UNIVERSITÀ DEGLI STUDI DI PADOVA

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## SCUOLA DI MEDICINA E CHIRURGIA


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## TITOLO TESI

PERCEVAL SUTURELESS PERICARDIAL BIOPROSTHESIS VALVE: CLINICO-PATHOLOGICAL AND EXPERIMENTAL OBSERVATIONS

BIOPROTESI PERICARDICHE SUTURELESS PERCEVAL: ASPETTI CLINICO-PATOLOGICI ED OSSERVAZIONI SPERIMENTALI


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**ABBREVIATIONS**

AS: Aortic Stenosis

AVR: Aortic Valve Replacement

CPB: Cardiopulmonary By-Pass

EOA: Effective Aortic Orifice

MIS: Minimally Invasive Surgery

PPM: Patient-Prosthesis Mismatch

TAVI: Transcatheter Aortic Valve Implantation

TIP: Time in Place

STS: Society of Thoracic Surgeons

SVD: Structural Valve Deterioration

SEM: Scanning Electron Microscope

SAVR: Surgical Aortic Valve Replacement

# SUMMARY


**Background**

Excellent performances have been demonstrated in haemodynamic outcomes, safety, and versatility of use in the sutureless Perceval aortic valve (LivaNova, London, UK). However, several questions remain unanswered, especially regarding the effects of the "collapsing" during the reduction of the dimensions of the bioprostheses before implantation, and long-term durability: the design of this prosthesis closely resembles that of the Freedom Solo stentless prosthesis that was associated with a significant incidence of Structural Valve Deterioration (SVD) in different studies. Our research focused on understanding the impact of the "collapsing" in the pericardial structure and the modality of failure of this bioprosthesis when implanted in humans.

**Materials and methods**

To analyse the collapsing impact, 12 collapsed at 15 min (surgical procedure collapsing time), 60 and 180 min duration, and 4 uncollapsed (controls) LivaNova Perceval S prostheses were morphologically studied. Gross, histology and scanning electron microscopy (SEM) analysis were performed. Multiple sections of pericardial cusps have been stained with Hematoxylin-Eosin (HE), Azan Mallory, Elastic Van Gieson and Picrosirius Red, where a morphometrical analyses was performed by measuring the length of the collagen period.

SVD was investigated in 33 Perceval bioprosthesis explanted in different European centres, from July 2007 to January 2017, participating to PIVOT TRIAL V10601, PIVOTAL TRAIL V10801, and CAVALIER TRIAL TPS001. In all the explants gross, histology (HE, Azan Mallory, Elastic van Gieson, Von Kossa, Gram stains), were performed. To assess a potential reduction of the effective orifice area (EOA) due to fibrous tissue overgrowth, the ratio expressed in percentage between the EOA area and the total area of the bioprosthesis on ventricular side was measured.

**Results**

Gross examination after collapsing and deployment revealed optimal cusp cooptation and absence of tears, perforation or folding. Moreover, prosthetic frame showed a preserved shape without distortion. Histology and SEM exhibited neither breaks nor differences in waviness periodicity of the fibrosa collagen fibers when compared to controls. Collagen wavelength periodicity measurement data did not reveal any statistically significant differences among the study groups (15 min collapse: 16.55±2.89 μm; 60 min collapse: 17.01±3.11 μm; 180 min collapse: 16.45±2.13 μm) and the un-collapsed controls (16.51±2.65 μm) and with un-mounted pericardium (17.47±2.50 μm) (P=NS).

Thirtythree bioprosthesis implanted in humans were examined. Endocarditis was diagnosed in 36% of all, which was similar to that reported for bioprosthesis valves, SVD by dystrophic calcification in 12% (only 4 cases), fibrous pannus overgrowth in 12% and paravalvular leak in 12%. Fibrous tissue overgrowth (on the valve and on the stent) was 61%, with and incidence of almost 83% in the bioprostheses with time in place more than one month. This alteration involved the valve as main

pathology, causing mainly orifice stenosis, or was associated to other failure modalities, as endocarditis, calcific dystrophy, or paravalvular leak. Its distribution was in the valve, in valve and nitinol stent or climbing the sole stent, occluding sometimes the spaces of nitinol network.

**Conclusions**

Pre-implantation collapse and ballooning procedures do not affect the structural integrity of the collagen fibers of the pericardial cusp tissue of Perceval S sutureless valve bioprosthesis.

In 4 cases early SVD by dystrophic calcification occurred at time in place of 5-6 years, questioning the efficacy of the anticalcification treatment of the pericardium. Progressive fibrous tissue overgrowth, invading the valve orifice, was the cause of the bioprosthesis stenosis even in absence of calcific dystrophy and did not spare the stent and nitinol network.

Despite the evolution on new technologies, design and pericardial treatment, the fibrous tissue overgrowth remains a major concern of this new generation bioprostheses.

# 1. AORTIC VALVE DISEASE

## 1.1 AORTIC VALVE STENOSIS

### 1.1.1 Introduction

After decades of relative quiescence, the management of patients with aortic valve (Figure 1) disease is again gathering interest due to several factors. Disease prevalence is increasing as the global population age (1). Many patients with severe symptomatic aortic stenosis remain untreated and many with aortic regurgitation develop left ventricular dysfunction before the onset of symptoms. Finally, the emergence of less-invasive transcatheter aortic valve implantation (TAVI) as an alternative to surgical aortic valve replacement (SAVR) for patients with aortic stenosis has created new opportunities for treatment, although also raising several challenges (2).

### 1.1.2 Pathophysiology

The symptom triad of angina, syncope, and dyspnea represents a late-stage consequence of chronic progressive left ventricular overload caused by worsening aortic stenosis, which usually has developed over several decades. Compensatory changes to maintain cardiac output, including increases in left ventricular wall thickness and contractility, are ultimately overwhelmed, resulting in the typical pathobiology of severely decreased diastolic compliance, sub endocardial ischemia, exhausted myocardial contractile reserve followed by irreversible myocardial fibrosis and baroreceptor-activated vasodilation. These changes contribute to further reductions in cardiac output and pulmonary congestion. Sudden cardiac death, a devastating complication of aortic stenosis, might be the result of a

multifactorial interplay of low cardiac output, ischemia, and arrhythmias culminating in a downward spiral of unrecoverable hypotension (3).

Figure 1. Leonardo da Vinci - The aortic valve, from the Royal Collection © Her Majesty Queen Elizabeth II. (*http://www.royalcollection.org.uk/collection/919082/the-aortic-valve)*

### 1.1.3 Epidemiology

In the past, the most common cause of aortic stenosis worldwide had been rheumatic heart disease; nowadays, degenerative calcific disease of the native tricuspid senile or congenitally bicuspid aortic valve predominates, especially in developed countries (1). The prevalence of aortic stenosis increases with increasing average lifespan, and population-level echocardiography studies estimate that it is moderate or severe in 5% of patients older than 75 years in the USA. The age of symptom onset varies dependent on the cause. People with congenitally bicuspid aortic stenosis might present in their 50-60s, whereas those with senile calcific aortic stenosis can present as late as their 70-80s. Although all forms of aortic stenosis seem to have a male predominance, men and women are similarly distributed among patients older than 75 years (3).

### 1.1.4 Natural history and diagnosis

Ross and Braunwald (4) memorably described the natural history of aortic stenosis in 1968, based on retrospective post-mortem data from patients with mostly rheumatic and calcific bicuspid aortic stenosis. A protracted symptom-free latent period with “increasing obstruction and myocardial overload” was followed by the abrupt onset of severe symptoms (angina, syncope, and dyspnea), with nearly uniform mortality within 5 years of onset (average age 63 years). Several later retrospective studies confirmed this disease course, although the age of symptoms onset and mortality were found to be substantially delayed in senile calcific aortic stenosis. The extremely high early mortality of untreated severe disease was confirmed prospectively in the PARTNER trial (5) which showed 50% mortality at 1 year and more than 90% at 5 years (Figure 2). These findings underscore the need for accurate diagnosis, appropriately timed intervention, and meticulous follow-up.

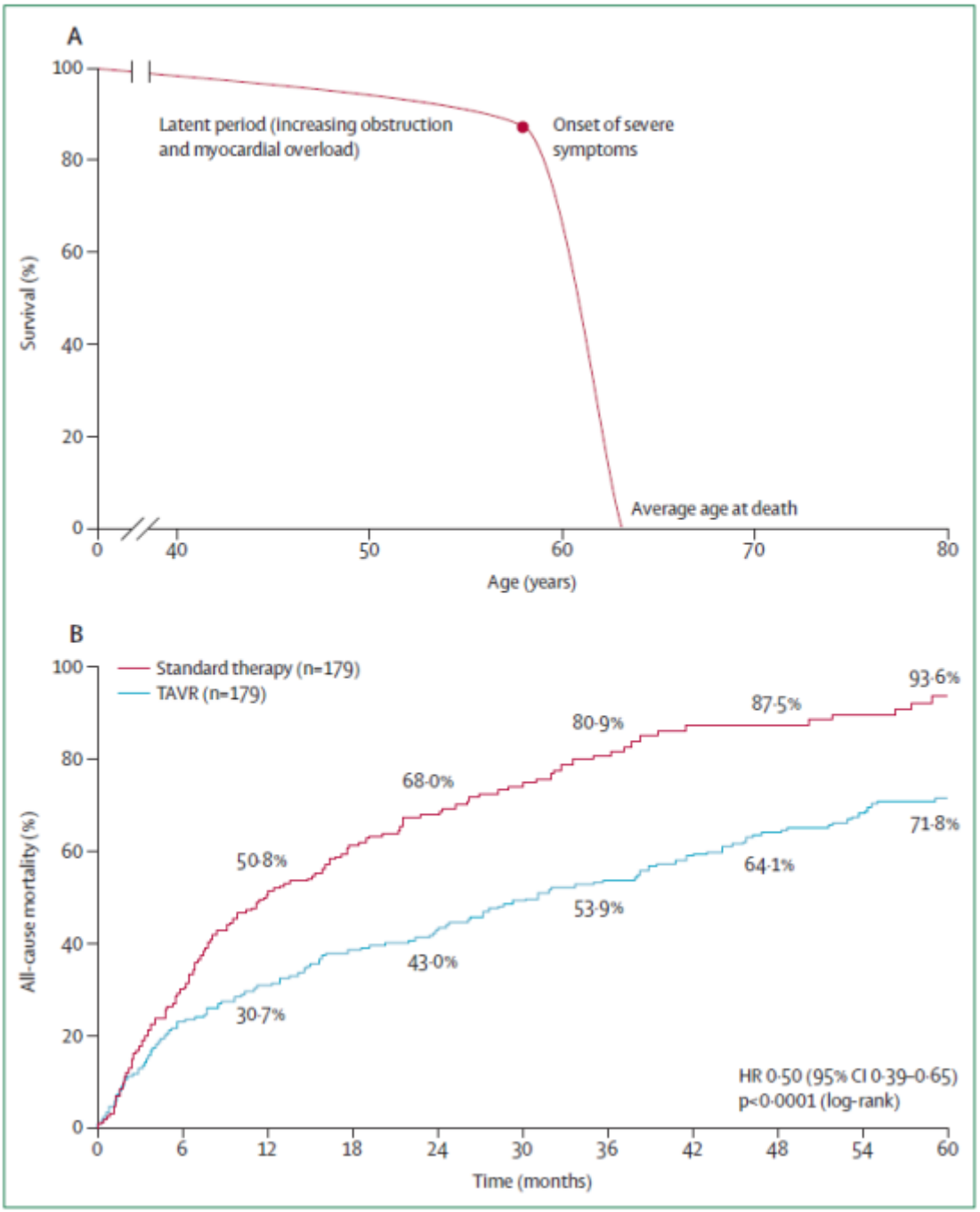



Figure 2: Natural history of aortic stenosis and effects of diagnosis, intervention, and follow-up (A) Survival at different stages of aortic stenosis. (B) Mortality in the PARTNER 1B trial (inoperable cohort). TAVI=transcatheter aortic valve implantation. HR=hazard ratio. *(Bonow RO, et al. 2016 Lancet; 387(10025):1312-23)*

Aortic stenosis is usually diagnosed incidentally by cardiac auscultation or on echocardiography for other indications. Echocardiography is the primary diagnostic

test and should be done to assess valve anatomy, valve haemodynamics, the presence of concomitant valvular lesions, and left ventricular function. The European and US echocardiography guidelines (6) define severe aortic stenosis as a mean gradient greater than 40 mm Hg, peak aortic jet velocity greater 4·0 m/s, and aortic valve area smaller than 1 $cm^2$ (aortic valve area index less than 0·6 $cm^2/m^2$). Around 20% of patients with severe aortic stenosis present with low left ventricular stroke volume and low gradients. Administration of an inotropic agent, such as dobutamine, augments cardiac output and allows a more accurate assessment of the severity of aortic stenosis. Cardiac catheterization is no longer a primary diagnostic test for aortic stenosis, but can be very useful to clarify severity when echocardiography is non-definitive. It is also helpful in identifying the presence of pulmonary hypertension and delineating concomitant coronary disease in patients who are candidates for aortic valve replacement. Other useful diagnostic tools are carefully supervised exercise tests to ascertain symptoms or high-risk features (6) cardiac CT to quantify severity and progression of aortic valve calcification, and serial measurements of brain natriuretic peptide concentrations as a biomarker of disease severity and progression.

