Persistent Diastolic Dysfunction Late After Valve Replacement in Severe Aortic Regurgitation

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Background—Regression of left ventricular (LV) hypertrophy with normalization of diastolic function has been reported in patients with aortic stenosis late after aortic valve replacement (AVR). The purpose of the present study was to evaluate the effect of AVR on LV function and structure in chronic aortic regurgitation early and late after AVR.

Methods and Results—Twenty-six patients were included in the present analysis. Eleven patients with severe aortic regurgitation were studied before, early (21 months) and late (89 months) after AVR through the use of LV biplane angiograms, high-fidelity pressure measurements, and LV endomyocardial biopsies. Fifteen healthy subjects were used as controls. LV systolic function was determined from biplane ejection fraction and midwall fractional shortening. LV diastolic function was calculated from the time constant of LV relaxation, peak filling rates, and myocardial stiffness constant. LV structure was assessed from muscle fiber diameter, interstitial fibrosis, and fibrous content. LV muscle mass decreased significantly by 38% early and 55% late after surgery. Ejection fraction was significantly reduced preoperatively and did not change after AVR \((P=NS)\). LV relaxation was significantly prolonged before surgery \((89\pm28\text{ ms})\) but was normalized late after AVR \((42\pm14\text{ ms})\). Early and late peak filling rates were increased preoperatively but normalized postoperatively. Diastolic stiffness constant was increased before surgery \((22\pm6\text{ versus }9\pm3\text{ in control subjects}; \ P=0.0003)\) and remained elevated early and late after AVR \((23\pm4; \ P=0.002)\). Muscle fiber diameter decreased significantly after AVR but remained increased at late follow-up. Interstitial fibrosis was increased preoperatively and increased even further early but decreased late after AVR. Fibrosis was positively linearly correlated to myocardial stiffness and inversely correlated to LV ejection fraction.

Conclusions—Patients with aortic regurgitation show normalization of macroscopic LV hypertrophy late after AVR, although fiber hypertrophy persists. These changes in LV myocardial structure late after AVR are accompanied by a change in passive elastic properties with persistent diastolic dysfunction.

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Key Words: diastole ■ hypertrophy ■ regurgitation ■ structure ■ surgery

Pressure and volume overload induce different adaptations of the left ventricle,1-3 the first generating concentric and the latter eccentric left ventricular (LV) hypertrophy. These changes in macroscopic LV anatomy are accompanied by alterations in the microscopic structure and consequently LV dysfunction.4,5 Diastolic dysfunction has been demonstrated to precede the alteration in systolic function in patients with LV hypertrophy from aortic valve disease.6,7 After successful aortic valve replacement (AVR), regression of LV hypertrophy with normalization of LV diastolic dysfunction has been reported in patients with severe aortic stenosis; an early decrease in muscle hypertrophy with a relative increase in interstitial fibrosis followed by a late decrease in collagen constant was found in these patients.8,9 Several investiga-
Inclusion Criteria

Inclusion criteria included severe AR (regurgitant fraction ≥0.45), no concomitant aortic stenosis (pressure gradient ≤15 mm Hg), no postoperative prosthesis mismatch, no coronary artery disease, sinus rhythm, no severe comorbidities (chronic pulmonary disease, history of stroke, renal failure), willingness to undergo postoperative heart catheterization, and no left or right bundle-branch block. In women of childbearing age, a pregnancy test was carried out to exclude pregnancy. Written informed consent was obtained from all patients.

Exclusion Criteria

Exclusion criteria were unwillingness to undergo postoperative cardiac catheterization, insulin-dependent diabetes mellitus, arterial hypertension (>160/90 mm Hg), bleeding disorder, active cancer, or pulmonary hypertension.

