Aus dem Institut für Geschlechterforschung in der Medizin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

in Kooperation mit dem

Deutschen Herzzentrum Berlin

DISSERTATION

Einfluss des Geschlechts auf Hypertrophie, ihre Regression und das Überleben nach Aortenklappenersatz bei PatientInnen mit Aortenklappenstenose

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Tabea Marie Schulze

aus Hannover

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Inhaltsverzeichnis

Zusammenfassung

Ziel: Das Ziel dieser Studie war, zu untersuchen, ob adaptives oder maladaptives Remodeling Einfluss auf das Überleben von Frauen und Männern nach Aortenklappenersatz (AKE) nimmt.

Hintergrund: Frauen mit isolierter Aortenklappenstenose (AS) entwickeln eine mehr konzentrische linksventrikuläre Hypertrophie (LVH) als Männer in ähnlichen Krankheitsstadien. Kürzlich berichteten wir über eine geringere Hochregulation von pro-fibrotischen Genen zum Zeitpunkt des AKE und eine schnellere postoperative LVH-Regression bei Frauen im Vergleich zu Männern, was darauf hindeutet, dass es geschlechtsspezifische Unterschiede bei den Anpassungsreaktionen an Druckbelastung und deren Rückbildung gibt.

Methoden: Die Studienkohorte umfasste 128 Patienten mit AS (70 ± 9,6 Jahre, 49% Frauen), die sich einem AKE unterzogen. Eine Echokardiographie wurde vor und 4 ± 1,6 Jahre nach der Operation durchgeführt. Mittels Faktorenanalyse wurde die LVH als adaptiv (Kombination von geringerer/m linksventrikulärer/m [LV] Masse/Durchmesser und größerer relativer Wandstärke) oder maladaptiv klassifiziert. Während des AKE wurden myokardiale Gewebeproben vom LV-Septum gewonnen, um die Myokardfibrose und damit verbundene molekulare Schlüsselregulatoren zu analysieren.

Ergebnisse: Vor dem AKE wurde eine LVH bei 62% der Frauen und 45% der Männer ($p < 0,050$) als adaptiv klassifiziert. Vier Jahre nach AKE wurde adaptive LVH bei 75% der Frauen und 49% der Männer ($p < 0,031$) beobachtet. Zum Zeitpunkt der Operation zeigten Männer mehr Myokardfibrose als Frauen ($p < 0,05$). Eine höhere transforming growth factor beta 1 [TGFβ1]-Expression ($p < 0,01$), SMAD2-Phosphorylierung ($p < 0,001$) und Periostin-Expression ($p < 0,05$) wurden bei Männern im Vergleich zu Frauen gefunden. Frauen mit maladaptiver LVH hatten schlechtere Überlebensraten als Frauen mit adaptiver LVH ($p < 0,050$), während das Muster der LVH keinen Einfluss auf das Überleben der Männer hatte ($p < 0,307$).

Schlussfolgerung: Frauen zeigen häufiger als Männer adaptives LV-Remodeling mit geringerer Fibrose. Maladaptive LVH ist mit schlechteren Überlebensraten bei Frauen verbunden. Folglich sollte das Geschlecht als starker modulierender Faktor berücksichtigt werden, wenn das Management von Patienten mit AS diskutiert wird.

Abstract

Objective: The purpose of this study was to test whether adaptive or maladaptive remodeling is associated with survival in women and men after aortic valve replacement (AVR).

Background: Women with isolated aortic valve stenosis (AS) develop more concentric left ventricular hypertrophy (LVH) than men in similar disease states. We recently reported less up-regulation of pro-fibrotic genes at AVR and faster LVH regression post-operatively in women than in men, suggesting that there are sex differences in the adaptation to pressure overload and its regression.

Methods: The study cohort included 128 patients (70±9.6 years, 49% women) undergoing AVR for AS. Echocardiography was obtained before and 4±1.6 years after surgery. Factor analysis was used to classify LVH as adaptive (combining smaller left ventricular [LV] mass/diameters and greater relative wall thicknesses) or maladaptive. Myocardial tissue samples from the LV septum were obtained during AVR to analyze cardiac fibrosis and associated key molecular regulators.

Results: Before AVR, LVH was classified as adaptive in 62% of women and 45% of men ($p<0.050$). Four years after AVR, adaptive LVH was observed in 75% of women and 49% of men ($p<0.031$). At surgery, more cardiac fibrosis was present in men compared with women ($p<0.05$). Higher levels of transforming growth factor beta 1 [TGFβ1] ($p<0.01$), SMAD2 phosphorylation ($p<0.001$), and periostin expression ($p<0.05$) were found in men than in women. Women with maladaptive LVH had worse survival than women with adaptive LVH ($p<0.050$), whereas the pattern of LVH did not affect survival in men ($p<0.307$).

Conclusion: Women more frequently exhibit adaptive LV remodeling with less fibrosis than men. Maladaptive LVH is associated with worse survival in women. Thus, sex should be considered as a strong modulating factor when management of patients with AS is discussed.

Eidesstattliche Versicherung

„Ich, Tabea Marie Schulze, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema - **Einfluss des Geschlechts auf Hypertrophie, ihre Regression und das Überleben nach Aortenklappenersatz bei PatientInnen mit Aortenklappenstenose** - selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
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<u>Ausführliche Anteilserklärung an der erfolgten Publikation</u>

Publikation: Petrov G[*], Dworatzek E[*], Schulze TM[*], Dandel M, Kararigas G, Mahmoodzadeh S, Knosalla C, Hetzer R, Regitz-Zagrosek V. **Maladaptive remodeling is associated with impaired survival in women but not in men after aortic valve replacement.** JACC Cardiovasc Imaging. 2014;7(11):1073-80.

Beitrag im Einzelnen:

1) Klinischer Teil: 2010-2012 am Deutschen Herzzentrum Berlin (DHZB):
- Auswahl der geeigneten StudienpatientInnen anhand der Ein- und Ausschlusskriterien.
- Einschluss der StudienpatientInnen in die Studie, Eingabe der Patientendaten in die Studien-Datenbank (4H-Datenbank) sowie regelmäßige Aktualisierung der Patientendaten.
- Mitwirkung bei der Weiterentwicklung der Studien-Datenbank.
- Organisation und Koordination der intraoperativen Entnahme der Myokardbiopsien im DHZB: hierfür Kontaktaufnahme mit OP-Büro und Operateuren, Entgegennahme der Biopsien im OP, Transport und Lagerung der Proben zur molekularbiologischen Untersuchung.