### 1.1.5 Management: timing of intervention

The onset of symptoms is associated with poor prognosis. Thus, patients with severe aortic stenosis who develop angina, dyspnea, light-headedness, or syncope have a class I indication for aortic valve replacement. However, identification and interpretation of symptoms is not always straightforward. Interpretation of mild dyspnea might be difficult in elderly and deconditioned patients because many reduce activity levels to avert symptoms. Exercise testing can be helpful to uncover symptoms in seemingly asymptomatic patients. US and European guidelines recommend aortic valve replacement in patients with severe aortic stenosis who have abnormal blood pressure responses during exercise (class IIa indication) and those with left ventricular systolic dysfunction (class I), because systolic dysfunction is presumed to represent severe afterload excess.

Physicians are increasingly recommending aortic valve replacement in asymptomatic patients with severe aortic stenosis, particularly if there is evidence of rapid progression. This approach is based on a high likelihood of patients developing symptoms within 5 years if the peak velocity is greater than 4·0 m/s and within 3 years if the peak velocity is greater than 5·0 m/s (7). Guidelines also recommend aortic valve replacement in patients with very severe asymptomatic aortic stenosis, defined as peak velocity higher than 5·0 m/s in the US or 5·5 m/s in Europe (class IIa indication), and in those with evidence of progressive disease and low operative risk. Operative risk pertains not only to patients with low surgical risk but also to the ability of the surgical team to keep risks of the procedure to a minimum. Appropriate risk assessment can be difficult and requires clear decision making by a multidisciplinary heart team.

Additional factors to consider in asymptomatic patients are findings suggestive of severe myocardial overload, although data so far are too preliminary for strong recommendations, as increasing concentrations of circulating brain natriuretic peptide, severe left ventricular hypertrophy, and an increase in mean aortic valve gradient more than 20 mm Hg during exercise are class IIb indications for aortic valve replacement.

### 1.1.6 Surgical aortic valve replacement

The gold standard intervention for severe aortic stenosis has been surgical aortic valve replacement (SAVR) for more than 50 years. Traditionally, SAVR involves a median sternotomy on full cardiopulmonary bypass: with an arrested heart, the aorta is opened, the diseased aortic valve and annular calcification are excised, and the prosthetic valve is inserted (Figure 3). Real-world clinical outcomes of this conventional procedure have been well described. Contemporary data from large registries in Europe (8) and USA (9) indicate that the overall 30-day mortality with isolated SAVR (i.e., without concomitant procedures) is 2–3%. In octogenarians, 30-day mortality has declined from more than 12% in the 1990s to less than 6%. Risk of surgical mortality is increased in patients with various clinical comorbidities and specific anatomical features. Surgical risk scores (including the EuroSCORE and the

Society of Thoracic Surgeons [STS] score) are well established and are useful for defining or comparing populations of patients with aortic stenosis. They are less useful, however, for predicting risk in individual patients, especially those in high-risk groups, because several risk factors are either poorly represented or not included in the algorithms (3). A multidisciplinary heart team should make clinical decisions.

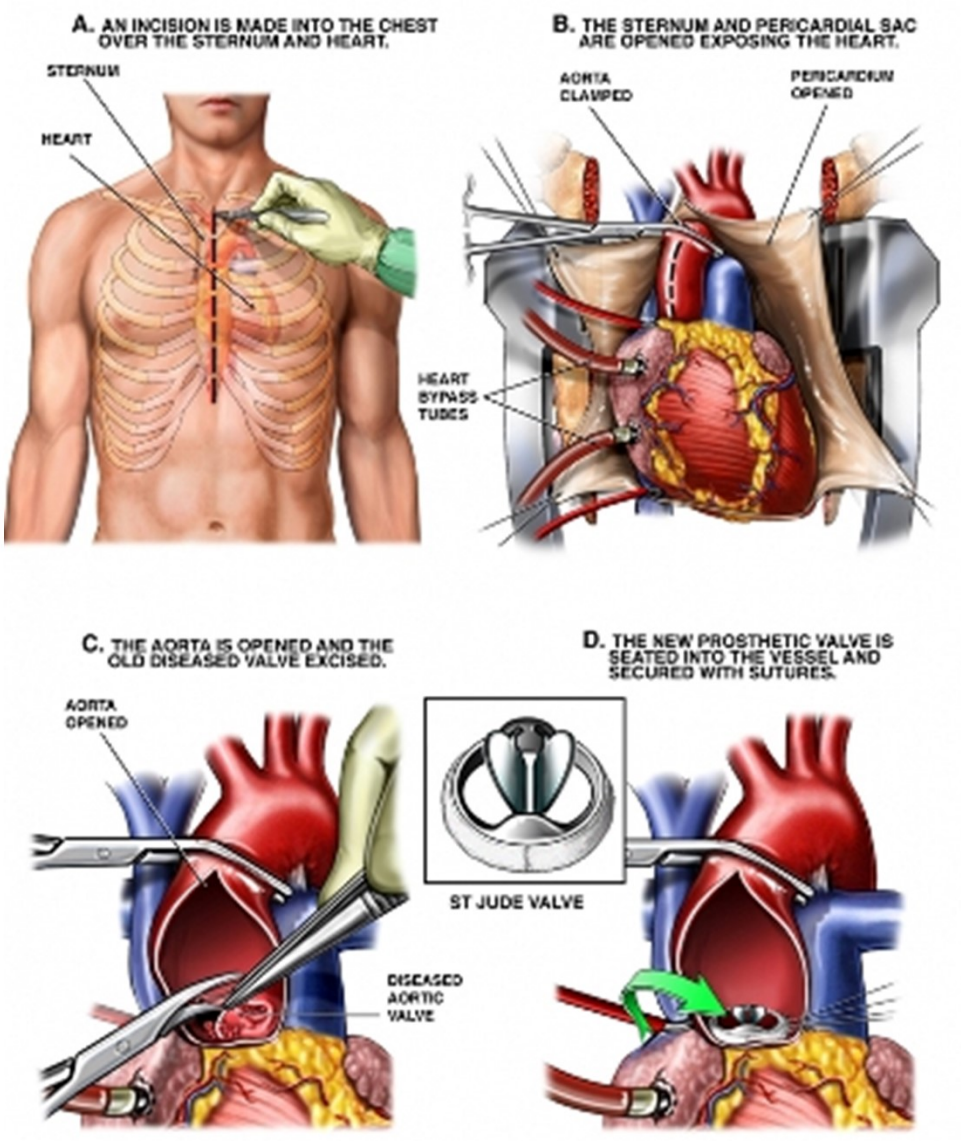



Figure 3. Surgical aortic valve replacement steps with i.e. a mechanical bileaflet aortic prosthesis. *(http://www.cvtsa.com/resources/aortic-valve-disease-and-surgery)*

Prosthetic valves are available in two general classes: mechanical and biological. Most of the current generation of mechanical valves are bileaflet and are made of

pyrolytic carbon. They generally remain free from structural failure for the lifetime of any patient and, therefore, they are most commonly used in those younger than 65 years who have good haemodynamics; the biological valves in patients with an age inferior than 65 years have early structural valve degeneration (SVD) due to calcific dystrophy. Mechanical valves are thrombogenic and require formal anticoagulation, which is most frequently achieved with warfarin. The limitations imposed by lifelong oral anticoagulation with warfarin cannot be underestimated, especially the risks of bleeding in the elderly.

In the past decade, the use of biological valves has increased strikingly, including in many patients younger than 65 years. For instance, in 1997 in the USA, biological valves were used in 43% of aortic valve replacements, compared with 78% in 2006.

The most frequently used biological valves are xenografts, usually made from a native porcine aortic valve or root or from bovine (and occasionally porcine) pericardium. Each valve is mounted on a rigid stent to facilitate implantation. Stented valves might have suboptimum forward flow haemodynamics, especially if the internal orifice is small. Of note, the valve label size is not necessarily an accurate or consistent predictor of the internal orifice size, and information should be sought from the manufacturer. Biological valves may be implanted without the use of a rigid stent, stentless bioprostheses, although they are generally more difficult to implant than stented valves. However, the haemodynamics are superior, especially if the patient's aortic annulus is small, and some data suggest that valve durability is better (3).

Bioprosthetic valves do not require long-term anticoagulation, although whether patients benefit from short-term anticoagulation is debated. All bioprosthetic valves are subject to structural degeneration, although the rate of structural valve deterioration is age-related and slows with advancing age. Thus, with the trend of implanting more bioprostheses in younger patients, the need for further intervention because of structural deterioration is likely to increase substantially (10) (Figure 4). Repeat SAVR that involves a repeat sternotomy owing to bioprosthetic valve failure might not be straightforward. Many patients are elderly at the time of the first implantation, and often 8–20 years pass before the need for

repeat implantation. A substantial proportion present with accelerate degeneration, acute new severe aortic regurgitation, and severe haemodynamic compromise.

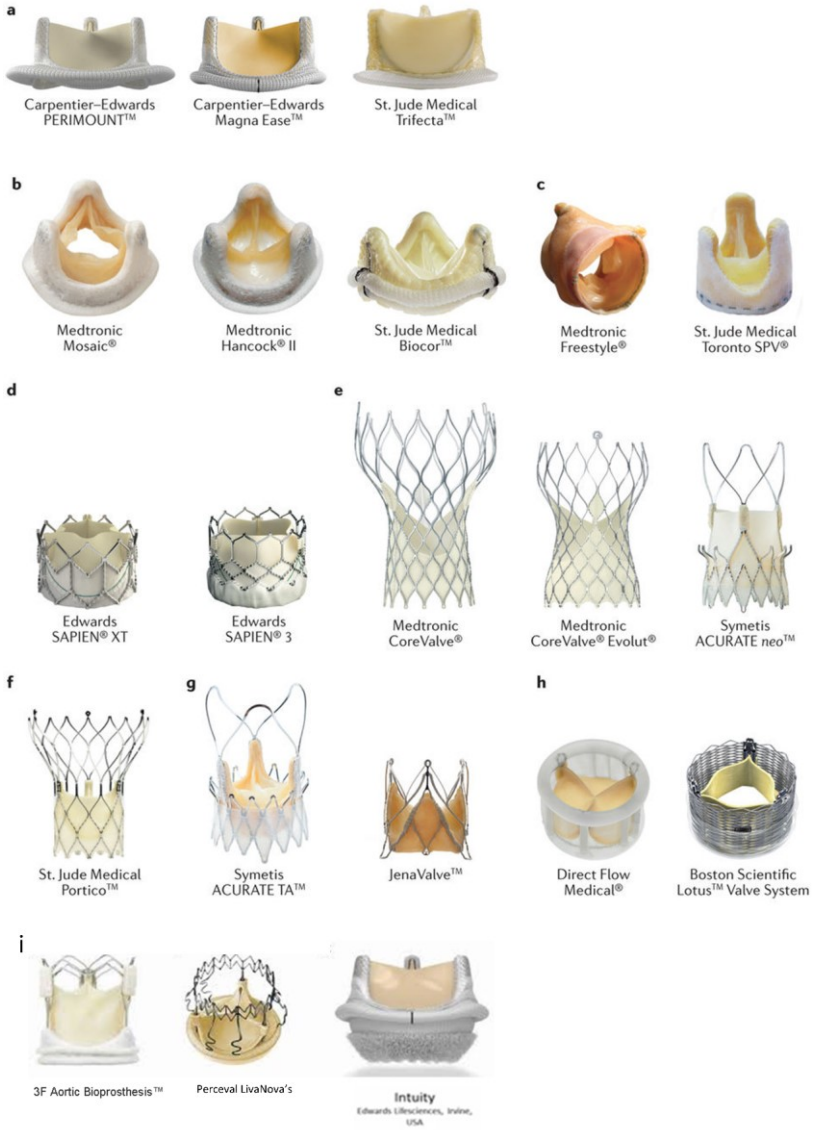



Figure 4. Schematic illustration of biological heart valves. a) Stented pericardial bovine surgical aortic valve bioprostheses. b) Stented porcine surgical aortic valve bioprostheses. c) Stentless surgical aortic valve bioprostheses. d) Balloon-expandable bovine pericardial tissue transcatheter bioprostheses. e) Self-expanding porcine pericardial tissue transcatheter bioprostheses. f) Self-expanding bovine pericardial tissue transcatheter bioprosthesis. g) Self-expanding native porcine cusps transcatheter bioprostheses. h) Alternative expansion design bovine pericardial tissue transcatheter bioprostheses. i) Sutureless bioprosthesis.

