Causes and consequences of cardiac fibrosis in patients referred for surgical aortic valve replacement

Arnault Galat¹,²,³,⁴†, Aziz Guellich¹,²,³,⁴†, Diane Bodez¹,²,³,⁴, Larissa Lipskaia¹,³,⁴, Stéphane Moutereau¹,⁵, Eric Bergoend¹,³,⁶, Sophie Huel¹,⁷, Julien Ternacle¹,²,³,⁴, Dania Mohty⁸, Jean-Luc Monin¹,²,³,⁴, Geneviève Derumeaux¹,²,³,⁴, Costin Radu¹,²,³,⁶ and Thibaud Damy¹,²,³,⁴,⁹*

¹UPEC, AP-HP, Henri-Mondor Teaching Hospital, Créteil, France; ²Department of Cardiology, AP-HP, Henri-Mondor Teaching Hospital, Créteil, France; ³Département Hospitalo-Universitaire Ageing Thorax-Vessels-Blood (DHU ATVB), Créteil, France; ⁴Department of Biochemistry, AP-HP, Henri-Mondor Teaching Hospital, Créteil, France; ⁵Department of Cardiovascular Surgery, AP-HP, Henri-Mondor Teaching Hospital, Créteil, France; ⁶Department of Immunology, AP-HP, Henri-Mondor Teaching Hospital, Créteil, France; ⁷Department of Cardiology, Dupuytren Hospital, CHU Limoges, Pôle Cœur-Poumon-Rein, Limoges, France; ⁸Inserm, Clinical Investigation Centre 1430, AP-HP, Henri Mondor Teaching Hospital, Créteil, France

Abstract

Aims Cardiac fibrosis is associated with left ventricular (LV) remodelling and contractile dysfunction in aortic stenosis (AS). The fibrotic process in this condition is still unclear. The aim of this study was to determine the role of both local and systemic inflammation as underlying mechanisms of LV fibrosis and contractile dysfunction. The diagnostic values of 2D-strain echocardiography and serum biomarkers in the evaluation of cardiac fibrosis in this condition were assessed through correlation analyses.

Methods and results Patients with AS referred for surgical valve replacement were prospectively and consecutively included. They all had a comprehensive echocardiography including 2D strain. Blood samples were collected to measure cytokines and inflammatory biomarkers using Luminex bead-based assays. A per-surgical myocardial biopsy of the basal antero-septal segment (S1) was performed. Serial sections of each biopsy were stained with Sirius red. Digital image analysis was used to quantify fibrosis. Immunostainings using specific antibodies against macrophage, glycoprotein (gp) 130, and interleukin 6 (IL-6) were also performed. Patients were divided into tertiles reflecting the severity of fibrosis: mild, moderate, and severe load (TF1 to TF3). The mean age of the S8 included patients was 73 ± 11 years. Twenty-four (43%) were in New York Heart Association III–IV. Mean aortic valve area was 0.8 ± 0.2 cm². Mean aortic stenosis peak velocity and mean gradient were respectively 4.5 ± 0.8 m/s and 54 ± 15 mmHg. The mean LV ejection fraction was 54 ± 12%, and the global LV longitudinal strain was −15 ± 4%. The mean S1 strain, corresponding to the biopsied region, was −10 ± 6% and was strongly correlated to fibrosis load (R = 0.83, P < 0.0001). TF3 was associated with higher mortality (P = 0.009), higher serum C-reactive protein and IL-6, and lower gp130 compared with the other tertiles (P < 0.05). IL-6 and gp130 were expressed in the heart and respectively in the plasma membrane of macrophages and in the cytoplasm of both macrophages and cardiomyocytes. During follow-up, three patients died and were all in the third fibrosis tertile.

Conclusions We found a positive correlation between elevated inflammatory markers and degree of fibrosis load. These two parameters were associated with worse outcomes in patients with severe AS. Our results may be of interest especially in patients for whom a transcatheter aortic valve implantation is indicated and myocardial biopsy is not possible. Strategies aiming at preventing inflammation might be considered to decrease or limit the progression of cardiac fibrosis in patients followed for AS.

Keywords Aortic stenosis; Cardiac fibrosis; Inflammation; IL-6

Received: 17 September 2018; Revised: 2 March 2019; Accepted: 21 April 2019

*Correspondence to: Thibaud Damy, Department of Cardiology, Henri Mondor University Hospital, 51 Av. de Lattre de Tassigny, Créteil 94000, France. Tel: +33 149 812 253; Fax: +33 149 812 805. Email: thibaud.damy@gmail.com

†The two first authors have contributed equally to this work.

