence of fibrosis is very appealing and deserves further investigation.

### *Determinants of clinical improvement after surgical replacement or transcatheter aortic valve implantation for isolated aortic stenosis*

In this study we found that, at 6 months, TAVI patients had a better hemodynamic result and greater clinical improvement than those submitted to SAVR, but LV reverse remodeling was of a less significant degree than in SAVR patients.

A greater decrease in transvalvular gradients and increase in effective orifice area was seen after TAVI. It would be expected that patients undergoing this procedure had faster remodeling if load was the most important determinant of mass regression, but in our study EAOI increase was not associated with changes in LV mass and volumes after AVR. We can speculate that the patients' baseline differences could have contributed to this result, as TAVI patients were "sicker", with worse diastolic dysfunction and worse functional class, despite similar severity of AS and EF, possibly due to longer time of LV overload exposure. This could explain a restricted ability of the myocardium to recover from pre-intervention changes.

Various groups have focused on the hemodynamic and structural effects of TAVI [66-70], with consistent results in afterload reduction and symptomatic improvement, but with conflicting results on reverse remodeling. Previous reports comparing the impact of TAVI and SAVR on LV remodeling addressed

heterogeneous populations, including patients with coronary artery disease, different levels of EF, and several types of aortic prosthesis, including mechanical, stented and stentless bioprosthesis [71-73].

In the only randomized trial performed in high-risk patients, LV mass regression was faster in the SAVR group, although there were no significant differences after 6 months [71]. These results are similar to those reported in our study, suggesting that reverse remodeling can also occur in TAVI patients, but the process is slower than after SAVR, even after matching for age and major comorbidities.

In our study, the presence of paravalvular aortic regurgitation had no correlation with the variation in LV volumes or mass. Once only 5 patients had moderate regurgitation, the lack of association with ventricular remodeling could be due to the small sample size. Older age, comorbidities and the existence of a more extensive myocardial disease, as suggested by worse diastolic dysfunction and worse functional class in TAVI patients, despite similar severity of AS and EF, could explain a restricted ability of the myocardium to recover, even after load relief.

# 7

# LIMITATIONS

# 7. LIMITATIONS

This was a single center observational study and the limited size of our cohort, although similar to those reported on literature about this subject, limits our statistical power. Moreover, the limited number of events can result in overfitted multivariate models.

We were unable to achieve myocardial biopsies fibrosis determination in all patients. Still these patients were randomly chosen and are believed to be representative of the overall study group.

The use of strain (S) and strain rate (SR) for detection of early and subclinical systolic LV dysfunction would be of value, but, at the time we began patient inclusion, this imaging technics were not available at our center.  Moreover, the systematic use of CMR with LGE to evaluate replacement fibrosis would have complemented our results. In fact, we were able to achieve this data in 40 patients but software limitations have delayed their analysis and, therefore, these data was not included.

# 8

# CONCLUSIONS

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- In patients with severe aortic stenosis, inappropriate LV hypertrophy can identify patients with worse diastolic and systolic function and lower survival free of events after AVR, probably due to more advanced and irreversible myocardial disease.

- Among AS patients, women have higher excess in LV hypertrophic response than men under similar workload conditions, and female gender is an independent predictor of residual hypertrophy after AVR. A gender-specific ECM remodeling, favoring interstitial fibrosis in women, might help to explain these differences.

- Residual LVH late after AVR is associated with a worse prognosis, in particular in women. The presence of more severe myocardial disease, as suggested by higher LVM and worse LV diastolic and systolic function, can help to explain the poorer clinical outcome of these patients.

- The coexistence of HT with AS is associated with ECM remodeling favoring collagen deposition and higher LVM. Moreover, HT impairs LV mass regression and reverse remodeling after AVR. This data reinforces the concept of modulation of the RAAS system in AS, particularly if HT is present.