The technical aspects of the procedure might be especially challenging in patients who had stentless valves implanted or who underwent aortic root replacement. High risks of early death or severe morbidity and long recovery times are associated with repeat procedures. Increases were also seen for combined operative mortality and major morbidity and stroke, aortic regurgitation, and the need for a pacemaker after surgery (3).

Minimally invasive SAVR, in which access is usually achieved through a partial upper sternotomy, results in less pain, shorter hospital stays, less postoperative atrial fibrillation, and an earlier return to full daily activities (Figure 5). Nevertheless, although this approach might be attractive to patients, it is technically more difficult and there is little scientific evidence of important benefits in major clinical outcomes.

To assess a potential reduction of the effective orifice area (EOA) due to fibrous tissue overgrowth, the ratio between the EOA area and the total area of the bioprosthesis considering the inner stent diameter on ventricular side were measured (Figure 29). The ratio was expressed in percentage. The measurements were performed with the same image analysis system reported in section 1 methods. The modality *measurement/area* was chosen.

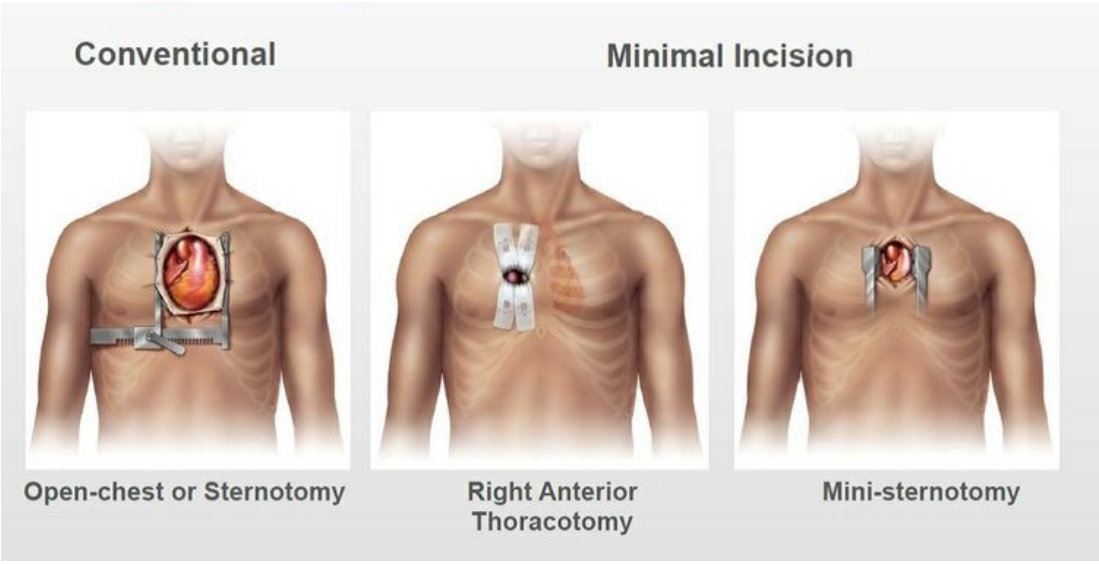



Figure 5. Aortic valve replacement: surgical options.

An alternative to sutured stented or stentless valves is rapid deployment sutureless valves, which are a hybrid of conventional surgical and transcatheter valves. They have sufficient radial force to allow annular implantation without sutures, and facilitate minimally invasive SAVR; the tissue of the native diseased valve needs to be excised.

Despite enthusiasm for minimally invasive SAVR and sutureless valves, they remain substantially more invasive than TAVI, which casts uncertainty on their role in the future.

### 1.1.7 Balloon aortic valvuloplasty

The first transcatheter therapy for aortic stenosis was balloon aortic valvuloplasty. This procedure fractures leaflet calcification to improve mobility, stretches the annulus, and separates fused commissures (Figure 6). Despite notable early recoil, this procedure provides short-term haemodynamic improvement often sufficient to ameliorate symptoms. Recurrence, however, is about 80% after 6–12 months, mandates further treatment, and does not improve survival. Indications for balloon aortic valvuloplasty in patients with severe aortic stenosis are palliation in patients not suitable for SAVR or Transcatheter ortic valve implantation (TAVI), management of acute haemodynamic decompensation, facilitation of percutaneous coronary intervention, bridging to SAVR or TAVI if definitive treatment needs to be delayed, and discernment of the contribution of symptoms such as dyspnoea from intrinsic lung disease rather than heart failure related to aortic stenosis.

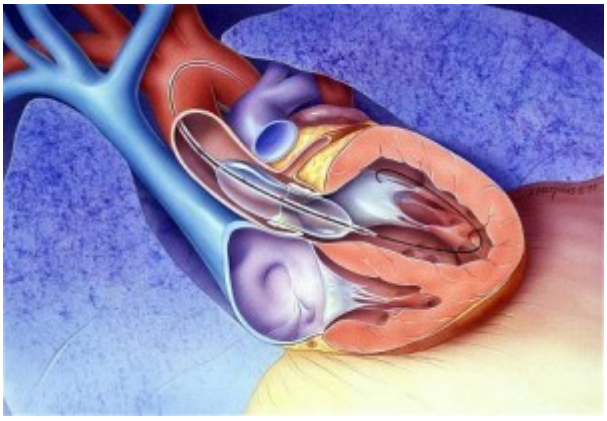


Figure 6. Balloon aortic valvuloplasty.

### 1.1.8 Transcatheter aortic valve implantation

TAVI was initially conceived as a niche therapy for patients with severe aortic stenosis who were not surgical candidates because of multiple comorbidities. Since Cribier and colleagues reported the initial proof-of-concept case in 2002 (11), the use of TAVI has expanded substantially, with more than 200 000 patients having undergone procedures in almost 1000 centres in around 65 countries. TAVI is now widely included as a treatment option for patients with severe aortic stenosis. The success of TAVI has been explained by five main factors: multidisciplinary approaches to selection of patients, case planning, and valve implantation; commitment to evidence-based research; rapid evolution of technology; reductions in procedure-related complications; and simplification of the procedure.

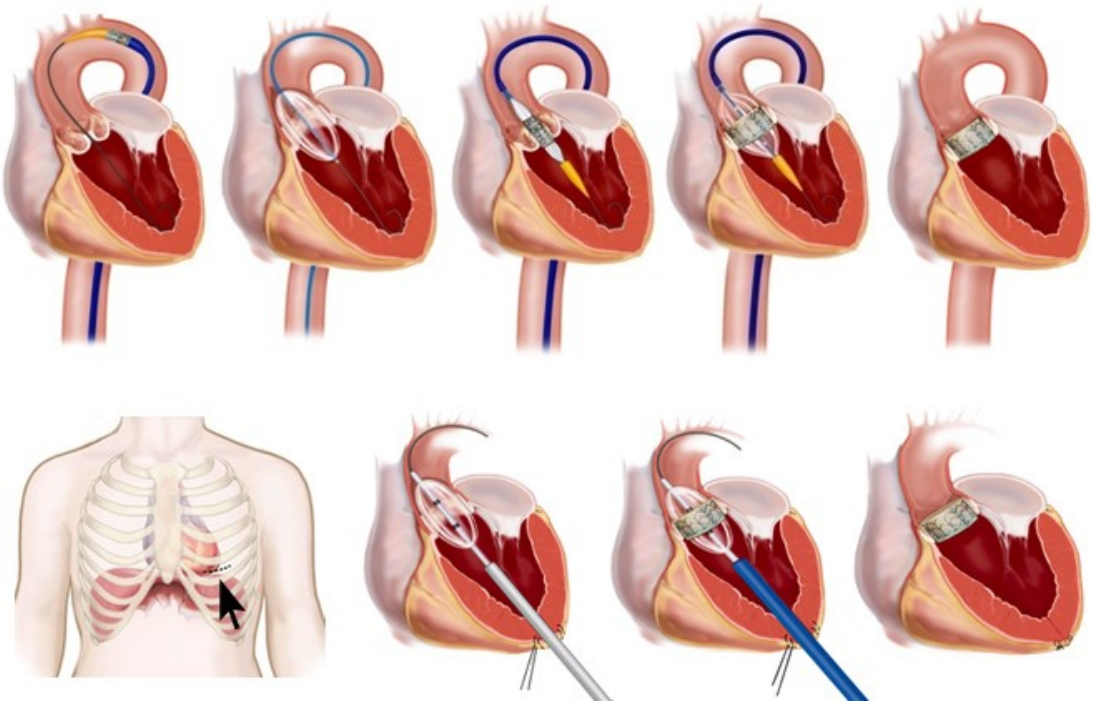


Figure 7. Transcatheter Aortic Valve Replacement Procedure via transfemoral approach (upper panel) and via transapical approach (lower panel) (http://www.raneyzusman.com/).

A TAVI system consists of a bioprosthetic trileaflet heart valve (made from bovine or porcine pericardium) sewn within a rigid or semi-rigid expandable frame that is secured to a catheter-based delivery system in a crimped low-profile state. The

transcatheter heart valve is deployed within the aortic annulus by an expansion mechanism (usually balloon inflation or self-expanding Nitinol) to displace the diseased native aortic valve. The preferred route of vascular access is via the common femoral artery, but in patients with severe peripheral vascular disease other routes are possible, including transapical and direct aortic (both requiring small thoracotomy incisions), subclavian or axillary artery, and carotid artery (Figure 7). Over the past 5 years, various different TAVI systems have been introduced in the clinical practice with enhancements that have improved the procedure and outcomes. Most candidates for TAVI present high-risk anatomical or clinical features and, therefore, careful selection of patients is important, even more so than for SAVR. Multidisciplinary heart teams should develop individualized screening and assessment plans. Although surgical risk scores can be helpful and should be calculated routinely, they have limited usefulness for patients with high-risk anatomical features and possibly those who are frail, have dementia, or both.

On the basis of the clinical evidence, the European Society of Cardiology and European Association for Cardio-Thoracic Surgery (6) recommend that TAVI is a class I indication for patients with severe symptomatic aortic stenosis with a predicted survival less than 1 year who are not candidates for SAVR. The PARTNER study (5) compared balloon-expandable TAVI with standard therapy in patients not suitable for surgery, and found a reduction in all-cause mortality at 1 year in the TAVI group. 5-year follow-up showed a sustained 20% reduction in mortality in the TAVI group, with no structural degeneration of the valve bioprosthesis (5). Guidelines also recommend TAVI as a class IIa indication for patients with severe symptomatic aortic stenosis who are at high risk of death (or severe complications) after SAVR (6). The consensus from experts worldwide is that TAVI is the preferred therapy and should be the standard of care for patients with aortic stenosis who are not candidates for surgery, and should be the preferred alternative to SAVR in high-risk patients, especially elderly patients, who are good candidates for TAVI (6).

In the early TAVI studies in high-risk patients, in which first-generation devices were implanted and operator experience was limited, the risk of periprocedural complications was problematic, especially strokes, major vascular access bleeding events and paravalvular regurgitation (2). More conduction abnormalities also

occurred after TAVI than SAVR (10–30% vs 7–16%), for which new permanent pacemakers were needed (2). In later TAVI studies, clinical outcomes improved because of improved technology, increased operator experience, more refined selection of patients, and improved procedural methods.

With TAVI procedure standardization, the focus has shifted in many centres towards a so-called minimalist strategy, which includes percutaneous transfemoral vascular access, conscious sedation with no general anaesthesia, reduction or elimination of transoesophageal echocardiography for guidance during the operation, reduced predilatation of the balloon before valve implantation, and the use of care plans that encourage rapid ambulation and early hospital discharge. Most centers, however, employ a hybrid of standard and minimalist strategies, using the minimalist approach in straightforward cases and more conventional approaches in patients with high-risk anatomy or ambiguous cases. The latter benefits from the use of trans esophageal echocardiographic guidance during the procedure.