© 2019 The Authors. ESC Heart Failure published by John Wiley & Sons Ltd on behalf of the European Society of Cardiology. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
Introduction

Aortic stenosis (AS) is the leading cause of valvular heart disease worldwide. Its prevalence increases substantially with age, reaching 25% in 75 year olds.\(^1\) AS is caused by limited opening of the aortic valve due to restricted leaflets motion that obstructs left ventricular (LV) outflow. In the long term, this leads to LV hypertrophy and tissue remodelling such as cardiac fibrosis.\(^2\)

AS is associated with poor prognosis in symptomatic patients. Aortic valve replacement (AVR) is the only effective treatment. It can be achieved by surgery or by transcatheter AVR in symptomatic patients at high surgical risk.\(^1\) Several studies have shown the beneficial effect this replacement has on LV function, functional status, and survival.\(^2\) However, survival and quality of life varies from patient to patient and depends on the preoperative status of the heart. Different studies have indicated that fibrosis could play a role as an underlying determinant of outcomes; i.e. improving functional status and survival after AVR for AS depends on the fibrotic load of the myocardium.\(^3,4\) However, there is still not much research on the mechanisms underlying myocardial fibrosis in humans and how they might affect regional longitudinal function.

Cardiac fibrosis involves multiples pathways such as hormonal, mechanical, and inflammatory mechanisms.\(^5\) Inflammation has been shown to be involved in the remodelling of the aortic valve.\(^5\) In response to inflammation, fibroblasts proliferate in the heart and differentiate to myofibroblasts. This results in the excessive production and deposition of extracellular matrix proteins. In addition to this, intercellular interactions between myofibroblasts and cardiomyocytes contribute to the adverse structural and functional abnormalities observed in different heart conditions including AS.\(^6\)

Among inflammatory mediators, interleukin 6 (IL-6) is one of the major cytokines involved in valvular calcification and AS progression.\(^7\) IL-6 seems to also play a critical role in cardiac remodelling as a response to pressure overload. In a mouse model, deleting IL-6 has been shown to attenuate LV fibrosis and prevent excessive remodelling and LV dysfunction.\(^8\) Prior studies have shown that pro-inflammatory cytokines such as IL-6 are elevated in chronic heart failure and associated with poor functional status and poor prognosis.\(^9\) In various diseases, different components of the IL-6/glycoprotein (gp) 130 receptor signalling pathway are impaired and fibrosis increases.\(^10\) The role of IL-6/gp130 receptor signalling pathways in myocardial fibrosis and its impact on contractile function are not well elucidated.

The aim of this study was to investigate the correlation between myocardial fibrosis and regional contractile dysfunction and to highlight the link between systemic and local inflammation and myocardial fibrosis in LV dysfunction in AS.

Methods

Patient population and follow-up

Starting in December 2013 to October 2014, eligible patients with severe AS undergoing surgical AVR at the Henri Mondor Teaching Hospital were prospectively and consecutively included in the study. We enrolled patients who met the following criteria for inclusion: severe aortic stenosis (aortic-valve area \(\leq 1\) cm\(^2\) on echocardiography) and indication for surgical AVR according to the current recommendations validated by the heart team. Fifty-eight patients with severe aortic stenosis were included. Clinical follow-up was carried out either by clinical visits or by phone calls.

The investigation conforms with the principles outlined in the Declaration of Helsinki. The protocol was approved by the institutional review board of Ile-de-France VI (CPP 05/09/2013). All participants gave written informed consent.

Echocardiography

Echocardiography was carried out during the month preceding surgery using a Vivid E9 system (GE Vingmed, Horten, Norway). All acquisitions were recorded over three consecutive cycles. Data were analysed offline using ECHOPac software (GE Healthcare). LV dimensions as well as septal and posterior wall thicknesses were measured from M-mode parasternal long-axis views. LV ejection fraction (LVEF) was measured using the Simpson biplane method from two-chamber and four-chamber apical views. LV mass was determined from M-mode echocardiograms. Tricuspid and mitral annular plane systolic excursion were obtained from the M-mode apical four-chamber view. Mitral inflow recordings were obtained with pulsed Doppler and used to quantify early and late wave peak velocities and the early-to-late diastolic flow ratio. Tissue Doppler imaging at the lateral and septal mitral annulus was performed to measure the ratio between early transmitral flow and mean lateral and septal early diastolic peak tissue velocity used as an estimate of LV filling pressure. Systolic pulmonary arterial pressure was estimated by applying the modified Bernoulli equation. Longitudinal strain (LS) was computed from the standard LV apical views (two, three, and four chambers) using 2D speckle-tracking echocardiography analysis by automated function imaging (ECHO-Pac, GE Healthcare). The LS values, as defined by the American Heart Association, were obtained from each of the 17 LV segments. LV global LS was also measured as the average of all the 17 segments for each patient.
**Biopsy, histology, and immunostaining**