Cardiac Catheterization

Informed consent was obtained from all patients. Cardiovascular medications were withheld for 24 hours before the procedure. Premedication consisted of 10 mg chloralhydrate orally 1 hour before catheterization. Right and left heart catheterizations were carried out in all patients postoperatively. Biplane left ventriculography was performed in the right anterior oblique (30°) and left anterior oblique (60°) projections (Cardioscope, Siemens-Albis, Zurich, Switzerland) at a filming rate of 50 frames per second. LV angiographic silhouettes were drawn manually from adequately opacified sinus beat, excluding extrasystolic and postextrasystolic beats. LV volumes were determined on a frame-by-frame basis with the area-length method.1,3 Dimensional and volumetric data were filtered with the moving average technique. Pressure and volume data were digitized (time resolution, 20 ms) every 20 milliseconds for 1 cardiac cycle. End diastole was defined as the time point of the rapid upstroke of dP/dt; end systole was defined as the time of incisural pressure in the aortic pressure curve. LV mass was determined as previously described.1,3,8 Circumferential wall stress was calculated from a simplified version of a thick-wall model.3,8 Mean systolic circumferential wall stress was defined as mean wall stress occurring during systolic ejection period.

The ratio of LV end-diastolic volume to mass was calculated and used as a parameter for assessing chamber geometry of the LV. Postoperative recatheterization was performed from the right femoral vein with transseptal puncture and a Brockenbrough guiding catheter for access to the left ventricle. Pressure measurements, biplane LV angiography, and LV endomyocardial biopsies were obtained as described earlier. Calculations were carried out as described above, and morphometry of LV biopsies was performed according to the method of Villari et al.8 Postoperative pressure gradients across the aortic valve prosthesis were estimated from LV systolic pressure and brachial cuff pressure (noninvasive, peak pressure gradient). No patient showed >12-mm Hg pressure gradient with this semi-invasive pressure measurement. No complications occurred. Patients were sent home 2 to 3 hours after catheterization (venous puncture).

Aortic Valve Replacement

AVR was carried out under deep hypothermia with cold crystalloid cardioplegic arrest. Either a mechanical valve prosthesis (n=2) in younger patients (<40 years of age) or a biological valve prosthesis (n=9) in older patients (>40 years of age) was implanted successfully. Only patients with a good hemodynamic and clinical result were included. Patients with valve prosthesis mismatch (postoperative pressure gradient >15 mm Hg) or left bundle-branch block (including pacemaker implantation) were excluded from the study. Patients and referring physicians were asked by letter to agree for a first and, later, to a second cardiac recatheterization on an ambulatory basis. The study protocol was approved by the local ethics committee. There were no complications in regard to preoperative and postoperative catheterization.

Assessment of LV Function

Systolic function was determined from biplane LV ejection fraction, midwall fractional shortening, LV end-diastolic pressure, and mean systolic wall stress as previously described.8,9 Diastolic function was evaluated from isovolumic relaxation, peak diastolic filling rates, and passive elastic properties. LV relaxation was determined from the time constant of isovolumic pressure decline, which was calculated as the negative reciprocal of the slope of the linear relation between LV pressure and −dP/dt.3 The isovolumetric relaxation period was defined as the time interval beginning immediately after maximal negative dP/dt and ending when pressure had decreased to 5 mm Hg above LV end-diastolic pressure.3,8 From this time interval, usually 7 to 14 points were available for calculation of the time constant of isovolumic pressure fall in the individual patient.

Peak diastolic filling rate was defined as the largest value of diastolic inflow (milliliter per meter squared per second) during the first half (early peak filling rate) and the second half (late peak filling rate) of diastole. The filling phase was considered to begin 20 ms before the first frame showing the entry of opacified blood into the left ventricle and to finish at end diastole.13 To minimize error resulting from random noise, raw data were filtered with the fifth-grade moving average. Diastolic filling rate (FR) was calculated from the following equation: FR(t)=[V(t−20)−V(t+20)]/0.04, where t is time and V is volume.

Diastolic passive elastic properties were determined during the period from minimum ventricular pressure to end diastole.8 LV myocardial properties were evaluated from the diastolic stress-strain relation using an elastic model with shifting asymptote: S=a·e^{k·t}+c or dS/dF=b(S−c), where S is LV circumferential wall stress (kilodynes per centimeter squared), a is an elastic constant (kilo- dynes per centimeter squared), b is the myocardial stiffness constant, F is diastolic strain (Lagrangian definition), c is asymptote of the stress-strain relation (kilodynes per centimeter squared), and dS/dF is instantaneous myocardial stiffness (kilodynes per centimeter squared). The 3 constants (a, b, and c) were determined by an iteration procedure.8 The constant of myocardial stiffness is mathematically represented by the slope of the stress-strain curve, and the