Clinical controversies might be considered for TAVI, like extention to people who would currently be treated with conventional SAVR and others who represent untested clinical situations. In fact, several European TAVI registries have acknowledged a drift towards assigning this procedure to lower-risk patients.

Other controversies to consider are the bioprosthetic valve failure. Early valve-cusp thrombosis and thickening associated with bioprosthetic heart valves has gained increasing attention. A multicentre report from 12 sites showed clinically important valve-cusp thrombosis in 26 (0·6%) of 4266 patients an average of 6 months after TAVI, which was associated with heart failure symptoms and increased transvalvular gradients in most patients (12). Treatment with warfarin for an average of 2 months improved symptoms and reduced valvular gradients 88% of patients.

Further studies will hopefully improve understanding of the importance of reduced cusp motion and contribute to developing a strategy for optimum adjunctive pharmacotherapy after TAVI.

## 1.2 AORTIC VALVE REGURGITATION

### 1.2.1 Introduction

Aortic regurgitation is much less prevalent than aortic stenosis and represents a lesser public health concern. Patients generally present at younger ages than those with aortic stenosis. Aortic regurgitation is mainly related to congenitally bicuspid valve disease or primary disease implantation of the aortic root or ascending aorta. The diastolic murmur is often difficult to discern and, therefore, the flow-related systolic murmur might be the more prominent auscultatory finding. Echocardiography is essential to establish the cause and severity of aortic regurgitation and its effect on left ventricular volume and function.

### 1.2.2 Management strategies: Timing of interventions

Deciding the optimum timing for surgical intervention in patients with aortic regurgitation remains challenging and controversial. SAVR is a class I indication in patients with severe aortic regurgitation who develop symptoms (usually effort dyspnoea or worsening heart failure) (6). However, the combined pressure and volume overload of aortic regurgitation can cause left ventricular systolic dysfunction before symptoms develop (Figure 8). This pattern differs from that with aortic stenosis, in which left ventricular systolic function is preserved in most symptomatic patients. Hence, by the time symptoms develop, many patients have myocardial dysfunction, placing them at high risk of postoperative heart failure and death. Left ventricular ejection fraction and end systolic dimension (or volume) are important prognostic factors (3). Long-term post operative studies indicate that survival is improved if patients with left ventricular systolic dysfunction undergo aortic valve replacement without waiting for symptom onset.

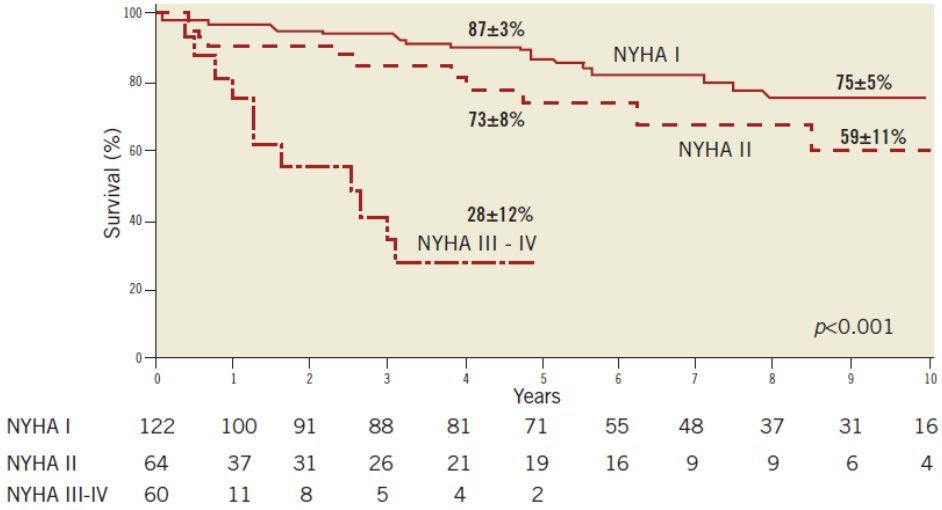



Figure 8. Survival of untreated patients with native aortic valve regurgitation after the onset of symptoms by New York Heart Association (NYHA) class. This figure shows the poor prognosis associated with NYHA Class III or IV symptoms. *(Roy D, et al. Native aortic valve regurgitation: transcatheter therapeutic options. EuroIntervention. 2013 Sep 10;9 S55-62)*

Guidelines also recommend SAVR if ejection fraction is subnormal (class I) and left ventricular end systolic dimension is larger than 50 mm (class IIa) (6). In asymptomatic patients with preserved systolic function, the volume load must be taken into account (left ventricular end diastolic dimension of 65–70 mm is a class IIb indication) (6).

### 1.2.3 Surgical aortic valve replacement

Compared with calcific aortic stenosis, the anatomical features of aortic regurgitation are more diverse, including more complex morphology of the valve and aortic root. Non-calcified leaflets, aortic annular dilation, and anatomical distortion and enlargement of the aortic root with associated aneurysmal dilatation of the ascending aorta are frequently involved. Thus, various surgical procedures may be required, including valve repair, conventional SAVR, full aortic root replacement (incorporating mechanical or biological prostheses), valve sparing root

replacement, and others that are tailored to the individual pathology. Several important surgical advances have been made in aortic valve repair, especially for young patients with bicuspid aortic valves. Anyway, durability of aortic valve repair remains a concern.

### 1.2.4 Transcatheter aortic valve implantation

The use of TAVI to treat patients with predominant aortic regurgitation and non-calcified aortic leaflets has been challenged because of the anatomical complexities (Figure 9). The need for large valve sizes and the management of associated aortic root disease are particular concerns. Few patients have thus far been treated for aortic regurgitation with self-expanding TAVI systems (13).

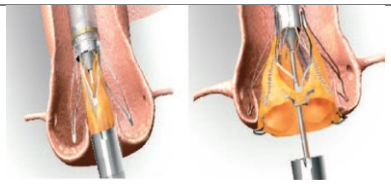
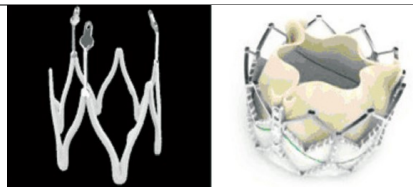
| Registry report | Number of patients | TAVI device | 30-day mortality | 30-day stroke | Dislocation or second valve | Residual AR ≥grade II |
|---|---|---|---|---|---|---|
| Sarkar et al 2012[37] | 4 | CoreValve | 0% | 0% | NR | NR |
| Roy et al 2013[32] | 43 | CoreValve | 9.3% | 4.7% | 18.6% | 16.3% |
| Testa et al 2013[34] | 23 | CoreValve | 23% | 0% | 19.2% | 73.6% |
| Seiffert et al 2013[35] | 5 | JenaValve | 0% | 0% | 0% | NR |
| Holzamer et al 2013[38] | 6 | CoreValve | 16.7% | 0% | 33.3% | NR |
| Pasupati et al 2013[36] | 4 | Edwards HELIO Transcatheter Dock (transapical-transfemoral) | 0% | 25% | 0% | 0% |

| Case reports | Valve | Case details | Outcome |
|---|---|---|---|
| Olsen et al 2009[18] | CoreValve | Previous homograft | "Native" in that it was treatment of homograft rather than degenerative bioprosthesis. Successful implant |
| Ducrocq et al 2010[19] | CoreValve | Radiation-induced NAVR – sternal malignancy | Successful implant |
| Dhillon et al 2010[20] | CoreValve | Radiation-induced NAVR | Successful implant |
| Krumsdorf et al 2011[21] | CoreValve | Inoperable | Valve dislocation before complete deployment requiring retrieval and repositioning. Successful final implant |
| Pacchioni et al 2011[22] | Edwards SAPIEN | Porcelain aorta | Valve dislocation and need for valve-in-valve with CoreValve |
| D'Ancona et al 2011[23] | Edwards SAPIEN | LVAD | Successful implant |
| Santini et al 2012[24] | CoreValve | LVAD | Successful transfemoral implant |
| Rossi et al 2012[25] | CoreValve | Ascending aortic aneurysm | Successful implant |
| Bleiziffer et al 2012[26] | JenaValve | Inoperable | Successful implant |
| Hildebrandt et al 2012[27] | CoreValve | Inoperable | Successful implant |
| Dumonteil et al 2012[28] | CoreValve | Inoperable | Successful implant |
| Nakamura et al 2013[29] | CoreValve | Previous native aortic valve repair | Successful implant |
| Lavee et al 2013[30] | CoreValve | LVAD | Successful transfemoral implant |
| Webb et al 2013[31] | Edwards HELIO Transcatheter Dock | Transfemoral-transfemoral | Successful implant |

Figure 9. Summary of published experience of treating native aortic valve regurgitation with TAVI *(Roy D, et al. Native aortic valve regurgitation: transcatheter therapeutic options. EuroIntervention. 2013 Sep 10;9 S55-62).*

Some dedicated TAVI devices for aortic regurgitation have been developed (Medtronic Engager™, Medtronic, Minneapolis, MN, USA; Jena Valve, Jena Valve Technology GmbH, Munich, Germany; Edwards HELIO Transcatheter Dock, Edwards Lifesciences, Irvine, CA, USA) (Table 1) and one (Jena valve), which clips to the native leaflets, has already been granted commercial approval in Europe (Figure 10) (14).

Table 1. Available devices for TAVI in pure native aortic regurgitation.

| Type of TAVI | Characteristics | Picture |
|---|---|---|
| **Medtronic Engager™ (Medtronic, Minneapolis, MN, USA)** | – Self-expanding<br>– Anatomical orientation with the commisures<br>– Arms trap valve leaflets to prevent movement/dislocation<br>– Leaflet trapping minimises paravalular regurgitation<br>– Transapical with transfemoral being developed |  |
| **JenaValve (JenaValve Technology GmbH, Munich, Germany)** | – Arms trap valve leaflets to prevent movement/dislocation<br>– Leaflet trapping minimises paravalular regurgitation<br>– Anatomical orientation with the commisures<br>– Transapical with transfemoral being developed |  |
| **Edwards HELIO Transcatheter Dock (Edwards Lifesciences, Irvine, CA, USA)** | – New approach to balloon-expandable TAVI<br>– HELIO "dock" placed behind valve leaflets just before valve deployment<br>– Pinning of leaflets prevents movement/dislocation<br>– No oversizing necessary<br>– Minimises paravalvular regurgitation |  |
| **Devices with "clipping" or "docking" mechanisms as well as devices which are fully retrievable and repositionable may hold the future for the use of TAVI for Native Aortic Valve regurgitation.** | | |

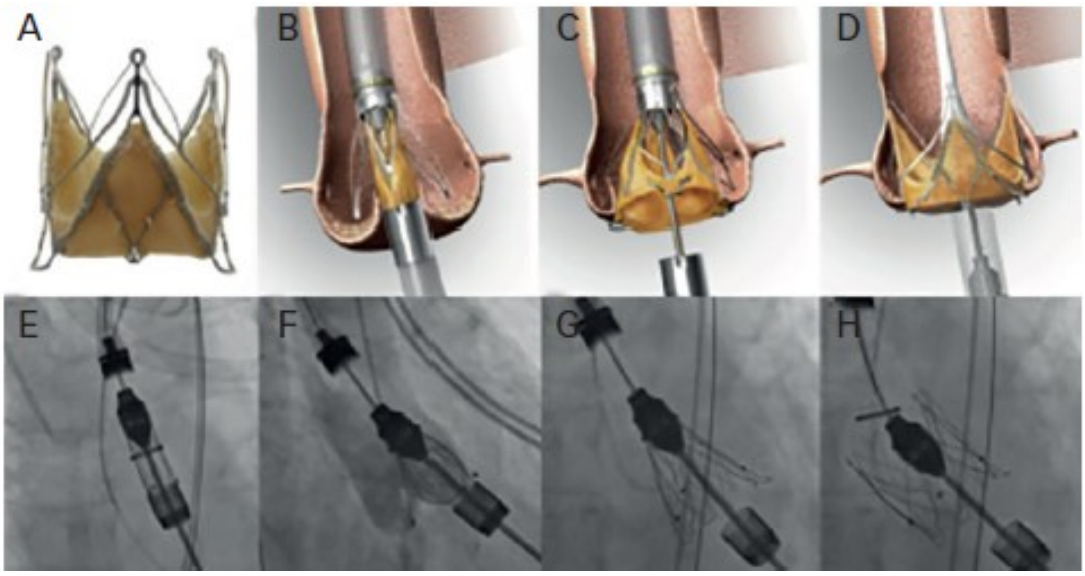



Figure 10. The Jena valve transcatheter heart valve (A) and its implantation illustration (B to D) and fluoroscopy (E to H). Release of the positioning feelers and placement into the aortic sinuses enables anatomic orientation (B and F). After correct orientation has been verified in two different fluoroscopic angulations, release of the lower stent part facilitates the clipping of the native aortic leaflets to the device and expansion of the stent allowing foe secure anchoring even in the absence of valve calcium (C and G). Release of the upper stent part completes deployment of the valve prosthesis (D and H). *(Sleffert et al., J Am Coll ardiovasc Interven, Vol 7, 2014).*

The assessment and management of patients with aortic valve disease are rapidly changing with new insights into the natural history of the disease, advances in imaging capabilities, and evolving minimally invasive surgical and transcatheter solutions for valve replacement. In aortic stenosis, TAVI is expanding to address the needs of higher-risk patients, with new indications for valve-in-valve treatment in patients with failing bioprosthetic valves. Concurrent advances in surgical techniques are being seen, including valve replacement with sutureless bioprosthesis for intemediate-risk patients and associated procedures programmed, in order to reduce surgical times. Guidelines for SAVR and TAVI are challenged to remain up to date with this rapidly evolving field.