After aortic cross-clamping, an aortotomy and excision of aortic valve leaflets, a myocardial biopsy of the basal LV was performed, and the sample was immediately fixed in formalin and processed for histological analysis. Fibrosis measurements were not taken for 23 patients: 8 patients had confounding factors (myocardial infarction, n = 3; severe mitral regurgitation associated with AS, n = 4; and cardiac amyloidosis, n = 1). Three patients had chronic kidney disease stage ≥4 making the biomarker analysis inaccurate, 8 patients had an inconclusive biopsy (small or limited to the endocardium), and 4 patients could not have a septal biopsy (judged too risky or ethically unacceptable by the surgeon).

Paraffin-embedded 3-μm-thick sections were cut and stained with Sirius red. Images covering the whole section were taken at ×10 magnification using an optical microscope equipped with an AxioCam MRm camera. Fibrosis was quantified using ImageJ software (NIH) and expressed as % of the total area of the section. Groups were divided into tertiles of fibrosis (TF1: mild; TF2: moderate; and TF3: severe fibrosis load). Triple immunostaining was performed using the following primary antibodies (mouse monoclonal anti-IL-6 antibodies, 1/100, Santa Cruz; rabbit polyclonal anti-gp130 antibodies, 1/50, Santa Cruz; and rat anti-macrophages/macrophages/monocytes antibodies, 1/50, Merck Millipore), and the corresponding secondary antibodies conjugated to Alexa-660, Alexa-555, and Alexa-488, respectively. DAPI was used to visualize nuclei. Slides were viewed on a Zeiss 510 Meta confocal microscope in multi-tracking mode.

**Blood collection and biomarkers**

Venous blood was acquired in the month prior to surgery. Blood samples were centrifuged immediately at 3000 rpm for 10 min, and serum was stored as aliquots at −80°C until time of assay. A Luminex bead-based assay was designed (Bio-tech, R&D systems) to measure serum mediators potentially involved in the fibrosis process including IL-6, gp130, ST2, osteopontine, and periostone. Galactin-3 was measured using a human ELISA kit (Abcam) according to the manufacturer’s instructions. Usual biomarkers were measured using turbidimetry [C-reactive protein (CRP)] or an automatized immunochromiluminescence assay (N terminal pro brain natriuretic peptide, troponin). All kits were from Roche (Roche™, Meylan, France).

**Statistical analysis**

Continuous variables are expressed as mean ± standard deviation. For exponential continuous variables, values were expressed as the median (25th and 75th percentiles). Dichotomous variables were expressed as absolute values and percentages. For continuous variables, a Kruskal–Wallis test was used when comparing more than two groups. Pearson correlation coefficient (r) was used to investigate relationships between parameters. P-values less than 0.05 were considered significant. The diagnostic performance of IL-6 to predict a high fibrosis load (TF3) was measured by calculating the area under the receiver-operating characteristic curve (AUC). A Kaplan–Meier analysis and log rank test were used to compare curves of patients with mild (T1) and moderate (T2) fibrosis vs. those with severe fibrosis (TF3). All statistical analyses were performed using SPSS software (SPSS v19.0 for Windows 2010 Inc.).

**Results**

**Characteristics and cardiac function of patients undergoing surgical AS**

Fifty-eight patients with severe AS undergoing surgical AVR were included. Their characteristics are outlined in Table 1. Thirty-three (59%) were men. The mean age was 73 ± 11 years. Twenty-four (43%) patients were in New York Heart Association class III–IV, 32 (57%) had hypertension, 27 (48%) had a history of ischaemic heart disease, and 4 (7%) had atrial fibrillation. The median (25th and 75th quartiles) of N terminal pro brain natriuretic peptide was 613 (264; 1208) ng/L and of high sensitive cardiac troponin T (Hs-cTnT) was 13 (9; 25) ng/L. The average of peak aortic velocity was 4.5 ± 0.8 m/s, and the mean gradient was 54 ± 15 mmHg for a mean aortic valve area of 0.8 ± 0.2 cm². The mean LVEF was 54 ± 12%. Global LV-LS was −15 ± 4%. It was −10 ± 6% in the basal antero-septal segment (S1) corresponding to the biopsied and fibrosis-quantified area.