[*] geteilte Erstautorenschaft; die Autoren haben in gleichem Maße an der Erstellung der Arbeit mitgewirkt

- Organisation des Langzeit-Follow-Ups (FU); Versand eines Anschreibens und Fragebogens an die StudienpatientInnen sowie Organisation der Echokardiografie im DHZB, hierfür telefonische Kontaktierung der FU-PatientInnen, Koordination der Terminvergabe zur Echokardiografie bei Herrn PD Dr. Dandel; Eingabe der FU-Daten in die 4H-Datenbank.
- Auswertung der klinischen Daten, graphische Darstellung sowie statistische Analyse.

2) Molekularbiologische Untersuchungen: 01-08/2012 (Urlaubssemester) am Institut für Geschlechterforschung in der Medizin der Charité:
- Anfertigung von Gefrierschnitten der humanen Myokardbiopsien; histologische Färbung der Schnitte (Hämatoxylin-Eosin, Sirius Red); Ermittlung des Fibrosegrades (Mikroskopie und Fotografie der gefärbten Schnitte sowie Auswertung mittels geeigneter Software).
- Aufarbeitung der Myokardbiopsien zur Vorbereitung auf die proteinbiochemische Analyse; Durchführung der Western Blots und der immunologischen Detektion der Proteine.
- Datenanalyse, graphische Darstellung und statistische Tests.

3) Auswertung und Interpretation der erhobenen Daten; Mitarbeit bei der Erstellung des Manuskripts.

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☑	1	JACC-CARDIOVASC IMAG	1936-878X	4390	7.188	6.754	2.022	89	3.6	0.02465	2.775
☐	2	RADIOLOGY	0033-8419	48908	6.867	7.259	0.935	369	>10.0	0.07700	2.546
☐	3	NEUROIMAGE	1053-8119	78028	6.357	7.289	1.500	1033	6.5	0.17251	2.253
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☐	7	CIRC-CARDIOVASC IMAG	1941-9651	2786	5.316	6.000	1.367	98	3.3	0.01761	2.628
☐	8	ULTRASCHALL MED	0172-4614	1476	4.924	3.468	0.621	58	3.5	0.00380	0.805
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☐	10	INVEST RADIOL	0020-9996	5463	4.437	4.418	0.745	102	6.8	0.01294	1.525
☐	11	RADIOTHER ONCOL	0167-8140	12939	4.363	4.502	0.963	300	5.8	0.03171	1.381
☐	12	INT J RADIAT ONCOL	0360-3016	39876	4.258	4.359	1.148	427	7.6	0.07698	1.389
☐	13	SEMIN RADIAT ONCOL	1053-4296	1924	4.029	4.124	0.351	37	7.9	0.00412	1.530
☐	14	EUR RADIOL	0938-7994	13516	4.014	3.735	0.560	377	6.1	0.03398	1.239
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☐	17	MED IMAGE ANAL	1361-8415	4058	3.654	4.454	0.647	102	6.5	0.00848	1.316
☐	18	BIOMED OPT EXPRESS	2156-7085	3804	3.648	3.752	0.656	346	2.8	0.01636	1.082
☐	19	AM J NEURORADIOL	0195-6108	19657	3.589	3.870	0.577	378	8.1	0.03609	1.267
☐	20	MAGN RESON MED	0740-3194	26697	3.571	3.751	0.763	426	9.8	0.03841	1.212

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ORIGINAL RESEARCH

Maladaptive Remodeling Is Associated With Impaired Survival in Women But Not in Men After Aortic Valve Replacement

George Petrov, MD, MSc,*† Elke Dworatzek, PhD,†‡ Tabea Marie Schulze, MD (cand),* Michael Dandel, MD,*† Georgios Kararigas, PhD,†‡ Shokufeh Mahmoodzadeh, PhD,†‡ Christoph Knosalla, MD,*† Roland Hetzer, MD,*† Vera Regitz-Zagrosek, MD*†‡

ABSTRACT

OBJECTIVES The purpose of this study was to test whether adaptive or maladaptive remodeling is associated with survival in women and men after aortic valve replacement (AVR).

BACKGROUND Women with isolated aortic valve stenosis (AS) develop more concentric left ventricular hypertrophy (LVH) than men in similar disease states. We recently reported less up-regulation of profibrotic genes at AVR and faster LVH regression post-operatively in women than in men, suggesting that there are sex differences in the adaptation to pressure overload and its regression.

METHODS The study cohort included 128 patients (age 70.0 ± 9.6 years, 49% women) undergoing AVR for AS. Echocardiography was obtained before and 4.0 ± 1.6 years after surgery. Factor analysis was used to classify LVH as adaptive (combining smaller left ventricular [LV] mass/diameters and greater relative wall thicknesses) or maladaptive. Myocardial tissue samples from the LV septum were obtained during AVR to analyze cardiac fibrosis and associated key molecular regulators.

RESULTS Before AVR, LVH was classified as adaptive in 62% of women and 45% of men ($p < 0.050$). Four years after AVR, adaptive LVH was observed in 75% of women and 49% of men ($p < 0.031$). At surgery, more cardiac fibrosis was present in men compared with women ($p < 0.05$). Higher levels of transforming growth factor beta 1 ($p < 0.01$), SMAD2 phosphorylation ($p < 0.001$), and periostin expression ($p < 0.05$) were found in men than in women. Women with maladaptive LVH had worse survival than women with adaptive LVH ($p < 0.050$), whereas the pattern of LVH did not affect survival in men ($p < 0.307$).

CONCLUSIONS Women more frequently exhibit adaptive LV remodeling with less fibrosis than men. Maladaptive LVH is associated with worse survival in women. Thus, sex should be considered as a strong modulating factor when management of patients with AS is discussed. (J Am Coll Cardiol Img 2014;7:1073–80) © 2014 by the American College of Cardiology Foundation.