<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics</th>
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<tbody>
<tr>
<td>Control Subjects</td>
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<tr>
<td>Age, y (n=15)</td>
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<td>ΔP, mm Hg (n=11)</td>
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<td>Ejection fraction, %</td>
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<td>Cl, L·min⁻¹·m⁻²</td>
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<td>Aortic valve</td>
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<td>Aortoannular dilatation</td>
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ΔP indicates mean aortic gradient; FAO, aortic regurgitant fraction; and Cl, cardiac index.
Endomyocardial Biopsies

LV endomyocardial biopsies were performed with the King’s College biotome (Olympus), which was introduced into the LV through the 11.5F Brockenbrough catheter. In each patient, 2 to 4 biopsy samples were taken from the anterolateral wall of the LV. Immediately after biopsy, specimens were fixed in glutaraldehyde, cut into semithin sections, and evaluated by light microscopy. Control biopsy samples were obtained from pretransplantation donor hearts as previously reported. Biopsy samples could not be taken from control subjects because of a negative vote by the ethics committee.

Assessment of Cellular Hypertrophy and Interstitial Nonmuscular Tissue

Morphometric analyses were carried out in glutaraldehyde-fixed specimens. The following 4 parameters were determined: muscle fiber diameter, which is the average fiber diameter of at least 100 measurements determined at the level of the nucleus from several randomly chosen cross sections with the use of a mechanical-optical pen (Kontron, Zürich, Switzerland); interstitial nonmuscular tissue, which is determined with the point-counting system excluding areas of arterioles and perivascular tissue as previously described (We used the term interstitial fibrosis [as did others] for this tissue because fibrous tissue is the predominant component of the interstitial space); volume fraction of myofibrils, which is evaluated at a magnification of 1000:1 with oil immersion and phase-contrast microscopy; and fibrous content, which is an index calculated as normal distribution of all continuous variables was tested with the 1-sample Kolmogorov-Smirnov test. Statistical comparisons between patients and control subjects were carried out with an unpaired Student t test with Welch correction to account for different variances. Preoperative and early and late postoperative data were compared by use of an ANOVA for repeated measurements. Posthoc tests were performed with the Bonferroni method (Tables 2 through 4). The test was considered statistically significant at values of P<0.05. A linear regression analysis was used for comparing stiffness data with myocardial fibrosis, LV ejection fraction, and LV muscle mass. The degree of linear relationship was measured by the Pearson correlation coefficient. All statistical calculations were performed with SPSS for Windows, release 12.0 (SPSS Inc, Chicago, Ill).

Results

Patient Characteristics

The mean age was similar in the 2 groups (Table 1). In patients with AR, the aortic regurgitation fraction was 61% and mean systolic pressure gradient 7 mm Hg. Cardiac index was slightly, although not significantly, reduced in preoperative patients with AR compared to control subjects. After valve replacement, AR was absent in all patients at the early and late follow-up examinations.

Hemodynamics

Heart rate was comparable in the different groups (Table 2). LV peak systolic pressure was comparable in the study groups, whereas end-diastolic pressure was significantly increased preoperatively and late after operation in patients with AR compared to control subjects. End-diastolic and late postoperative data were compared by use of an ANOVA for
end-systolic volumes were significantly larger preoperatively and early after AVR than in control subjects but were normalized late postoperatively. Ejection fraction and mid-wall fractional shortening were slightly reduced in preoperative patients with AR and did not change after operation. LV wall thickness was significantly increased before surgery and was normalized after AVR. LV muscle mass was significantly elevated in patients with AR and remained increased early after surgery, although there was a 38% decrease in mass. Late after valve replacement, there was a further decrease by 17%. LV end-diastolic volume-to-mass ratio was comparable in patients with AR and control subjects. Mean systolic stress and end-diastolic stress were significantly increased preoperatively and early after valve replacement compared to control subjects but were normalized late after operation.

Diastolic Function Data
LV relaxation was significantly prolonged before surgery and returned toward control levels early and late after valve replacement (Table 3). Early and late peak filling rates were increased preoperatively and normalized early after valve replacement. Myocardial stiffness constant was increased in all patients with AR preoperatively but decreased significantly after valve replacement. However, it remained increased in all patients early and in 7 of 10 patients late after AVR (Figure 1). The time constant of LV relaxation (Figure 2) was significantly increased preoperatively in AR patients compared to control subjects. It remained slightly elevated during early but was normalized during late follow-up.