# 2. SUTURELESS OR RAPID RELEASE AORTIC BIOPROSTHESIS

## 2.1 Introduction

At present, the standard treatment for aortic stenosis surgery is SAVR using conventional prosthesis through median sternotomy. Moreover, the proportion of biological prostheses to mechanical prostheses has increased over the last decade primarily due to the predominance of elderly patients with this disease. The durability of conventional bioprostheses is sufficiently acceptable in the elderly, with 90% of patients over the age of 65 free of dysfunction at 15 years (15); e.g. actuarial and actual freedom from structural valve deterioration (SVD) at 18 years of the Carpentier-Edwards supra-annular aortic porcine bioprosthesis for the 61- to 70-year age group was 77.6% and 90.5%, respectively and for >70 years age group, it was 94.6% and 98.2%, respectively. The mortality reported by the various societies, hospitals and published series on isolated SAVR is low (<3%). In the last 20 years, the mortality rate has even continued to decrease (15). The incidence rate of early complications from the implantation of traditional aortic prostheses is low, with a 4.2% rate of periprosthetic leaks >1/4 (16), 1.45% rate of stroke (16) and a 4% rate of re-examinations due to bleeding, with an intermediate risk in terms of the need for pacemaker implantation (<7%) (17) and severe patient–prosthesis mismatch (PPM; 9.8%) (18). However, we have less information on other results of the SAVR that could be of interest for analysis, such as the repercussion of

ischaemia times and of certain associated comorbidities, costs, stays and long-term survival in various age groups.

In order for the newly marketed prosthetic valves to replace traditional prostheses, the former must be easy to implant; reduce the ischaemia times; exceed the haemodynamics of the latter; have a low incidence of perivalvular leaks (<2%), PPM <5% and stroke or embolism <2%; provide a durability that is at least as good as the current valve prostheses; and demonstrate a nearly absence of thrombosis and endocarditis (19).

## 2.2 Manufactured models

Due to the greater comorbidity and increased surgical risk for candidates of SAVR and in order to expand the operability and decrease surgical trauma, techniques have been developed that include minimally invasive surgical (MIS) approaches, as well as new valves, such as sutureless prostheses, which are characterized by their ease quick of implantation during cardiac surgery. Although the concept of sutureless prostheses is >50 years (20), this conceptual sutureless prosthesis model had not been implemented in practice until the advent of the recent TAVI. The currently marketed prostheses include the 3f Enable, the LivaNova Perceval S and the Edwards Intuity (Figure 11).

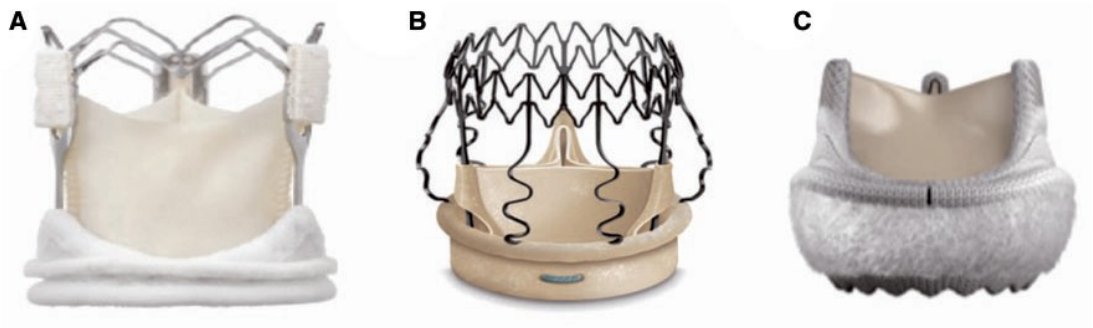



Figure 11: Sutureless aortic valve prostheses. (A) 3F Enable (Medtronic, Ninneapolis, USA). (B) Perceval S (LivaNova, London, UK). (C) Intuity Elite (Edward Lifesciencies, Irvine, USA).

These biological valves are radically different from conventional ones. The valves are mounted on a stent that expands and attaches to the aortic ring through controlled release under direct vision, after having excised and decalcified the aortic valve. These prosthetic valves have been on the market for a short time, and the majority of studies on the valves are observational with an occasional randomized clinical trial. However, the evidence in favour of these prostheses is fairly clear. Of these, the Perceval S valve has the most studies, over the longest term and the most publications. Table 2 shows a comparison of the manifactured available sutureless or rapid deployment bioprostheses.

Table 2. Comparison of the principal characteristics of the manifactured available sutureless prostheses.

| | **Perceval S** | **Edwards Intuity** | **3F Enable Model 6000** |
|---|---|---|---|
| **Tissue** | Bovine pericardium (based on LivaNova Solo bioprosthesis) | Bovine pericardium (based on Perimount bioprosthesis) | Equine Pericardium (based on 3F bioprosthesis) |
| **Ring sizes (mm)** | S (19-21), M (21-23), L (23-25), XL (25-27) | 19, 21, 23, 25, 27 | 19, 21, 23, 25, 27 |
| **Permanent sutures (nr)** | No | 3 | 1 |
| **Deployment** | Collapsible for 15 min; fits with the the aortic root | Not collapsible or foldable; fits with the annulus | Foldable; fits with the annulus and the aortic root |
| **Anchoring system** | No | The anchor is mainly subannular | No |
| **Mount material** | Nitinol stent | Chromium-cobalt alloy stent, stainless steel skirt | Nitinol stent coated in poliester in the upper part and at the ring |
| **Fixation** | Glutaraldehyde | Glutaraldehyde | Glutaraldehyde |
| **Decalcification treatment** | HAT (homocysteic acid treatment) | ThermaFix process (heat, ethanol, and surfactant treatment) | None |
| **Publications** | ➢ 100 | ➢ 20 | ➢ 20 |
| **Published with a longer term follow-up (years)** | 6 | 3 | 5 |

The Perceval S, which has a similar design to the LivaNova Solo, is a bovine pericardium valve mounted on an anchor device, which (thanks to the nitinol memory of the metallic cage in the ring and of the sinotubular junction) is able, through a compression device, to first collapse and then self-expand to anchor itself in the ring and sinotubular junction (Figure 12). Three guiding threads need to be placed in the nadirs of the sinuses (the threads are subsequently removed) to position the prosthesis in the ring. Using a release system, the prosthesis self-expands and is subsequently ballooned to ensure its correct deployment (21).

Figure 12. Perceval Sutureless Bioprosthesis components. Stentless Solo tissue valve (left) in the anchoring device (middle) creates the Perceval S sutureless valve (right).

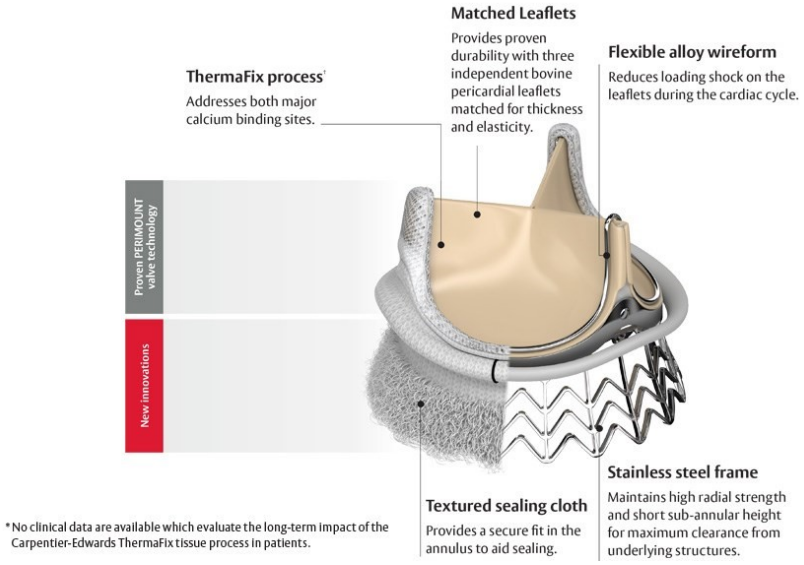



Figure 13. Edwards Intuity Bioprosthesis components. Perimount tissue valve in the anchoring device created by stainless steel frame sealed by a textured cloth.

The Edwards Intuity, using a design based on the Perimount, has a chromium–cobalt stent with a polyester-coated steel skirt that expands with a balloon and is anchored in the subannular region (Figure 13). The prosthesis requires 3 sutures and is therefore considered a rapid-deployment valve and not strictly sutureless (21). In the latest generation of prosthesis, the skirt has been improved, reducing the narrower part of the sealed area (which theoretically translates into fewer rhythm abnormalities), and the prosthesis now has a flexible release system (which mostly facilitates MIS).

The 3f Enable Model 6000 prosthesis has a design that is very similar to the 3f (Figure 14). The cusps are made of equine pericardium sutured to a self-expanding nitinol frame coated in polyester on the upper part and at the ring, the latter of which is precisely where the prosthesis is fixed. The surgical technique is very similar to that of the Perceval S. The available sizes range from 19mm to 27mm (22).

Figure 14. 3F Enable Bioprosthesis components. 3f stentless valve Model 1000 tissue (left) and Nitinol Frame (middle) creates the 3F Enable Model 6000 (right).

The ease of implanting these sutureless prostheses is therefore highly reproducible and the learning curve is short. There are clear similarities between AVR using sutureless prosthesis and conventional prostheses. The 2 implantations are performed through surgical incisions, which can be performed using median sternotomy or MIS. Similarly, the 2 implantations require cardiopulmonary bypass (CPB) and aortic clamping. The excision of the valve is the same as the conventional

surgery to properly anchor the valve under direct vision and to minimize periprosthetic leaks.

There are differences between the different prosthesis. The nature of the expandable sutureless prosthesis means that numerous sutures are not required, which generally results in reduced surgical times, especially when employing the minimally invasive surgery (MIS) approach.

## 2.3 Outcome and complications

### 2.3.1 LivaNova Perceval S

There are numerous observational studies; however, the broadest and most recently published is that of Shrestha et al. (23) in which more than 700 patients participated consecutively, with a 5-year follow-up. The patients came from 25 European centres in the Perceval Pilot, Perceval Pivotal and CAVALIER prospective studies. The patients had a mean age of almost 80 years and an intermediate-high risk profile (EuroSCORE 10.9). MIS was performed in only 25% of the cases and concomitant surgeries 33%. The ischaemia times and CPB for isolated AVR were 33 and 56 min, respectively, which is practically half that of a conventional AVR. The early mortality rate was 3.4%. The clinical and haemodynamic results were excellent considering the patient population treated. The incidence rate of stroke was 1.6%, 1.4% for perivalvular leakage and 6% for the need for pacemaker implantation due to third-degree atrioventricular block. There was no migration, the haemodynamics was excellent, and there was no structural dysfunction at 5 years.

### 2.3.2 Edwards Intuity

There is less literature analysing the results of this prosthesis. We will focus on the 3 published trials (Triton, Transform and Cadence MIS studies) (24-26); of these, the Transform study has the largest number of patients (839). In general, the mean age in these studies was almost 75 years, and the mean EuroSCORE was a little lower

than that of the Perceval study by Shrestha et al. (23) The clinical and haemodynamic results were also excellent, with a better than expected mortality (2.1–3.3%), low incidence of stroke (1.7–3.2%), few perivalvular leaks (0–1.4%) and a need for pacemaker implantation (4.3–11.9%) similar to that of conventional surgery.