Aortic stenosis (AS) is a common valvular disease that affects both sexes. More than 12,000 patients undergo aortic valve replacement (AVR) in Germany per year; at least 70% of them are age ≥60 years (1). In AS, left ventricular hypertrophy (LVH) develops as a response to pressure overload (PO). It is considered primarily a compensatory response to reduce wall stress and to maintain systolic

From the *Department of Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, Berlin, Germany; †DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, Germany; and the ‡Institute of Gender in Medicine and Center for Cardiovascular Research, Charité Universitaetsmedizin Berlin, Berlin, Germany. This work was supported by the German Research Foundation (DFG Re662/9-1, FG1054, and DW70/1-1) and DZHK (German Center for Cardiovascular Research). All authors have reported that they have no relationships relevant to the contents of this paper to disclose. Dr. Petrov, Dr. Dworatzek, and Ms. Schulze contributed equally to this work.

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ABBREVIATIONS AND ACRONYMS

AS = aortic stenosis

AVR = aortic valve replacement

LV = left ventricle

LVH = left ventricular hypertrophy

$LVID_{(d)}$ = left ventricular internal diameter at end-diastole

PO = pressure overload

RWT = relative wall thickness

SMAD2/3 = SMAD family member 2 or 3

TGF-ß1 = transforming growth factor beta 1

function of the heart. However, pressure-induced LVH also initiates a series of molecular events that may lead to a vicious circle and finally to myocardial fibrosis, dilation, and heart failure (2). LVH is not uniform in patients with a similar degree of AS, and its regression after surgical correction is variable. Genetic predisposition of the patient probably interferes with hemodynamic load to determine the pattern of LVH, its regression, and clinical course after surgery (3,4).

Sex has been described to have a major impact on the development of LVH in pressure overload (5,6). Women with AS develop a more concentric form of LVH with smaller ventricular diameters, greater relative wall thicknesses, and a better systolic function, whereas men with comparable degree of pressure overload are characterized by a more eccentric form of LVH with more ventricular dilation and fibrosis, decreased ejection fraction, and heart failure (7). Our own previous investigations in patients with AS showed at the time of AVR higher messenger ribonucleic acid expression of fibrosis-associated genes, that is, collagen I and III, MMP-2, and MMP-9, in men compared with women (8).

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Relief of mechanical obstruction by AVR leads to hemodynamic improvement and reversal of LVH. Sufficient evidence exists that LVH regression not only is a passive process, triggered by reduction in afterload, but also requires an activation of a specific gene expression program (9). Regression of LVH starts early after AVR but may go on for years (10). Even though the time course of hypertrophy regression has been extensively studied, few studies have considered the influence of sex on the post-operative reversibility of LVH, and the overall evidence of systematic sex differences remains limited (8,11). We recently demonstrated that early LVH regression after AVR is more pronounced in women than in men (8).

Survival after AVR depends on a number of clinical variables, including pattern of pre-operative LVH, severity of myocardial dysfunction, and cardiac fibrosis (12). There are only few studies analyzing sex differences in the post-operative survival after AVR (13–15). Linking sex differences in pre-operative LVH to regression and survival after AVR may improve our understanding of their relevance and offer new hints for optimal timing of interventions. We, therefore, continued our previous investigations on sex-specific LVH regression after AVR (8). The present study was undertaken to assess whether the pattern of myocardial adaptation in women and men with AS is associated with long-term survival after AVR.

METHODS

We included patients who were hospitalized at the German Heart Institute Berlin for AVR due to isolated AS. Exclusion criteria were emergency surgery, second AVR, transcatheter aortic valve implantation, left ventricular (LV) ejection fraction ≤40%, coronary artery narrowing ≥50%, coincident significant congenital or acquired valvular defects other than AS, pre-existing inflammatory heart diseases, cardiomyopathies, uncontrolled hypertension (blood pressure ≥160/≥100 mm Hg) or other extracardiac comorbidities (i.e., human immunodeficiency virus, metabolic disorders, malignancies, and so on), or language problems that might affect the clinical outcome or limit informed consent. The study was started in 2005; patients were followed prospectively and invited between 2011 and 2012 for follow-up echocardiography. The initial response rate was 83%. In follow-up responders, reasons for refusal to participate in echocardiography included residency abroad or in other federal states of Germany (n = 10), physical invalidity (n = 5), death before follow-up was conducted (n = 19), or refusal without indication of reasons (n = 4). Cardiac morphology and function were assessed about a week before AVR and 4.0 ± 1.6 years post-operatively, following the recommendations of the American Society of Echocardiography (16). Follow-up echocardiography was obtained in a representative subgroup of 68 survivors (43% women; $p < 0.097$ for sex difference; see Online Table S1-A for patients' characteristics), and the cumulative hazard to have an echocardiography at follow-up did not vary between women and men ($p < 0.117$). Written informed consent was obtained from all patients, and the study was approved by the ethical committee of Charité Universitaetsmedizin Berlin.

Mean overall follow-up was 3.2 years in women (95% confidence interval [CI]: 2.7 to 3.7 years) and 2.9 years in men (95% CI: 2.5 to 3.4 years, $p < 0.362$). Primary clinical endpoints were LVH regression and death. Before the study database was closed in December 2012, a query to all local residents' registration offices (all residents in Germany are registered) was performed to assign unknown deaths.

Myocardial tissue samples were harvested from the LV septum during AVR in a representative subgroup of 43 AS patients (49% women; see Online Table S1-B for patients' characteristics) and compared with LV septum biopsies from nondiseased donor hearts (n = 23; 52% women, see Online Table S2 for nondiseased

subjects' characteristics) that could not be used for transplantation because of age or logistic problems with the recipients. Fibrosis was quantitated by histology; transforming growth factor beta-1 (TGF-β1), SMAD family member 2 or 3 (SMAD2/3), and periostin protein expression by western blotting (see the Online Appendix for details). Detailed methodology has been described previously (17,18).