**Cardiac fibrosis quantification**

Correlations between amount of cardiac fibrosis and LV systolic and diastolic parameters, LV remodelling, and aortic stenosis severity are shown in Table 2. Both global LV (LVEF and LV-LS) and regional (S1-LS) dysfunction were significantly correlated to the amount of fibrosis (Table 2 and Figure 2). There was no difference in the severity of aortic stenosis between the tertiles. However, LVEF, global LV-LS basal, mid and S1 segment strain were significantly lower in the highest tertile (Table 3).
As summarized in Table 4, TF3 patients had higher serum levels of inflammatory markers (including IL-6 and CRP) than those in the other tertiles. gp130 serum was lower in TF3 patients when compared with the other tertiles. Serum IL-6 was good at distinguishing patients with high fibrotic load (TF3) from TF1 and TF2 [AUC 0.74, 95% confidence interval (0.56–0.93)]. Interestingly, IL-6 and gp130 were highly expressed in the heart and respectively in the plasma membrane of macrophage and in the cytoplasm of both macrophages and cardiomyocytes as shown in Figure 2.

**Discussion**

This prospective study provided us with new insights into the underlying mechanisms of cardiac remodelling, which lead to heart failure in patients with AS. We showed that there was a correlation between cardiac fibrotic load and the severity of cardiac contractile dysfunction and that this was associated with poor outcome but not the severity of AS. We also showed that there was a correlation between systemic inflammation (IL-6 and CRP) and myocardial fibrosis. Local IL-6 and gp130 could have played a role in this, as they were expressed in macrophages and cardiomyocytes. These data highlight the link between fibrosis and inflammation in LV remodelling and dysfunction in patients with AS.

---

**Table 1** Clinical, electrocardiological, echocardiographic, and biological characteristics of patients undergoing surgical AS

| Characteristics | All patients (N = 58) |
|-----------------|-----------------------|
| **Clinical data** |                       |
| Age (years)     | 73 ± 11               |
| Gender, men, n (%) | 33 (59)            |
| IHD, n (%)      | 27 (48)               |
| MI, n (%)       | 3 (5)                 |
| Diabetes, n (%) | 18 (32)               |
| Hypertension, n (%) | 32 (57)     |
| HR (bpm)        | 71 ± 11               |
| Systolic blood pressure (mmHg) | 129 ± 9          |
| Diastolic blood pressure (mmHg) | 70 ± 5           |
| NYHA III-IV vs. I-II, n (%) | 24 (43)       |
| **Medications** |                       |
| Beta-blockers   | 23 (41)               |
| Angiotensin II receptor blocker | 17 (30)      |
| Angiotensin-converting enzyme inhibitor | 14 (25) |
| Diuretics       | 28 (50)               |
| **ECG**         |                       |
| PR duration (ms) | 169 ± 36             |
| QRS duration (ms) | 103 ± 33           |
| AF, n (%)       | 4 (7)                 |
| **Echocardiography** |                   |
| LVEF (%)        | 54 ± 12               |
| LV-LS (%)       | −15 ± 4              |
| S1-LS (%)       | −10 ± 6              |
| Strain, basal (%) | −12 ± 6            |
| Strain, mid (%) | −13 ± 6              |
| Strain, apical (%) | −17 ± 8           |
| ITV (cm)        | 21 ± 4               |
| IVST (mm)       | 13 ± 2               |
| LVM indexed (g/m²) | 139 ± 45         |
| E/e             | 12 ± 8               |
| sPAP (mmHg)     | 32 ± 12              |
| MAPSE (mm)      | 21 ± 5               |
| Mean GP (mmHg)  | 54 ± 15              |
| Vmax (cm/s)     | 4.5 ± 0.8            |
| AVA (cm²)       | 0.8 ± 0.2            |
| **Biology**     |                       |
| NT-proBNP (ng/L) | 613 (264; 1208)     |
| Hs-CtTnT (ng/L) | 13 (9; 25)           |
| Creatinine (µmol/L) | 86 ± 27       |
| CRP (mg/L)      | 3.6 ± 5.7            |

AF, atrial fibrillation; AVA, aortic valve surface area; GP, gradient pressure; HR, heart rate; Hs-CtTnT, high sensitive cardiac troponin T; IHD, ischaemic heart disease; IVST, interventricular septum thickness; LVEF, left ventricular ejection fraction; LV-LS, left ventricular longitudinal strain; LVM, left ventricular mass; MI, myocardial infarction; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; S1-LS, longitudinal strain of segment 1 according to the American Heart Association model (the segment from where the biopsy has been taken); sPAP, systolic pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion.