### 2.3.3 3F Enable

The most relevant long-term study is that of Englberger et al. (27) in which 10 European centres participated. The study included 141 patients with a maximum and mean follow-up of 5 years and 2.76 years, respectively. Interestingly, the ischaemia and CPB times were similar to those of conventional valves. The authors tentatively explained this by the fact that 30% were concomitant procedures, and because it was a study of initial experience, it had an incomplete learning curve. Other subsequent studies obtained significantly shorter ischaemia and CPB times than this study. There were 3 patients with severe perivalvular leakage. The haemodynamics of the prosthesis was excellent from the start, with a mean gradient peak of 15mmHg at 5 years. No relevant prosthesis-related complications were associated, and in this study no thromboembolic event or structural deterioration was recorded.

Due to the finding of sporadic cases of valve migration, this valve is not commerciable actually (28-31).

### 2.3.4 Scientific evidence to support sutureless valve bioprosthesis

#### 2.3.4.1 Studies that encompass sutureless prostheses as a whole

The most representative study is the systematic review and meta-analysis by Phan et al. (32), which included 12 relevant articles on all sutureless valves with a total of 1037 patients. The incidence rate of early complications was low, the mortality at 30 days was 2.1%, with a rate of 4.3% of periprosthetic leaks and the incidence rate of stroke was 1.9%. Up to 1-year follow-up, the all-cause mortality was 4.9%, 5.6%

for pacemaker implantation and 2.2% for endocarditis. Apart from these clinical results, it is worth noting that the ischaemia and CPB times in mean terms were half that of conventional isolated AVR. Moreover, the haemodynamics were also especially good, showing peak gradients of 20mmHg and mean gradients of 10mmHg at discharge, which remained stable over time.

#### 2.3.4.2 Sutureless valve prosthesis versus conventional AVR

To analyse the evidence of these new prostheses in comparison with the conventional AVR, there are several relevant retrospective studies, a single completed randomized study (CADENCE) (26), a currently underway study (PERSIST-AVR) and a recent meta-analysis (33). In all of the studies, the ischaemia and pump times were always significantly shorter with the sutureless prosthesis. The incidence of post-surgical complications was similar with the 2 technologies in the majority of studies. A number of studies however, associated the sutureless prosthesis with a significant reduction in the need for transfusions, a shorter stay in intensive care unit, shorter intubation times and a lower incidence of post-surgical atrial fibrillation and respiratory failure. The 2 types of aortic valve replacements demonstrated similar long-term survival, except in the octogenarian patient group who could benefit more from sutureless prosthesis, with a greater survival with this new technology, which is likely related to the increased importance of reducing surgical times in this age group (34). Moreover, studies have shown that there is a significant cost reduction of up to 25% with sutureless prostheses, mainly due to the savings in the diagnostic procedures performed during hospitalization and to shorter overall hospital stays (35). The more negative results regarding these new prostheses involve the greater than or equal incidence of needing a pacemaker implant after the procedure when compared with traditional AVR.

#### 2.3.4.3 Sutureless valve prosthesis versus TAVI

As with the previous section, there is recent evidence comparing the 2 types of prosthesis. All of the studies were retrospective and performed a pairing and propensity analysis of patients with similar risks and characteristics and a single meta-analysis that encompassed 7 major observational studies of 87 potential

studies (36). The sutureless prosthesis appears to have many advantages over TAVI including aspects such as perivalvular leaks, the need for pacemaker implantation, neurological events, vascular complications, costs and late mortality. Even in the meta-analysis by Takagi et al. (36), the sutureless prosthesis demonstrated significant improvement in early mortality compared with the TAVI (2.5% vs 7.3%) and a reduction in perivalvular leaks (3.5% vs 33.2%). The study by Santarpino et al. (37), which also employed the sutureless prosthesis, showed a reduction in costs, observing a total savings of approximately 10 000 euro is the price of the TAVI was included.

Most of the authors concluded that the sutureless prosthesis could be the first-line treatment for patients who lie within 'the grey area' between the indications for conventional AVR and TAVI (Figure 2).

### 2.3.5 Reasons to employ sutureless valve prostheses

#### 2.3.5.1 To reduce the aortic clamping time and cardiopulmonary bypass time

More than half of the aortic clamping time during a conventional AVR operation is spent implanting the sutures in the aortic ring and prosthesis and then tying them. These are phases of surgery that can be avoided with sutureless prostheses. Long aortic clamping and CPB times are considered independent predictors of morbidity and mortality in heart surgery. This association has been recently demonstrated, even in conventional AVR surgery (38), which itself is not usually a long procedure. Ranucci et al. showed that a reduction in the clamping time during conventional AVR produced lower morbidity, especially in those with impaired ejection fraction and in patients with diabetes mellitus (38). The meta-analysis by Phan et al. (32) showed that the mean clamping and CPB times in AVR with sutureless prosthesis were reduced to half that typically required for conventional AVR. Therefore, this reduction in times could translate into improved clinical results for potentially all patients but especially those with comorbidities or a medium-high surgical risk profile.

### 2.3.5.2 To facilitate AVR through minimally invasive surgery

Since the advent of MIS for AVR, similar results to conventional AVR surgery have been obtained, with a number of advantages such as reduced postoperative pain, earlier mobilization, shorter hospital stays, better aesthetics, a lower incidence of surgical wound infection and lower requirements for transfusions. However, MIS with conventional aortic prostheses has yet to spread throughout the world, because it is associated with greater technical difficulty due to reduced visualization from the tight spaces, increased aortic clamping and CPB time and a difficult and longer learning curve, all of which appear to counter the above-mentioned benefits (39). From lesser to greater complexity, there are essentially 3 access pathways for changing the aortic valve.

The first and most widely used is full median sternotomy. The other two pathways are by mini-sternotomy and right anterior mini-thoracotomy, which are considered MIS according to the 2008 definition of the American Heart Association (40), which defines MIS in cardiac surgery as 'a small incision in the chest wall that does not include conventional full median sternotomy'. The meta-analysis by Phan et al. (32) showed how the use of MIS has significantly and progressively increased since the advent of sutureless prostheses (Figure 15).

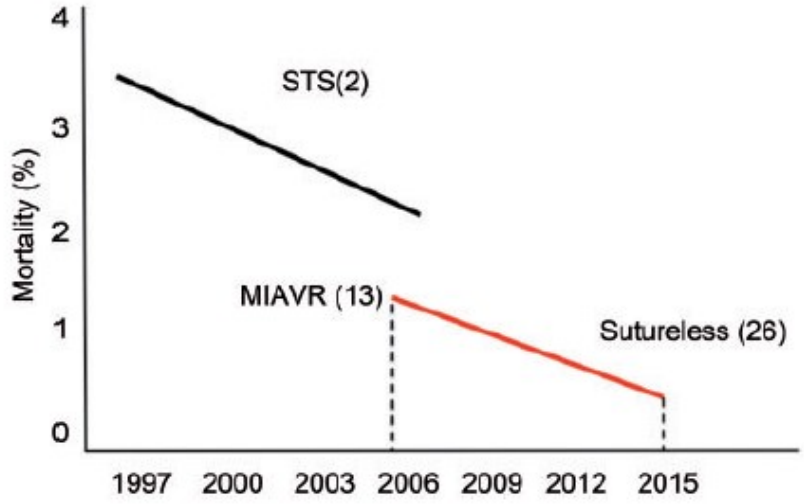



Figure 15. Aortic valve replacement mortality over time. Black line: mortality of the conventional AVR according to STS. Red line: mortality of the AVR with Mini invasive surgery since the insertion of the sutureless prosthesis. MIAVR: minimally invasive

aortic valve replacement; STS: Society of Thoracic Surgeons. *(Martínez-Comendador J. et al. Sutureless aortic bioprosthesis. Interact Cardiovasc Thorac Surg. 2017 Jul 1;25(1):114-121)*

These new valves have therefore helped strongly retake the MIS approach due to their reproducibility and ease of implantation in such a small surgical area. In addition, MIS mortality has gradually decreased since these prostheses were first implanted (41). There is also CADENCE (26), a clinical trial with only 100 patients that evaluated the results of the Intuity sutureless prosthesis using MIS compared with the implantation of the conventional prosthesis by median sternotomy, showing only a better ischaemia time and better haemodynamics. Sutureless prostheses enable standardization, simplification and MIS approaches that conventional prostheses have not yet made possible. A number of studies have analysed sutureless prostheses, comparing mini-sternotomy and right anterior mini-thoracotomy approaches. The studies have shown that the 2 approaches are safe and reproducible and provide good clinical results and significantly reduced clamping and CPB times compared with conventional AVR (42). Other studies have compared MIS with sutureless prostheses versus conventional prostheses, showing excellent results for these new prostheses, such as reduced surgical times, reduced mechanical ventilation times, lower morbidity and excellent haemodynamics, which ultimately result in better long-term survival (43).

#### 2.3.5.3 Excellent haemodynamics

Although it is a continuously debated topic, PPM has been recently associated with less symptom relief, less regression of the left ventricular hypertrophy, poorer haemodynamics at rest or during exercise, more heart events after the intervention and lower long-term survival [40]. Patients at particular risk of PPM are those with small aortic rings, which are more common in smaller patients, patients with obesity and elderly women with multiple comorbidities (44). A solution for preventing PPM in these patients is an enlargement of the aortic ring, which represents a long complex technical operation, entailing a greater surgical risk than

normal. The other solution is the sutureless prosthesis, due to its easy and rapid implantation. This patient group with a high risk of PPM is one of the groups that could benefit most from this new technology. The mean gradients of the conventional aortic prostheses (regardless of their size) are higher than those obtained with sutureless prostheses (45). The mean gradients of these new prostheses are usually almost 10mmHg, even below those achieved by TAVI (almost 15 mmHg) (Figure 16). An additional surprising fact is that these gradients are maintained and even improve over time during the first few years (46).

Table 3. Mean aortic gradients after the implantation of sutureless prosthesis and transcatheter aortic valve implant (TAVI) in different studies.

| **Parameters (mmHg)** | **Enable 3F** | **Perceval S** | **Intuity** | **TAVI** |
|---|---|---|---|---|
| **Mean gradient at 3 months** | | | | |
| **Santarpino et al., 2014** | | 13.3 ± 3.9 | | 14.2 ± 5.8 |
| **Borger et al., 2014** | | | 8.5 ± 3.4 | |
| **Leon et al., 2010** | | | | 11.1 ± 6.9 |
| **Mean gradient at 6 months** | | | | |
| **Martens et al., 2011** | 9.4 ± 3.6 | | | |
| **Mean gradient at 1 year** | | | | |
| **Kocher et al., 2011** | | 10.0 | | |
| **Martens et al., 2011** | 8.6 ± 3.2 | | 8.4 ± 3.5 | |
| **Eichsteadt et al., 2014** | 9.5 ± 3.8 | | | |

### 2.3.6 Controversies concerning sutureless prostheses

#### 2.3.6.1 Periprosthetic leaks

Periprosthetic leakage is a relevant complication that should always be considered when assessing the results of a valvular prosthesis implantation. We know that TAVI causes a greater number of moderate-to-severe periprosthetic leaks (12%) than conventional AVR, which at 2 years have been shown to be independent predictors

of mortality (47). In the meta-analysis by Phan et al. (32), sutureless prostheses had an incidence rate for perivalvular leakage of 3–4%. However, it was observed that after a short learning curve, the number of perivalvular leaks decreased even further. In the most noteworthy studies with the Perceval S and Intuity (23), the incidence rate for periprosthetic leaks that were greater than mild was <2%, which represents a figure that competes quite well with conventional aortic prostheses.

#### 2.3.6.2 Durability

Bioprosthetic valves in current use have well-known and very acceptable tissue durability. Tissue failure is multifactorial. Stress on the cusps is in part a cause of tissue degeneration. Decades have been spent perfecting the incorporation of tissue into the valve stent housing and using strut material with “spring” properties to reduce the tissue load during cusp closure.

Now we have the same tissue mounted into TAVI valves and self-expanding sutureless aortic bioprostheses. Cusp function can be determined *in vitro* and *in vivo* within a short period of time; however, these new valve designs are mounting the cusp tissue to stents and present a different stress pattern that can lead to premature calcification and cusp degeneration. It takes years to study these adverse events. Because these devices are used in lower-risk and younger patients, the clinical concerns shift from feasibility and function to durability.

In the multicentre study with the largest number of patients and longest term for the Perceval S (48), there was no recorded structural deterioration at 5 years, which a priori is a spectacular result for this time period. Nevertheless, 5 years of follow-up is still a short time compared with the data we have for other conventional prostheses with follow-ups of up to 20–25 years (15). Therefore, the results of these prostheses in the real long term are yet to be written. We should therefore be cautious when extrapolating results to the long term, weighing by way of example the poor results recently published on the durability of TAVI, which showed that 50% of these prostheses malfunctioned at 5 years (48).