Statistics were performed using SPSS Statistics, version 21 (IBM, Armonk, New York). All of the data in the tables are presented as mean ± SD or percentages unless otherwise indicated. Sex comparisons were performed using unpaired Student *t* test or Mann-Whitney *U* test as appropriate. Categorical variables were tested using Pearson chi-square test. Two-way analysis of variance with Bonferroni post hoc testing was used to assess sex differences in cardiac fibrosis and associated key molecular regulators of patients with AS versus samples from nondiseased hearts. Kaplan-Meier and Cox models were used to analyze the survival data. Comparisons of survival curves were on the basis of the Breslow test. A value of $p < 0.05$ was considered statistically significant.

Factor analysis including LV mass, left ventricular internal diameter at end-diastole ($LVID_{[d]}$), relative wall thickness (RWT), transvalvular or transprosthetic mean pressure gradient (P_{mean}) and peak pressure gradient (P_{peak}), as well as body size and mass was used to analyze LVH under conditions of chronic PO and its regression after AVR. The factor analysis identified 1 multidimensional index for LVH (LVH index), 1 for PO (PO index), and 1 for body size and mass. The LVH index was correlated with LV mass (factor loading 0.676) and $LVID_{(d)}$ (factor loading 0.937) and inversely correlated with RWT (factor loading −0.861). The PO index was correlated with P_{mean} (factor loading 0.971) and P_{peak} (factor loading 0.970). The body size and mass index was correlated with height (factor loading 0.856) and weight (factor loading 0.839). Because the factor analysis was conducted as a principal component analysis, the LVH, PO, and body size and mass indexes are orthogonal (uncorrelated). Thus, the observed variance in cardiac morphology that is included in the LVH index is statistically independent from differences in afterload, body size, and mass. We used the LVH index to classify LVH into adaptive or maladaptive patterns. Because the LVH index is a z-transformed variable, negative LVH index values reflect adaptive stages of LVH, with smaller LV mass and $LVID_{(d)}$ and greater RWT, whereas positive LVH index values reflect maladaptive stages of LVH with adverse alteration of LV morphology. The null value served as a parameterized reference to discriminate between adaptive and maladaptive LVH patterns. Kaiser-Guttman criterion was applied to ascertain the number of extracted factors (Online Tables S3-A and S3-B).

RESULTS

The study population comprised 128 patients (49% women). Women and men were at comparable age, had equal symptoms and New York Heart Association functional class at referral for AVR, and comparable cardiovascular risk profile as reflected by similar prevalence of diabetes mellitus, hypertension, and hyperlipidemia. Cardiac medications were equally administered in women and men. Coronary artery sclerosis, chronic obstructive pulmonary disease, or cerebrovascular accident were observed in a small portion of the study population, and there were no significant sex differences. Women compared with men had smaller body size and lower renal function. Mechanical and larger prosthetic valves were more frequently implanted in men than in women. Sex differences abolished after the effective orifice area of

TABLE 1 Baseline Characteristics of the Study Population

	Women (n = 63)	Men (n = 65)	p Value
Age at AVR, yrs	71 ± 9	70 ± 10	0.457
BSA, m^2	1.8 ± 0.2	2.0 ± 0.2	**<0.001**
Obesity	33	35	0.807
GFR (Cockroft-Gault), ml/min	69 ± 21	84 ± 26	**0.001**
Syncope	24	18	0.458
Dyspnea at exertion	83	82	0.883
Dyspnea at rest	11	6	0.317
NYHA functional class II/III	83	85	0.158
Hypertension	86	89	0.548
Hyperlipidemia	60	58	0.831
Diabetes mellitus	27	31	0.637
Coronary artery sclerosis*	2	11	0.062
Chronic obstructive pulmonary disease	13	17	0.502
Cerebrovascular accident	8	3	0.270
Beta-blockers	48	49	0.855
ACE inhibitors	44	40	0.611
Prosthesis type			
Mechanical	8	21	**0.050**
Biological	92	79	**0.018**
Prosthesis EOA, cm^2	1.3 ± 0.1	1.5 ± 0.4	**<0.001**
Prosthesis EOA/BSA, cm^2/m^2	0.7 ± 0.1	0.7 ± 0.2	0.209
Prosthesis-patient mismatch			
Nonsignificant	7	10	0.744
Moderate	72	63	0.323
Severe	21	27	0.461

Values are mean ± SD or %. **Bold values** indicate statistical significance. *Coronary artery sclerosis is defined as lumen obstruction ≤50%.

ACE = angiotensin converting enzyme; AVR = aortic valve replacement; BSA = body surface area; EOA = effective orifice area; GFR = glomerular filtration rate; NYHA = New York Heart Association.

the implanted prosthetic valves was indexed to body surface area, and there was no evidence of significant sex differences in the occurrence of prosthesis-patient mismatch after AVR (Table 1).

At the time of AVR, myocardial fibrosis, quantified in biopsies from the LV septum, was significantly more pronounced in men with AS compared with nondiseased men and women with AS (Figures 1A and 1B). We found in men, but not in women with AS, a significant increase in TGFβ-1 protein expression (Figure 2A) and phosphorylation of SMAD2 (Figure 2B). Phosphorylation of SMAD3 was not altered in a sex-specific manner (Figure 2C). Periostin was significantly increased in both sexes with AS, but was more highly expressed in men compared with women with AS (Figure 2D).

Before AVR, LVH morphology differed between women and men with AS. Absolute LV mass and $LVID_{(d)}$ were significantly larger in men, but sex differences were reduced after adjustment for body surface area. RWT had the tendency to be higher in women, but the sex difference was of borderline statistical significance (Table 2). We, therefore, used factor analysis to combine single normalized parameters of LVH into a composed LVH index. On the basis of the LVH index, LVH was classified as adaptive or maladaptive (see Methods section for more details). Pre-operatively, adaptive LVH prevailed in women (women: 62% vs. men: 45%) and maladaptive LVH in men (women: 38% vs. men: 55%, $p < 0.050$ for sex difference). This was reflected by slightly greater pre-operative mean LVH indexes in men than in women (women: −0.08, 95% CI: −0.35 to 0.20 vs. men: 0.09, 95% CI: −0.15 to 0.34; $p < 0.360$) (Figure 3).