Continuous variables are expressed as mean ± standard deviation and exponential variables as median (25th and 75th percentiles).
Fibrosis is the result of a variety of molecular processes in response to chronic pressure overload in AS where fibroblasts play a central role. Fibroblasts are present in the healthy myocardium and are essential for the regulation of the extracellular matrix to ensure normal heart function. In AS, decreased myocardial perfusion, increased systolic wall stress, oxidative stress, inflammation, geometric chamber alterations, and myocardial cellular structure modifications can all cause fibroblasts to turn into myofibroblasts. These cells alter the structure and function of cardiomyocytes through direct cell contact or indirectly via the extracellular matrix. They do this by replacing the contractile tissue with fibrosis as well as by releasing soluble mediators. Fibrosis is also recognized as an independent predictor of cardiac events following AVR.

Our study used endomyocardial biopsies to confirm these previous reports. Patients with a greater amount of fibrosis had the worst prognosis and lower contractile function recovery. Indeed, LV mass regression due to normalization of myocytes’ size is expected to happen early after the relief of the pressure overload following AVR. However, remodelling of the interstitial collagen matrix might take more time or even be irreversible if diastolic stiffness persists and myocyte contractility and electrophysiological properties alter due to fibroblast-derived mediators. Our study suggests that systemic inflammation (IL-6 and CRP) is associated with higher interstitial fibrosis load. Indeed, fibrosis could cause IL-6 to be released, in turn, IL-6 secretion could be a cause of fibrosis, creating a vicious circle. The link between IL-6 and CRP has been observed in acute myocardial infarction and heart failure. Both markers have been associated with higher cardiac-related mortality as well as with later clinical events in patients with coronary artery disease. IL-6 is known to promote activation and infiltration of mononuclear leukocytes and to induce the differentiation of monocytes into macrophages. The membrane-anchored form of gp130 is associated with IL-6R when IL-6 is present and is involved in the transduction of IL-6 signal. It has been suggested that soluble forms of gp130 have pleiotropic properties and may also have anti-inflammatory effects as they act as an inhibitor of the IL-6R/IL-6 complex. These forms could negatively regulate the IL-6 signal by binding the soluble serum IL-6R and the IL6 in a ternary complex. Thus, negative correlation of sgp130 and serum IL6 is as one would expect: serum sgp130 is supposed to eliminate systemic IL-6. Cardiac-restricted gp130 deficiency induced a pathological ventricular dilation on acute pressure overload. On the other hand, constitutive activation of gp130 induces myocardial hypertrophy. In a recent study, higher
levels of gp130 were suggested to be beneficial and protective in myocardial infarction. However, sustained gp130 activation may also cause excess inflammation, LV rupture, and heart failure after myocardial infarction. Therefore, it seems that unbalanced gp130 activity leads to maladaptation and heart failure.

In our study, myocardial immunostaining analyses revealed that IL-6 was localized in the macrophages and gp130 was expressed on both macrophages and cardiomyocytes’ plasma membranes, which supports the idea that there is a local interaction between both cells. Increased expression of cardiac gp130 seemed to have a correlation with macrophage infiltration of cardiac tissue, indicating inflammatory phenotype, in concordance with elevated systemic IL-6 level. These data also suggest that macrophages are involved in myocardial inflammation and might also have a direct impact on cardiomyocyte function. The paracrine interaction between fibroblasts and cardiomyocytes has been seen in the heart’s response to pressure overload in murine model, and various mediators, including IL-6, have been linked to the paracrine effects of cultured fibroblasts. Our results are the first to support this theory about AS in humans. The two major intracellular signalling pathways for IL-6 already found in other diseases are (i) through a cytokine-specific receptor complex with at least one subunit of the signal-transducing protein gp130 and (ii) the trans-signalling pathway involving