A prediction on the long-term durability of Perceval bioprostheses can be the literature that describes the pathology of LivaNova Solo stentless bioprostheses. The valve processing is the same with regard to decalcification treatment. Unfortunately, the results are discordant. In fact, Stanger and colleagues (50) in their single center experience evidenced that Freedom Solo stentless aortic valve (LivaNova London, UK) is safe to implant with excellent early and midterm haemodynamic performance. However, structural valve deterioration was observed in a substantial number of patients after only 5 to 6 years and the need for explantation increased markedly, suggesting lower-than expected durability (Figure 16). On the contrary, Repossini et al. (51) in their multicenter, retrospective study concluded for low rates of SVD, with 10-year survival free of SVD of 90.8%, and free of reoperation due to SVD 91.9% (Figure 17).

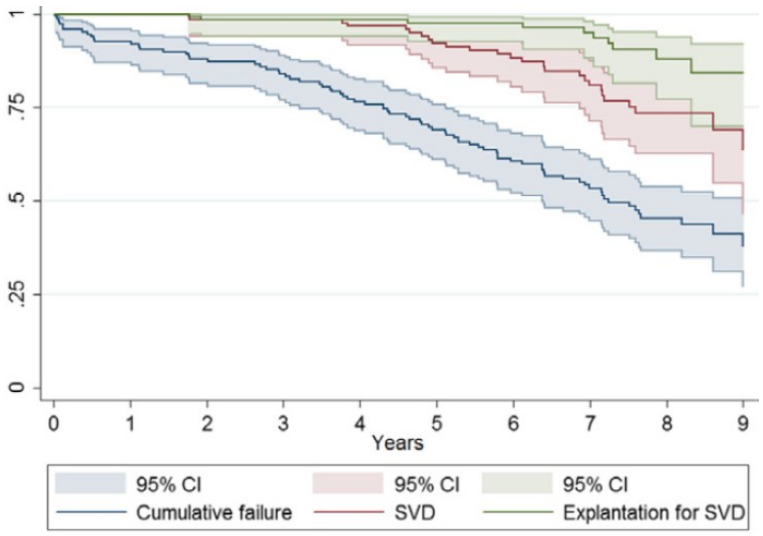



Figure 16. Freedom from SVD, explantation for SVD, and cumulative failure of the Freedom Solo pericardial stentless bioprosthesis *(Stanger O, et al. J Thorac Cardiovasc Surg. 2015 Jul;150:70-7).*

A comparative analysis of the durability of bioprostheses in different studies is influenced by several methodological problems, and a precise definition of SVD is necessary for the proper analysis of data. When SVD is diagnosed only in case of reoperation or death caused by malfunctioning prostheses, the true incidence of

SVD is likely underestimated (52). However, when criteria for SVD classification are arbitrary, a clear overestimation may occur. Additionally, it is important to assess long-term results only for properly implanted prostheses. Any early explant due to technical errors (i.e., inappropriate sizing or malpositioning) has to be considered nonstructural valve dysfunction, as well as aortic regurgitation due to sinotubular junction or annular dilatation.

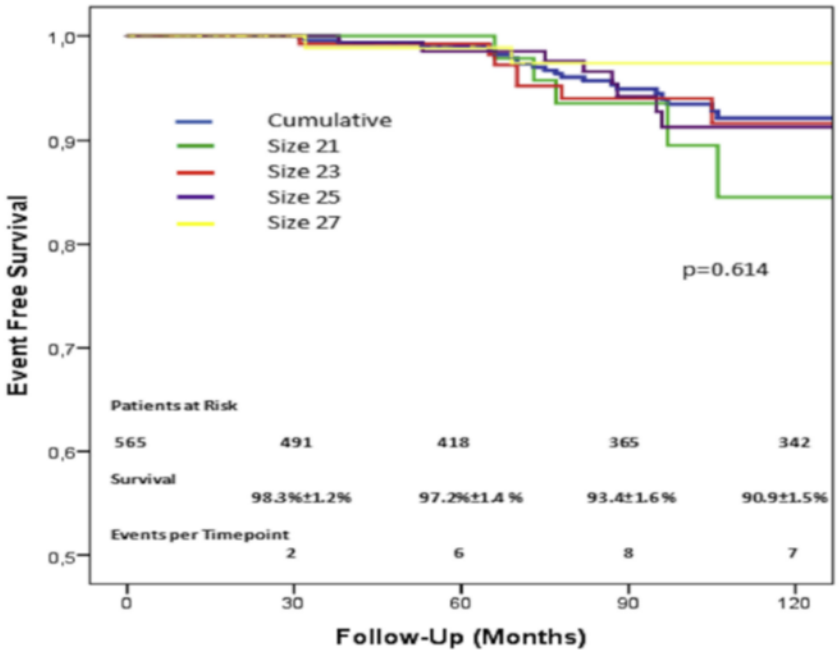



Figure 17. freedom Solo pericardial stentless valve bioprosthesis: Kaplan-Meier curves show outcomes regarding survival and structural valve deterioration-free survival by valve size *(Repossini A. et al., Ann Thorac Surg. 2016 Dec;102:1956-1965)*.

#### 2.3.6.3 Pre- implantation bioprosthesis "Collapsing"

Unlike the traditional pericardial valve xenografts, in which the pericardium is gently manipulated to mold cusps mimicking the native aortic valve, in TAVI, either through the trans-arterial or the trans-apical approach, the pericardial valve is folded ('crimped') to minimize the size while reaching the final set in the aortic root. In addition, in Sapien the prosthetic valve is dilated by ballooning, flattening the pericardium against the stent, to attach the stent itself to the aortic root without suturing, so as to avoid device escape and periprosthetic leak. This process may harm transcatheter valves. Crimping, however, is inevitable in order to be able to

insert transcatheter valves through small-diameter sheaths. The potential effect of crimping on the calcification, strength, and durability of the prosthesis, was evaluated systematically by Kiefer and collegues (53). They sought to analyze the effect of crimping on the Sapien valve in a short-term rat model. They found that precrimping of the Sapien valve seems to have no influence on the grade of calcification; however, it significantly influences the ultrastructure of the pericardial tissue, with higher rate of phagocytosis and cell disruption, with consequent multiple fragmentations of the collagen and elastic fibers (Figure 18).

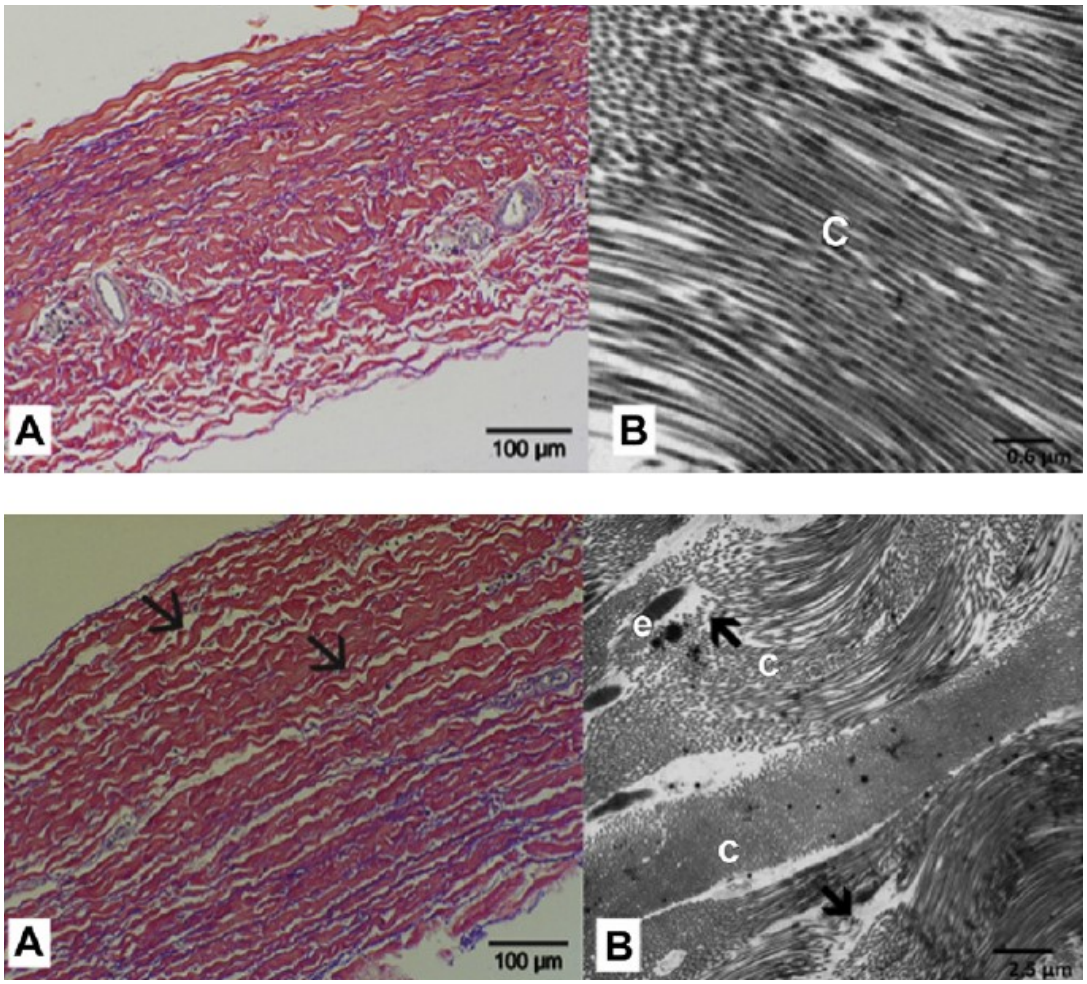



Figure 18. Upper pannel: Samples of uncrimped pericardium Sapien cusps. (A) Typical structure of bovine pericardium mainly composed of regularly structured collagen (red), a few elastic fibers, (purple), and single small vessels (picrosirius red stain) (B) Regular structure of collagen fiber bundles are visible with electron microscopy. Lower panel: Samples of 1-hour crimped pericardium Sapien cusps. (A) Wavy arrangement and single fragmentations of the collagen and elastic fibers

(arrows; picrosirius red stain), (B) Wavy arrangement and focal fragmentation (arrows) of collagen fibers (c) and single elastic fibers (e) are visible with electron microscopy. *(Kiefer et al.: Ann Thorac Surg 2011;92:155–60).*

Zegdi at al. processed for pathological analysis four Edwards Sapien valves (Edwards Lifesciences, Irvine, CA, USA) after implantation procedures (53). There was no macroscopic evidence of traumatic injury to the pericardial cusps of the percutaneous valves: there were no laceration, no dehiscence and no tears. However, pathologic microscopic findings were observed in all of them. These mainly consisted of collagen fiber fragmentation and disruption. The entire thickness of the cusps might be involved. Injury was found in each of the four prostheses evaluated, despite the manipulation of the prosthesis and the delivery catheter was performed by an adequately trained team with a large experience in TAVI (53). The traumatic lesions, however, were more pronounced at the level of the sub-mesothelium (Figure 19). Thiene et al. observed, however, that the meaning of collagen disruption is not clear, in particular as to whether collagen fascicles are really broken or simply interrupted in their natural wavy course. Collagen crimping length should be calculated and compared, to estimate the extent of deployment effect (Thiene et al. 2011). Zegdi et al. [3] should be congratulated for having drawn attention to this crucial issue of pericardial structural injury before TAVI implantation. Prompt and more sophisticated investigations are mandatory to extend these observations and to confirm the alarm.

The severity of the lesions also differed among cusps within the same prosthesis. These lesions may have occurred during the crimping and/or the deployment of the prosthesis. During the crimping process, the bovine pericardium is severely folded and compressed. During dilation of the prosthesis, the bovine tissue is subjected to compression and friction against the stent (53). This is probably why the observed lesions were found to predominate at the level of the ‘smooth’ surface of the pericardium, which is in direct contact with the stent (when the cusps are in an open position).

It is difficult to say at the present time whether these traumatic lesions will have an impact on prosthesis durability. This injury was associated with plasmatic insudation within the cusps, which might secondarily favor cusp calcification (Figure 20).

To what extent these changes correlate with medium- term and long-term durability is unknown so far; however, we deduce from the results of this study that extensive pre-crimping should be avoided (54). For the future, it will be interesting to examine the potential effect of even tighter crimping on durability. This will be of special clinical importance because smaller devices are being developed.