After AVR, LVH regression occurred in both sexes, but its pattern was different in women and men. Adaptive LVH was found after AVR more often in women (women: 75% vs. men: 49%), whereas maladaptive LVH characterized predominantly men (women: 25% vs. men: 51%, $p < 0.031$ for sex difference). The post-operative mean LVH index remained greater in men than in women (women: −0.44, 95% CI: −0.78 to −0.09 vs. men: 0.22, 95% CI: −0.12 to 0.57; $p < 0.012$) (Figure 3).

Post-AVR survival was similar in both sexes (overall mortality, women: 17% vs. men: 18%, $p < 0.883$). However, there was an interaction among sex, pattern of LVH, and survival (hazard ratio: 0.12, 95% CI: 0.02 to 0.89, $p < 0.038$). Women with maladaptive LVH at surgery had significantly worse survival than women with adaptive LVH ($p < 0.050$) (Figure 4B). In contrast, the pattern of LVH was not associated with survival in men ($p < 0.307$) (Figure 4A).

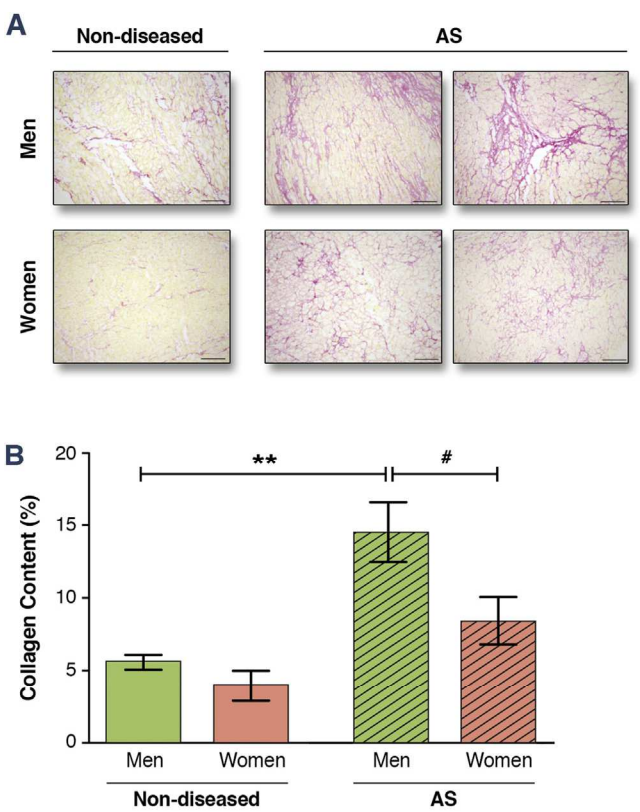

FIGURE 1 Cardiac Fibrosis in Women and Men With AS

Men with aortic stenosis (AS) exhibited increased cardiac fibrosis. **(A)** Representative Sirius Red staining of total collagen; **(B)** collagen content, quantified in patients with AS or nondiseased donor hearts. **$p < 0.01$ (nondiseased vs. AS), #$p < 0.05$ (women vs. men). Nondiseased, women: $n = 6$ versus men: $n = 6$; AS, women: $n = 11$ versus men: $n = 14$. Scale: 200 μm.

DISCUSSION

In aortic stenosis, maladaptive LV remodeling with eccentric LVH, more profibrotic gene expression, and fibrosis occurs with greater likelihood in men than in women with isolated AS (5–8,19). Our present study is well in agreement with these findings; however, it is the first study to link sex-specific patterns of PO-induced LVH to survival after AVR. An impaired survival was found in women with maladaptive LVH compared with women with adaptive LVH. In contrast, the pattern of LVH did not affect survival in men. This suggests that maladaptive LV remodeling has a stronger negative impact in women than in men.

Sex differences in the pre-operative LV remodeling have implications in the regression of LVH after