Table 3 Comparison of echocardiographic parameters and myocardial interstitial stratified by tertiles

| Variables                          | Interstitial fibrosis tertiles |
|------------------------------------|------------------------------|
|                                    | T1    | T2   | T3    | P     |
| Intertitial fibrosis (%)           | <15.5 | 15.5–19.2 | >19.2 |       |
| N                                  | 12    | 12   | 11    |       |
| Clinical parameters                |       |      |       |       |
| Age (years)                        | 73 ± 10 | 74 ± 12 | 69 ± 15 | 0.809 |
| Gender, male, n (%)                | 7 (58) | 8 (67) | 6 (55) | 0.830 |
| BP (mmHg)                          | 132 ± 11 | 132 ± 14 | 120 ± 16 | 0.115 |
| NYHA III–IV vs. I–II, n (%)        | 5 (42) | 6 (50) | 3 (27) | 0.534 |
| Systolic function                  |       |      |       |       |
| Global LV-LS (%)                   | –18 ± 2 | –15 ± 4 | –13 ± 4 | 0.009 |
| LS-S1 (%)                          | –15 ± 2 | –11 ± 4 | –4 ± 4  | 0.0001 |
| Basal LV-LS (%)                    | –17 ± 3 | –12 ± 4 | –9 ± 7  | 0.001 |
| Mid LV-LS (%)                      | –18 ± 2 | –14 ± 4 | –12 ± 5 | 0.007 |
| Apical LV-LS (%)                   | –21 ± 5 | –19 ± 6 | –16 ± 7 | 0.200 |
| LVEF (%)                           | 62 ± 5  | 52 ± 10 | 52 ± 14 | 0.025 |
| MAPSE (mm)                         | 1.4 ± 0.2 | 1.2 ± 0.2 | 1.2 ± 0.2 | 0.213 |
| SVI (mL/m²)                        | 45 ± 7  | 44 ± 9  | 43 ± 15 | 0.820 |
| Hypertrophy                        |       |      |       |       |
| IVST (mm)                          | 14 ± 2  | 13 ± 2  | 13 ± 1  | 0.281 |
| LVM (g)                            | 257 ± 92 | 259 ± 91 | 257 ± 72 | 0.994 |
| Aortic valve parameters            |       |      |       |       |
| AVA (cm²)                          | 0.7 ± 0.1 | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.338 |
| Mean GP (mmHg)                     | 53 ± 14 | 51 ± 13 | 56 ± 13 | 0.674 |
| Vmax (m/s)                         | 4.4 ± 0.6 | 4.4 ± 0.5 | 4.6 ± 0.5 | 0.808 |

Abbreviations as in Tables 1 and 2. P < 0.05, which means statistical significance.

Table 4 Comparison of serum markers according to interstitial fibrosis divided into tertiles in AS

| Variables                          | Interstitial fibrosis |
|------------------------------------|-----------------------|
|                                    | T1    | T2   | T3    | All  |
| Intertitial fibrosis (%)           | <15.5 | 15.5–19.2 | >19.2 |       |
| N                                  | 12    | 12   | 11    |       |
| CRP (mg/L)                         | 1.6 ± 1.2 | 3.1 ± 4.0 | 7.2 ± 11 | 0.031 |
| Hs-cTnT (ng/L)                     | 10 (8; 13) | 11 (9; 16) | 13 (9; 17) | 0.182 |
| NT-proBNP (ng/L)                   | 268 (144; 345) | 495 (152; 928) | 865 (248; 1250) | 0.053 |
| NT-proBNP (ng/L)                   | 268 (144; 345) | 495 (152; 928) | 865 (248; 1250) | 0.053 |
| ST2 (ng/mL)                        | 12.8 ± 4.6 | 12.9 ± 4.0 | 13.3 ± 5.9 | 0.977 |
| IL-6 (pg/mL)                       | 2.39 ± 0.9 | 7.8 ± 10.3 | 7.1 ± 8.0 | 0.014 |
| gp130 (ng/mL)                      | 131 ± 14 | 128 ± 8 | 106 ± 29 | 0.049 |
| Galectin-3 (ng/mL), N = 34         | 3.1 ± 1.2 | 3.9 ± 0.9 | 3.5 ± 1.6 | 0.529 |
| Periostin (ng/mL)                  | 104 ± 27 | 123 ± 54 | 81 ± 38 | 0.053 |
| Osteopontin (ng/mL)                | 23 ± 8  | 25 ± 13 | 36 ± 19 | 0.370 |

CRP, C-reactive protein; Hs-cTnT, high sensitive cardiac troponin T; gp130, glycoprotein 130; IL-6, interleukin 6; NT-proBNP, N terminal pro brain natriuretic peptide. Continuous variables are expressed as mean ± standard deviation and exponential variables as median (25th and 75th percentiles). P < 0.05, which means statistical significance.
soluble IL-6R that enables interaction with gp130. In our study, serum gp130 levels were lower in the severe cardiac fibrosis group. However, cardiac gp130 staining was detected at a significantly greater intensity in the severely fibrotic group. gp130 signalling mediates several cellular processes including cell survival, apoptosis, growth, proliferation, differentiation, and survival signalling through the gp130/Jak/STAT pathway. Therefore, targeting the IL-6 pathway could be a new therapeutic strategy to slow cardiac fibrosis accumulation and LV dysfunction and to improve post-AVR outcome. IL-6R antagonists, such as the monoclonal antibody tocilizumab, are already used in other inflammatory diseases.