Structural Data
Muscle fiber diameter was increased in patients with AR before and after surgery compared with control subjects (Figure 3). However, it decreased significantly early and late after valve replacement compared to preoperative data but remained hypertrophied even late after surgery (Table 4). Interstitial fibrosis was larger before surgery than in control subjects but increased even further early after operation, whereas it decreased significantly late after valve replacement (Figure 4). However, it was significantly increased with respect to controls at the late follow-up examination. Fibrous content (Figure 5) was significantly increased preoperatively and remained increased during early and late follow-up, although it decreased slightly after AVR. Interstitial fibrosis was positively linearly correlated to myocardial stiffness ($r=0.596$, $P=0.002$) and inversely correlated to LV ejection fraction ($r=-0.57$, $P=0.004$) but not to LV hypertrophy or other hemodynamic parameters (Figure 6).

Discussion
AVR has led to a dramatic change in the natural history of AR. According to the 2006 guidelines of the American...
AVR is indicated for symptomatic patients with severe AR regardless of LV systolic function and for asymptomatic patients with chronic severe AR and LV systolic dysfunction. AVR is also indicated for asymptomatic patients with severe AR with normal LV systolic function but with severe LV dilatation.

In the majority of patients, valve replacement results in a reversal of LV dilatation and an increase in LV systolic performance. The guidelines of the European Society of Cardiology on valvular heart disease suggest that LV systolic function is an important determinant of long-term prognosis in patients with chronic AR undergoing AVR. However, concerns remain about the long-term sequelae of chronic overload on the myocardium. Whether the regression of LV hypertrophy is inherently a salutary or deleterious process has been questioned. In patients with aortic stenosis, we have demonstrated that reversal of diastolic dysfunction takes years and is accompanied by a slow regression of interstitial fibrosis. Approximately 6 to 7 years after AVR, normalization of LV diastolic structure and function has been observed in aortic stenosis patients.

The present study demonstrates that a rapid and complete normalization of muscular tissue occurs in patients with AR followed by an incomplete regression of fibrotic tissue and delayed ventricular remodeling. These abnormalities in LV structure are accompanied by abnormal passive elastic properties with persistent diastolic dysfunction late after AVR.

LV Structure and Function After AVR
In a subset of patients with preoperative LV dysfunction, AVR is associated with a diminished reduction in LV diastolic volume and no demonstrable improvement in LV function despite correction of valvular regurgitation. This observation has led to the assumption that irreversible myocardial dysfunction with structural alterations has occurred before valve replacement; thus, negative remodeling parallel to the reduction in volume overload is no longer possible.

In the present study, we observed that changes in LV structure are similar to those in aortic stenosis with regard to the early postoperative period. There is a relative increase in interstitial fibrosis and a significant reduction in muscle fiber diameter in both pressure- and volume-overloaded ventricles. The increase in interstitial fibrosis is relative to the decrease in muscle mass because the total amount of fibrous tissue remains unchanged. In patients with pressure and volume overload, myocardial stiffness increased in the early postoperative period as a result of the relative increase in interstitial fibrosis.

In contrast to aortic stenosis, patients with AR do not show normalization of diastolic dysfunction late after valve replacement. Percent fibrosis was larger in AR than aortic stenosis mainly because of a diminished regression late after valve replacement. This could be explained by the higher wall stress with a slightly eccentric left ventricle, more than that in aortic stenosis patients late after valve replacement. Thus, eccentric remodeling seems to be worse than concentric remodeling.

Clinical Implications
Severe AR is a serious condition complicated by excess mortality and high morbidity; even when valve replacement is timely, regression of eccentric hypertrophy is often delayed, and LV remodeling is incomplete. Previous studies have indicated that microvascular dysfunction with changes in extracellular matrix may be responsible for adverse responses to chronic volume overload involving myocardial fibrosis with disproportionate increases in noncollagen tissue with changes in fibronectin gene expression and synthesis. In the present study, we observed similar changes with an increase in interstitial fibrosis and a persistent myocardial fiber hypertrophy that persisted 7 to 10 years after AVR. This indicates delayed or incomplete regression of the collagen tissue, which is different from that...
in chronic pressure overload. These changes are paralleled by alterations in diastolic but not systolic function, ie, a persistent increase in diastolic stiffness (Figure 1) late after valve replacement. The residual hypertrophy at the microscopic but not macroscopic level suggests persistent muscle fiber overload, although loading conditions are normal. This may be attributed to the mild residual pressure gradient associated with the artificial valve prosthesis.