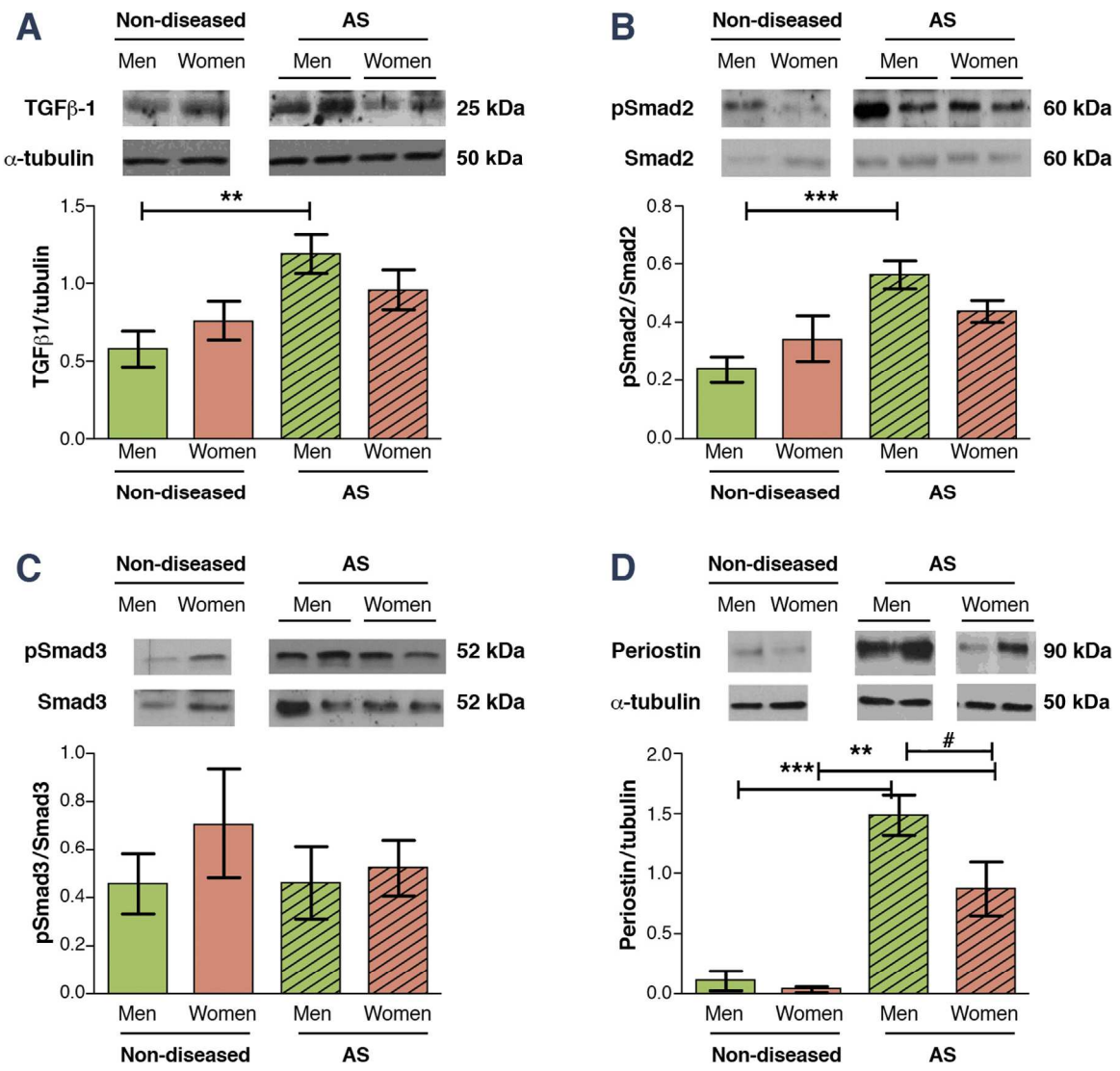

FIGURE 2 Sex-Specific Activation of Profibrotic Markers

(A) Transforming growth factor beta 1 (TGF-β1) and **(B)** phosphorylation of SMAD2 (pSMAD2) are significantly increased in men with aortic stenosis (AS), but not in women, compared with nondiseased subjects. **(C)** SMAD3 phosphorylation (pSMAD3) was similar in both sexes. **(D)** Left ventricular hypertrophy led to increased periostin expression in both sexes compared with nondiseased donor hearts. In AS, periostin was more highly expressed in men than in women. ***$p < 0.001$, **$p < 0.01$ (nondiseased vs. AS), and #$p < 0.05$ (men vs. women). Nondiseased, women: n = 12 versus men: n = 10; AS, women: n = 14 versus men: n = 21.

AVR. We showed in a former phase of the current study that development of more favorable LVH in women was associated with faster regression in the early follow-up after AVR (8). We now go beyond this finding and confirm a more pronounced LVH regression in the long-term follow-up. Furthermore, our study provides evidence that sex differences occurring early after AVR are sustained in the late post-operative course, and thus it documents systematic sex differences in the time course of LVH regression after AVR.

We also show that higher cardiac fibrosis in men with AS compared with women is associated with increased TGF-β1 protein expression and phosphorylation of SMAD2. Periostin, a down-stream target of TGF-β1 signaling and a key regulator of cardiac fibrosis (20), was more highly expressed in men as well. In human failing hearts, up-regulated

TABLE 2 LV Morphology and Function Before and Late After AVR

	Pre-AVR			Post-AVR		
	Women (n = 63)	Men (n = 65)	p Value	Women (n = 29)	Men (n = 39)	p Value
$LVID_{(d)}$, mm	48.0 ± 6.9	51.0 ± 5.3	**0.004**	46.0 ± 4.9	51.0 ± 5.8	**<0.001**
$LVID_{(d)}$/BSA, mm/m^2	26.0 ± 3.3	25.0 ± 3.1	0.073	25.0 ± 2.6	25.0 ± 2.4	0.717
$LVID_{(s)}$, mm	30.0 ± 7.8	33.0 ± 7.5	0.064	31.0 ± 5.6	34.0 ± 5.9	**0.019**
$PWT_{(d)}$, mm	12.0 ± 1.3	12.0 ± 1.3	0.245	12.0 ± 1.2	12.0 ± 1.1	0.121
$IVS_{(d)}$, mm	13.0 ± 1.4	13.0 ± 1.4	0.163	12.0 ± 1.3	13.0 ± 1.2	0.628
RWT	0.50 ± 0.08	0.48 ± 0.07	0.093	0.51 ± 0.07	0.48 ± 0.07	0.059
LV mass, g	227 ± 66	258 ± 56	**0.005**	207 ± 43	254 ± 51	**<0.001**
LV mass/BSA, g/m^2	125 ± 31	127 ± 27	0.621	114 ± 21	124 ± 24	0.092
LVEF, %	61.0 ± 6.2	58.0 ± 6.6	0.774	60.0 ± 7.8	58.0 ± 7.3	0.098
FS, %	37.0 ± 9.5	36 ± 10	0.680	33.0 ± 7.5	33.0 ± 9.0	0.881
P_{mean}, mm Hg	57 ± 13	55 ± 12	0.469	15 ± 10	13.0 ± 5.9	0.313
P_{peak}, mm Hg	80 ± 20	82 ± 17	0.509	25 ± 16	22.0 ± 9.0	0.287

Values are mean ± SD. **Bold values** indicate statistical significance.

BSA = body surface area; FS = fractional shortening; $IVS_{(d)}$ = end-diastolic interventricular septum thickness; LV = left ventricular; LVEF = left ventricular ejection fraction; LVID = left ventricular internal dimension at end-diastole (d) and end-systole (s); P_{mean} = mean pressure gradient; P_{peak} = peak pressure gradient; $PWT_{(d)}$ = end-diastolic posterior wall thickness; RWT = relative wall thickness.

periostin expression is associated with cardiac fibrosis (21). Higher periostin levels were found in blood from men compared with women with heart failure (22). Furthermore, periostin has already been described as a modulator of cardiac remodeling under PO in mice (23). We now demonstrated sex differences in PO-induced up-regulation of periostin and speculate that lower expression of periostin in the female heart is associated with less cardiac fibrosis, tentatively linked to an adaptive LVH.