- We have confirmed the prognostic relevance of myocardial fibrosis in severe AS, and extended this evidence to low risk patients with a less severe form of myocardial remodeling. Fibrosis is an ominous sign in AS in the continuous of myocardial structural changes and should be actively sought for risk stratification, not only in asymptomatic patients with preserved ejection fraction, but also in symptomatic patients undergoing AVR.

- At six months after aortic valve intervention, better hemodynamic result was seen after TAVI, but LV reverse remodeling was of a less significant degree than after SAVR. Older age, comorbidities and the existence of a more extensive myocardial disease, as suggested by worse diastolic function and worse functional class in TAVI patients, despite similar severity of AS and EF, could explain the restricted ability of the myocardium to recover after load relief. Mid-term clinical improvement was strongly related to the increase in EAOI and had no association with LV remodeling parameters. Thus, doubling the initial aortic valve area seems to be a key point to achieve clinical improvement after valve replacement, a particularly important endpoint in the elderly.

# 9

# CLINICAL IMPLICATIONS AND FUTURE RESEARCH

# 9. CLINICAL IMPLICATIONS AND FUTURE RESEARCH

Left ventricular hypertrophy in aortic stenosis results from the progressive increase in afterload and LV wall stress, and it is an adaptive response to avoid afterload mismatch and consequent LV dysfunction. However, the degree of LVH is only weakly related to the severity of valve obstruction [74-76] and patients who respond with a more severe degree of hypertrophy have worse prognosis after valve replacement. Thus, in some patients there seems to be a maladaptive response to chronic overload, dependent of non-hemodynamic factors, which induces a specific phenotype, impairs LVM regression and impacts prognosis.

The work included in this thesis brings additional evidence that excessive LV hypertrophy, in response to similar degrees of AS severity, is associated with a worse prognosis and a more advanced form of myocardial disease. Older age, comorbidities and the existence of worse pre-intervention diastolic function and functional class, as occurs with TAVI patients, can limit the ability of the myocardium to recover, even after load relief. The lack of normalization of LV mass after surgery helps to identify patients that might merit additional intervention, besides surgery.

We found that some specific subgroups of patients are particularly vulnerable to this maladaptive response, such as women and hypertensive patients, and these may benefit of additional studies addressing the impact of an earlier surgical intervention, independently of symptoms or ejection fraction.

At the structural and molecular level, we have described differences in ECM remodeling and higher degrees of interstitial fibrosis in those with impaired reverse remodeling and those with worse outcomes. Moreover, we found a more severe preoperative fibrosis and evidence of a specific ECM remodeling in women, which may justify the study of gender-specific therapeutic interventions.

Considering the pro-hypertrophic and pro-fibrotic effects of angiotensin II in pressure overload [60, 77, 78], the use of medical therapy modulating the renin-angiotensin-aldosterone system in addition to surgery, particularly in patients with non-invasive evidence of preoperative fibrosis, could be a game changer.

Our results have led us to new questions and on the road for answers. Two projects are ongoing trying to answer to the following question:

— *Do micro RNAs (miR) influence reverse remodeling?*

We performed a microarray analysis of miR expression in myocardial biopsies of 14 patients from our cohort and 5 explanted normal hearts that were used as controls. The expression of

selected miR genes will be measured in the beginning of 2015. Correlations with the degree of hypertrophy and LV reverse remodeling will be performed.

*— Can the block of RAA system lead to a faster and more complete reverse remodeling after load relief? At what level should the block occur?*

Using an animal model of banding and debanding, already implemented at our research unit, we will evaluate if pharmacologic blockade of RAA system impacts the hypertrophic response during banding and reverse remodeling after debanding. To evaluate if the level of RAA system blockade can influence results, we will use 3 groups: sham group, ACE inhibitor group and aldosterone receptors block group.

Finally, hypothesizing a beneficial effect of RAAS blockade on cardiovascular outcomes after AVR, we aim to conduct a national, multicenter and randomized trial, using RAAS blockade in patients with isolated aortic stenosis undergoing AVR.

# 10

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# 11

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