**Fibrosis and Myocardial Stiffness**

Because structural changes of the myocardium are associated with functional alterations of the heart, we plotted interstitial fibrosis versus myocardial stiffness and found a positive correlation; ie, the higher the stiffness is, the higher the myocardial fibrosis is \( r = 0.596, P = 0.002 \). Interstitial fibrosis is negatively correlated to LV ejection fraction \( r = -0.570, P = 0.004 \) but not to LV hypertrophy (Figure 6). Thus, the more fibrosis there is, the stiffer the myocardium is and the lower the LV ejection fraction becomes.

**Study Limitations**

One limitation of the present study is the small number of patients included in the analysis. This could have some influence on the statistical power of variables. However, the study protocol is complicated, including recatheterizations and endomyocardial biopsies over several years. The same patient had to undergo 3 different catheterizations, 1 or 2 of which were carried out on an ambulatory basis. All patients gave written informed consent to participate in the study, and no complications occurred in any patients. The family physician of each patient was contacted and informed about the nature of the procedure.

The number of patients is relatively small because of the invasive nature of the present study. Over a similar observation period, 8 to 10 patients with severe AR and without concomitant coronary artery disease were operated each year.21 Our study population was recruited from these patients and followed for up to 7 years. These patients represent roughly 5%–10% of patients undergoing AVR for severe AR in the time period studied.

The number of biopsy specimens obtained in a given patient per cardiac catheterization was 2 to 4 in the present study. It has previously been shown7 that at least 5 biopsy samples are necessary to establish correlations between ventricular structure and function in patients with cardiomyopathies. However, in patients with AR, morphological changes in the myocardium are more or less uniformly distributed over the entire ventricle,22 so 4 biopsy samples should adequately reflect myocardial collagen and myocyte size in this select cohort of patients. Although the relation between function and structure (cause and effect) is not known, our correlations suggest that relaxation is influenced by the extent of hypertrophy,22 whereas passive elastic properties are correlated to the changes of the nonmuscular tissue.

Only patients with severe AR were selected for evaluation. LV end-diastolic volume was severely increased with significant dilatation of the left ventricle (Table 2). Stroke volume was more doubled in preoperative patients (2.3 times normal) compared to control subjects.

Postoperative follow-up was not standardized, but 7 of 11 patients were restudied 2 years and 9 were restudied 7 years after AVR. The other 6 patients refused restudy at the allotted time.

**Conclusions**

Persistent diastolic dysfunction can be observed late after AVR in patients with chronic AR. This finding can be explained by the incomplete regression of the extracellular matrix late after AVR because interstitial fibrosis remained unchanged compared to the preoperative situation. Persistent diastolic dysfunction with maintained systolic ejection performance has been shown to be associated with increased filling pressures during strenuous exercise17 and signs of exertional dyspnea.

**Acknowledgment**

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**Disclosures**

None.
Patients with severe aortic regurgitation show eccentric left ventricular hypertrophy and structural changes of the myocardium. Reversibility of functional and structural changes after successful valve replacement may be limited. Persistent diastolic dysfunction was observed in the present study late after aortic valve replacement. This finding is explained by incomplete regression of the extracellular matrix 7 years after valve replacement. Interstitial fibrosis remains unchanged compared with the preoperative situation but was increased early after operation as a result of the reduction in left ventricular muscle mass. Regression of left ventricular hypertrophy was 38% after 2 years and 55% after 7 years of valve replacement. Myocardial muscle fibers decreased slightly but remained hypertrophied even late after operation. Interstitial fibrosis was found to be positively correlated to myocardial stiffness and inversely correlated to left ventricular ejection fraction. Thus, persistent diastolic dysfunction with maintained systolic ejection performance can be observed late after successful valve replacement in patients with severe aortic regurgitation.
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