The limitations of our study are that we used per-surgical biopsies that were limited to specific areas of the endomyocardium, not necessarily representative of the whole wall’s thickness. Another limitation is the study’s small sample size. The full signalling pathways and molecular mechanisms were not detailed in this study. Further experimental investigations are needed to explain our findings. A particular polymorphism in the promoter region of IL-6 was shown to lead to higher systemic levels of IL-6. This was not investigated in the present study. Only three patients died during follow-up. However, they were all from the TF3 group. Large-scale studies with longer follow-up are warranted to confirm our results and to perform Cox proportionate multivariable analyses.

In conclusion, myocardial fibrosis load is correlated with the serum level of IL-6 and gp130, and both proteins are expressed in the heart. Local cardiac inflammation may be a...
key mechanism in the pathophysiology of cardiac fibrosis and LV dysfunction in AS. Post-AVR outcome strongly depends on the preoperative risk profile, which is dictated at least in part by cardiac fibrotic load and the inflammatory status. Further investigations are necessary to explore the molecular mechanisms underlying cardiac fibrosis deposits, especially those involving macrophage. IL-6 could be used as a biomarker of cardiac remodelling, which could in turn improve monitoring of AS patients and determine the optimal timing for AVR. It could also be considered as a therapeutic target to prevent cardiac fibrosis in AS.

Acknowledgements

The authors would like to thank the nurses in the heart failure unit and the cardiovascular surgery department for their invaluable work.

References

1. Joint Task Force on the Management of Valvular Heart Disease of the European Society of C, European Association for Cardio-Thoracic S, Vahanian A, Alferi O, Andreotti F, Antunes MJ, Baron-Esquivias G, Baumgartner H, Borger MA, Carrel TP, De Bonis M, Evangelista A, Falk V, Jang B, Lancellotti P, Pierard L, Price S, Schafers HJ, Schuler G, Stepinska J, Swedberg K, Takkenberg J, Von Oppell UO, Windecker S, Zamorano JL, Zembala M. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J 2012; 33: 2451–2496.

2. 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: 90–95.

3. Weidemann F, Herrmann S, Stork S, Niemann M, Frantz S, Lange V, Beer M, Gattenlohner S, Voelker W, Ertl G, Strotmann JM. Impact of myocardial fibrosis in patients with symptomatic severe aortic stenosis. Circulation 2009; 120: 577–584.

4. Azevedo CF, Nigri M, Higuchi ML, Pomerantzeff PM, Spina GS, Sampaio RO, Tarasoutchi F, Grinberg M, Rochitte CE. Prognostic significance of myocardial fibrosis quantification by histopathology and magnetic resonance imaging in patients with severe aortic valve disease. J Am Coll Cardiol 2010; 56: 278–287.

5. Nicoletti A, Michel JB. Cardiac fibrosis and inflammation: interaction with hemodynamic and hormonal factors. Circ Res 1999; 84: 532–543.

6. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. Circulation 2013; 128: 388–400.

7. Fox CS, Guo CY, Larson MG, Vasan RS, Parise H, O’Donnell CJ, D’Agostino RB Sr, Keaney JF Jr, Benjamin EJ. Relations of inflammation and novel risk factors to valvular calcification. Am J Cardiol 2006; 97: 1502–1505.

8. Zhao L, Cheng G, Jin R, Afzal MR, Samanta A, Xuan YT, Girgis M, Elias HK, Zhu Y, Davani A, Yang Y, Chen X, Ye S, Wang OL, Chen L, Hauptman J, Vincent RJ, Dawn B. Deletion of interleukin-6 attenuates pressure overload-induced left ventricular hypertrophy and dysfunc tion. Circ Res 2016; 118: 1918–1929.

9. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesarinonone trial (VEST). Circulation 2001; 103: 2055–2059.

10. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011; 1813: 878–888.

11. Souders CA, Bowers SL, Baudino TA. Cardiac fibroblast: the renaissance cell. Circ Res 2009; 105: 1164–1176.

Conflict of interest

None declared.