Analysis of sex differences in the development and regression of LVH requires the estimation of LV mass, ventricular diameters, wall thicknesses, and their combined effect. We applied in the current study a factor analysis to operationalize LVH as a combined endpoint of LV mass, $LVID_{(d)}$, and RWT changes. Although the factor analysis is commonly used in social sciences, it has only recently been used in biomedical research (24). Factor analysis has the advantages of combining multiple variables into a single index at the patient level, facilitating the use of this index in more complex statistical analyses, and addressing the behavior of complex biological processes better than the use of single variables. With regard to statistical performance, factor analysis of echocardiography data was as efficient as a conventional approach, which we applied earlier, to compare patients' LV mass and RWT with prognostically-validated reference values from the general population (8). Notably, similar numbers of patients were needed to establish significant sex differences in the development and regression of LVH. Adequate model power beyond controlled study design and meticulous selection is an important prerequisite for the transferability of results.

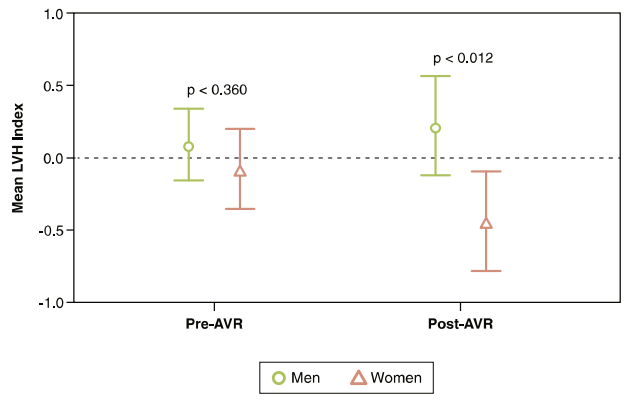

FIGURE 3 Development of LVH and its Regression After AVR in Women and Men

Mean left ventricular hypertrophy (LVH) index values with their corresponding confidence intervals are given before (n = 128, 49% women) and after (n = 68, 43% women) aortic valve replacement (AVR).

Clinicians are well aware of strong interindividual differences in the amount and pattern of LVH. We now demonstrate that consideration of sex partially reduces this variability. Adaptive LVH is more common in women's hearts, whereas maladaptive LVH and increased fibrosis are more characteristic of men's hearts. This is well in agreement with some previous observations from us and others (7,8). We link higher TGF-β1/SMAD2 signaling, periostin expression, and higher cardiac fibrosis level in men tentatively with maladaptive remodeling. A better understanding of the regulatory mechanisms

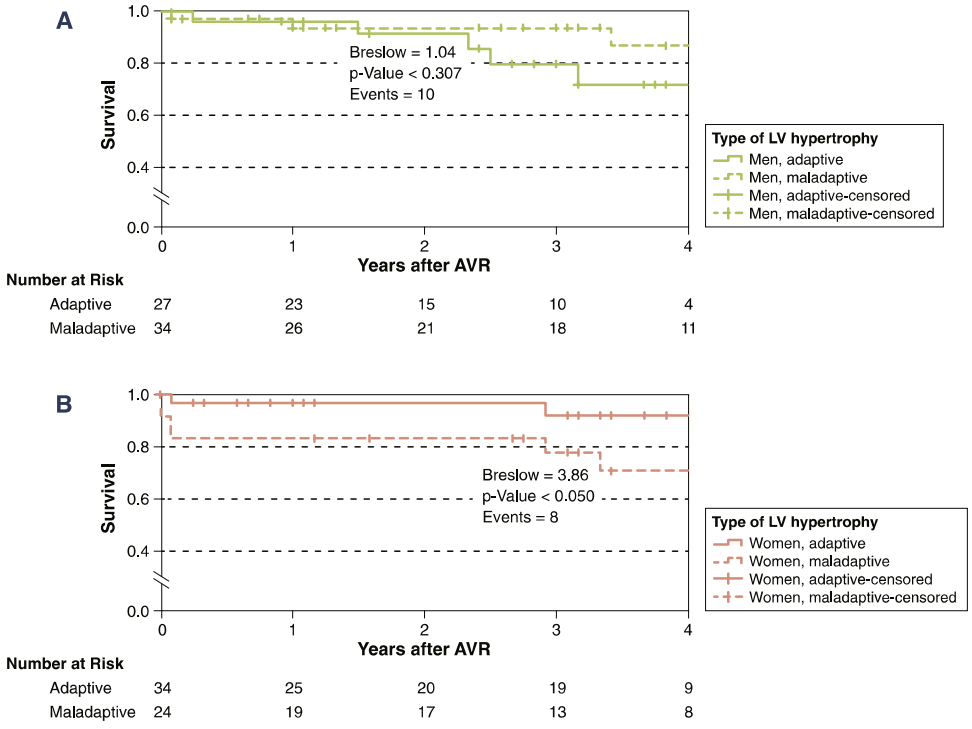

FIGURE 4 Impact of Sex and Pre-Operative Pattern of LVH on Late Post-AVR Survival

(A) Men with adaptive LVH have similar survival compared with men with maladaptive LVH; **(B)** women with adaptive LVH have significantly better survival compared with women with maladaptive LVH. Data from 128 patients (49% women) are summarized. LV = left ventricular; other abbreviations as in Figure 3.

underlying this sex difference offers the potential to identify a protective pathway in the response of the heart to PO. This is of particular interest, because maladaptive LVH in women is associated with worse survival. Our data suggest that determining whether earlier intervention in women—before maladaptive remodeling occurs—is beneficial may be worth evaluating. Prospective and retrospective analysis of large databases may be first steps. The same hypotheses may be tested in men, but may require higher numbers for testing.

Alternatively, sex-specific evidence should be sought to determine whether women and/or men with maladaptive remodeling benefit post-operatively from substances that counteract profibrotic mechanisms, such as angiotensin-converting enzyme inhibitors or aldosterone antagonists.

STUDY LIMITATIONS. This study has limited patient numbers and events, but the statistical impact on the power of the analysis is largely compensated by the duration of follow-up and meticulous patient characterization. A further limitation is the small number of patients at follow-up.

CONCLUSIONS

We show for the first time that pre-operative LVH is associated with survival in women. We also identified molecular mechanisms underlying sex differences in cardiac fibrosis that may contribute to sex-specific LV remodeling. The link between pattern of remodeling and survival raises the question of optimizing the management of AS in a sex-specific manner, and further evidence, obtained by analysis of suitable databases and well-designed clinical trials, is needed.

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REPRINT REQUESTS AND CORRESPONDENCE: Dr. George Petrov, Department of Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum Berlin, DHZB, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: george@docpetrov.info.

REFERENCES

1. Bruckenberger E. Herzbericht 2010. Hannover: Bruckenberger E, 2011.

2. Hill JA, Olson EN. Cardiac plasticity. N Engl J Med 2008;358:1370–80.

3. Kupari M, Turto H, Lommi J. Left ventricular hypertrophy in aortic valve stenosis: preventive or promotive of systolic dysfunction and heart failure? Eur Heart J 2005;26:1790–6.

4. Gavina C, Falcao-Pires I, Rocha-Goncalves F, Leite-Moreira A. Left ventricular hypertrophy in isolated aortic stenosis: primetime for the ventricle. Curr Pharm Biotechnol 2012;13:2503–14.

5. Carroll JD, Carroll EP, Feldman T, et al. Sex-associated differences in left ventricular function in aortic stenosis of the elderly. Circulation 1992; 86:1099–107.

6. Douglas PS, Otto CM, Mickel MC, Labovitz A, Reid CL, Davis KB. Gender differences in left ventricle geometry and function in patients undergoing balloon dilatation of the aortic valve for isolated aortic stenosis. NHLBI Balloon Valvuloplasty Registry. Br Heart J 1995;73:548–54.

7. Villari B, Campbell SE, Schneider J, Vassalli G, Chiariello M, Hess OM. Sex-dependent differences in left ventricular function and structure in chronic pressure overload. Eur Heart J 1995;16:1410–9.

8. Petrov G, Regitz-Zagrosek V, Lehmkuhl E, et al. Regression of myocardial hypertrophy after aortic valve replacement: faster in women? Circulation 2010;122:S23–8.

9. Stansfield WE, Charles PC, Tang RH, et al. Regression of pressure-induced left ventricular hypertrophy is characterized by a distinct gene expression profile. J Thorac Cardiovasc Surg 2009; 137:232–8, 238e1–8.

10. Sharma UC, Barenbrug P, Pokharel S, Dassen WR, Pinto YM, Maessen JG. Systematic review of the outcome of aortic valve replacement in patients with aortic stenosis. Ann Thorac Surg 2004;78:90–5.

11. Villar AV, Merino D, Wenner M, et al. Myocardial gene expression of microRNA-133a and myosin heavy and light chains, in conjunction with clinical parameters, predict regression of left ventricular hypertrophy after valve replacement in patients with aortic stenosis. Heart 2011;97:1132–7.

12. Milano AD, Faggian G, Dodonov M, et al. Prognostic value of myocardial fibrosis in patients with severe aortic valve stenosis. J Thorac Cardiovasc Surg 2012;144:830–7.

13. Hayashida K, Morice MC, Chevalier B, et al. Sex-related differences in clinical presentation and outcome of transcatheter aortic valve implantation for severe aortic stenosis. J Am Coll Cardiol 2012;59:566–71.

14. Stangl V, Baldenhofer G, Knebel F, et al. Impact of gender on three-month outcome and left ventricular remodeling after transfemoral transcatheter aortic valve implantation. Am J Cardiol 2012;110:884–90.

15. Hamed O, Persson PJ, Engel AM, McDonough S, Smith JM. Gender differences in outcomes following aortic valve replacement surgery. Int J Surg 2009;7:214–7.

16. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18: 1440–63.

17. Fliegner D, Schubert C, Penkalla A, et al. Female sex and estrogen receptor-beta attenuate cardiac remodeling and apoptosis in pressure overload. Am J Physiol Regul Integr Comp Physiol 2010;298:R1597–606.

18. Kararigas G, Bito V, Tinel H, et al. Transcriptome characterization of estrogen-treated human myocardium identifies myosin regulatory light chain interacting protein as a sex-specific element influencing contractile function. J Am Coll Cardiol 2012;59:410–7.

19. Villar AV, Llano M, Cobo M, et al. Gender differences of echocardiographic and gene expression patterns in human pressure overload left ventricular hypertrophy. J Mol Cell Cardiol 2009; 46:526–35.

20. Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. Circ Res 2009; 105:934–47.

21. Zhao S, Wu H, Xia W, et al. Periostin expression is upregulated and associated with myocardial fibrosis in human failing hearts. J Cardiol 2014;63:373–8.

22. Meyer S, van der Meer P, Massie BM, et al. Sex-specific acute heart failure phenotypes and outcomes from PROTECT. Eur J Heart Fail 2013;15:1374–81.

23. Stansfield WE, Andersen NM, Tang RH, Selzman CH. Periostin is a novel factor in cardiac remodeling after experimental and clinical unloading of the failing heart. Ann Thorac Surg 2009;88:1916–21.

24. Ullah S, Finch CF. Applications of functional data analysis: a systematic review. BMC Med Res Methodol 2013;13:43.

KEY WORDS aortic valve replacement, aortic valve stenosis, cardiac fibrosis, gender, hypertrophy regression, sex, survival

APPENDIX For supplemental materials and tables, please see the online version of this article.

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publikationsliste

Zeitschriftenartikel mit Peer-Review-Verfahren

Petrov G[*], Dworatzek E[*], **Schulze TM**[*], Dandel M, Kararigas G, Mahmoodzadeh S, Knosalla C, Hetzer R, Regitz-Zagrosek V. Maladaptive remodeling is associated with impaired survival in women but not in men after aortic valve replacement.
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Kongressbeiträge

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Petrov G, Dworatzek E, **Schulze TM**, Dandel M, Kararigas G, Mahmoodzadeh S, Hetzer R, Regitz-Zagrosek V. Adaptive Hypertrophy in Women With Aortic Valve Stenosis is Associated With Better Reversibility and Survival After Valve Replacement. American Heart Association Scientific Sessions, Dallas, Texas, USA. November 2013. Circulation. 2013;128:A16853

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