Funding

This work was supported by the Henri-Mondor Teaching Hospital, Créteil. Dr Galat is a recipient of fellowship from ‘Fondation pour la Recherche Médicale’.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Pre-operative characteristics of the three patients who died during follow-up after AVR.

12. Kakkar R, Lee RT. Intramyocardial fibroblast-myocyte communication. Circ Res 2010; 106: 47–57.

13. Villari B, Vassalli G, Betocchi S, Briguori C, Chiariello M, Hess OM. Normalization of left ventricular nonuniformity late after valve replacement for aortic stenosis. Am J Cardiol 1996; 78: 66–71.

14. Vasquez C, Mohandas P, Louie KL, Benamer N, Bapat AC, Morley GE. Enhanced fibroblast-myocyte interactions in response to cardiac injury. Circ Res 2010; 107: 1011–1020.

15. Tan J, Hua Q, Li J, Fan Z. Prognostic value of interleukin-6 during a 3-year follow-up in patients with acute ST-segment elevation myocardial infarction. Heart Vessels 2009; 24: 329–334.

16. Hirota H, Izumi M, Hamaguchi T, Sugiyama S, Murakami E, Kunisada K, Fujiyo Y, Oshima Y, Nakaoka Y, Yamachi-Takahara K. Circulating interleukin-6 family cytokines and their receptors in patients with congestive heart failure. Heart Vessels 2004; 19: 237–241.

17. McLoughlin RM, Hurst SM, Nowell MA, Harris DA, Horiiuchi S, Morgan LW, Wilkinson TS, Yamamoto N, Topley N, Jones SA. Differential regulation of neutrophil-activating chemokines by IL-6 and its soluble receptor isoforms. J Immunol 2004; 172: 5676–5683.

18. Chomarat P, Banchereau J, Davoust J, Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol 2000; 1: 510–514.
19. Jostock T, Mullberg J, Ozbek S, Atreya R, Blinn G, Voltz N, Fischer M, Neurath MF, Rose-John S. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur J Biochem* 2001; 268: 160–167.

20. Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y, Vancopoulou GD, Taga T, Kishimoto T. Soluble forms of the interleukin-6 signal-transducing receptor component gp130 in human serum possessing a potential to inhibit signals through membrane-anchored gp130. *Blood* 1993; 82: 1120–1126.

21. Hirota H, Chen J, Betz UA, Rajewsky K, Gu Y, Ross J Jr, Muller W, Chien KR. Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. *Cell* 1999; 97: 189–198.

22. Hirota H, Yoshida K, Kishimoto T, Taga T. Continuous activation of gp130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice. *Proc Natl Acad Sci U S A* 1995; 92: 4862–4866.

23. Moreno Velasquez I, Golabkesh Z, Kallberg H, Leander K, de Faire U, Gigante B. Circulating levels of interleukin 6 soluble receptor and its natural antagonist, sgp130, and the risk of myocardial infarction. *Atherosclerosis* 2015; 240: 477–481.

24. Hilfiker-Kleiner D, Shukla P, Klein G, Schaefer A, Stapel B, Hoch M, Muller W, Scherr M, Theilmeier G, Ernst M, Hilfiker A, Drexler H. Continuous glycoprotein-130-mediated signal transducer and activator of transcription-3 activation promotes inflammation, left ventricular rupture, and adverse outcome in subacute myocardial infarction. *Circulation* 2010; 122: 145–155.

25. Wollert KC, Drexler H. The role of interleukin-6 in the failing heart. *Heart Fail Rev* 2001; 6: 95–103.

26. Takeda N, Manabe I, Uchino Y, Eguchi K, Matsumoto S, Nishimura S, Shindo T, Sano M, Otsu K, Snider P, Conway SJ, Nagai R. Cardiac fibroblasts are essential for the adaptive response of the murine heart to pressure overload. *J Clin Invest* 2010; 120: 254–265.

27. Taga T, Kishimoto T. Gp130 and the interleukin-6 family of cytokines. *Annu Rev Immunol* 1997; 15: 797–819.

28. Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 1998; 334: 297–314.

29. Dhillon S. Intravenous tocilizumab: a review of its use in adults with rheumatoid arthritis. *BioDrugs* 2014; 28: 75–106.

30. Smith AJ, D’Auito F, Palmen J, Cooper JA, Samuel J, Thompson S, Sanders J, Donos N, Nibali L, Brull D, Woo P, Humphries SE. Association of serum interleukin-6 concentration with a functional IL6-6331T>C polymorphism. *Clin Chem* 2008; 54: 841–850.