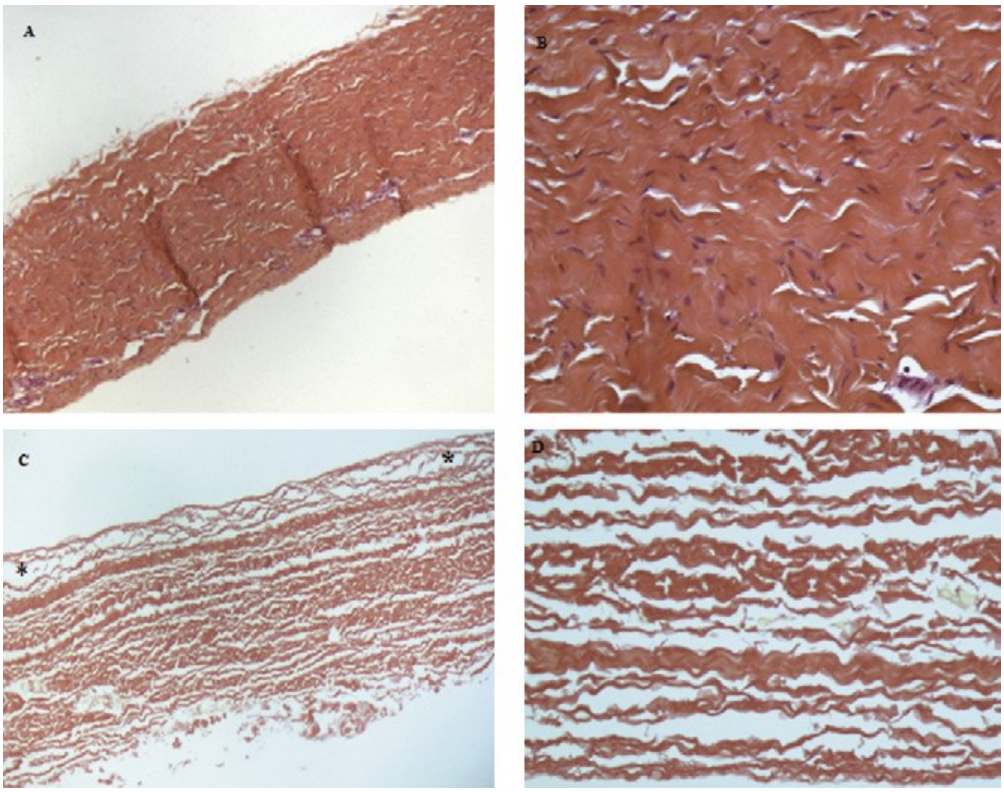



Figure 19. Typical microscopic aspect of pericardium from the control group (A and B) and a balloon-expandable Sapien-Edwards valve (C and D). In this latter, disruption (*) of the collagen fibers is present across the whole thickness of the cusp and predominates at the sub-mesothelium level. (Sirius red stain; x5 (A—C) and x20 (B—D). *(Zegdi et al.: Eur J Cardio-Thorac Surg 2011;40:257–60)*

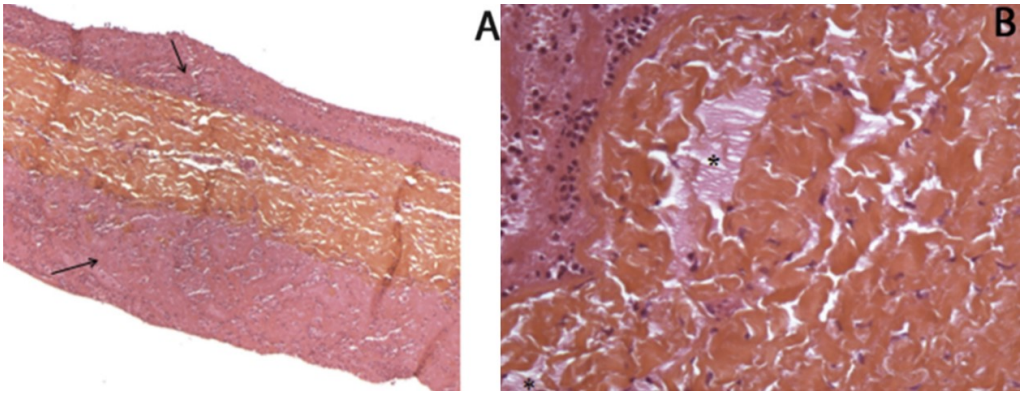



Figure 20. Microscopic aspect (at low (A) and high (B) magnification — H&E stain) of a pericardial cusp from a balloon-expandable Sapien-Edwards valve that migrated after its implantation in a patient. The cusp is covered by thrombosis (arrows). Areas of plasmatic insudation are seen close to the cusp's surface (*). *(Zegdi et al.: Eur J Cardio-Thorac Surg 2011;40:257–60)*

#### 2.3.6.4 Post-surgical need for implanting pacemaker

In the multicentre study by Shrestha et al. (7), the incidence rate for pacemaker implantation was 6%. Nevertheless, there is a wide range in the incidence (2–10%) among various institutions and studies. This variability might be explained by the possible influence of numerous variables involved in this complication such as patient-related factors, baseline conduction disorders (Figure 21), advanced age, annular calcification and reoperations, surgeon-related factors (excessive decalcification of the aortic ring, excess valve size and valve position) and the center's specific protocol for pacemaker implantation.

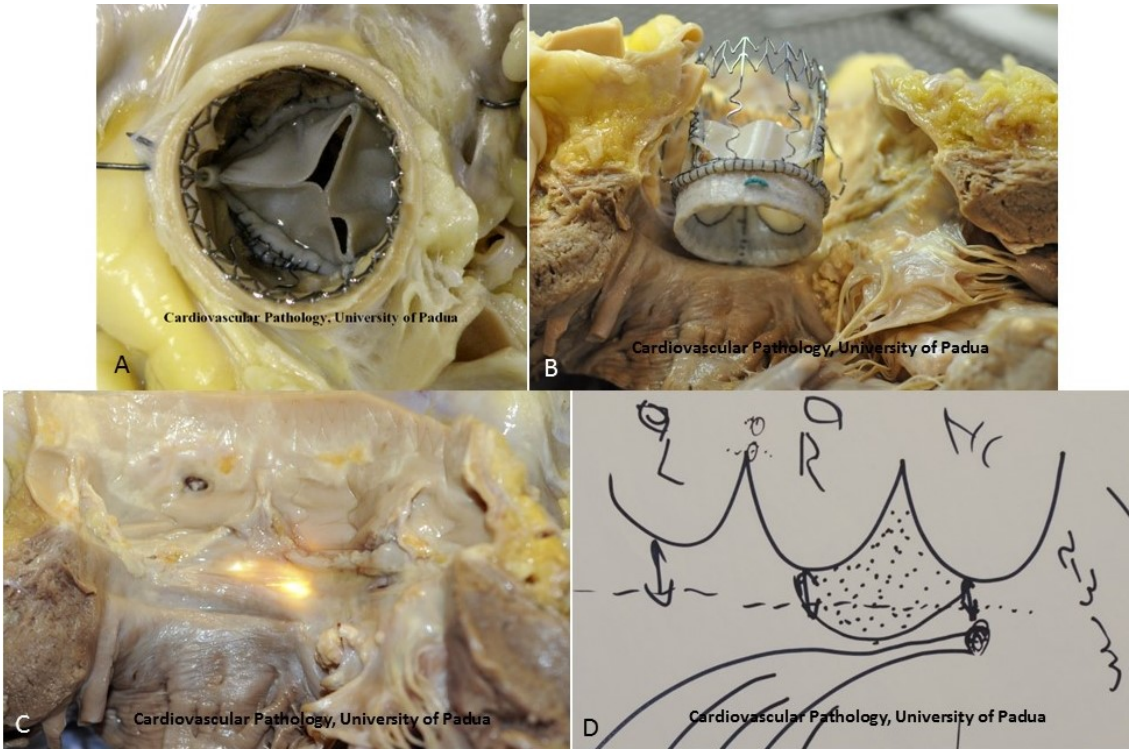



Figure 21. Implant of Perceval S aortic valve bioprosthesis in a cardiac specimen. A) Implant seen from the aorta. B) Perceval S in the aortic root. C) The aortic root with removed cusps, without the bioprosthesis D) Drawing of the distance from the aortic annulus at “nadir” and His bundle. Bioprosthetic annulus should stop at the nadir to avoid the risk of heavy block.

#### 2.3.6.5 Valve migration

There have been sporadic cases of valve migration, which have only been recorded with the 3F Enable prosthesis (16-18). The Perceval S and Intuity prostheses are therefore currently safe in this report.

#### 2.3.6.6 Possibility of performing a valve-in-valve

Percutaneous implantation of the aortic valve as treatment for a degenerated bioprosthesis (valve-in-valve) is a therapeutic alternative, not listed in the clinical practice guidelines but is increasingly being employed. The design of the Perceval and Intuity sutureless prostheses is compatible with valve-in-valve because the cusps are mounted in the interior of the metal frame. For the same size prosthesis, the Perceval S has an internal diameter greater than that of the Intuity because the former does not have the polyester-coated steel skirt of the latter, which is an advantage. Thus, for example, most percutaneous prostheses on the market can be

implanted without problem with the small S sized Perceval (which corresponds to 19 mm). For the small Intuity (19 mm), however, valve-in-valve can only be performed with the smaller Sapien percutaneous prosthesis.

#### 2.3.6.7 Concomitant heart surgery

One of the advantages of these sutureless valves is that they allow practically all types of concomitant heart surgery. In the multicentre study by Shrestha et al. (7), 32% of all implanted Perceval S sutureless prostheses were inserted during concomitant procedures, with good clinical results, low mortality (2.1%) and excellent gradients. The majority of procedures were coronary revascularization surgeries; however, mitral and tricuspid valve surgeries were also performed without complications. There are a number of specific technical considerations if concomitant heart surgery is performed. Proximal anastomosis needs to be performed for the grafts on the aorta, which is performed during the aortic clamping time to prevent a partial clamp and distortion of the sutureless prosthesis, especially with the Perceval S due to its high profile. In order to perform concomitant mitral surgery, we should ensure that there is sufficient aortomitral space so as to not distort the mitral valve and prevent its insufficiency.

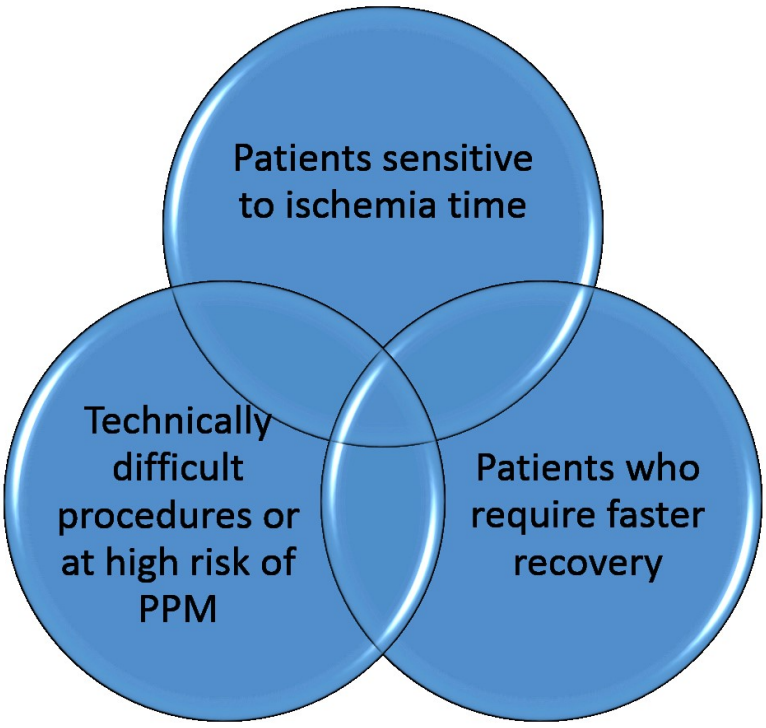


Figure 22. Factors favouring the implantation of a sutureless prosthesis. EF: ejection fraction; PPM: patient–prosthesis mismatch.

Therefore, in practically any surgery with an indication for aortic bioprosthesis implantation and another concomitant heart operation, the reduction in aortic clamping time and CPB time associated with the use of these sutureless prostheses would undoubtedly help minimize the morbidity and mortality of this patient group (Figure 22).

# 3. AIM OF THE STUDY

The sutureless Perceval aortic valve (LivaNova, London, UK) is a device increasingly used in many European cardiac surgery centers. Since the first reports evaluating implantation feasibility and valve safety in humans in 2007, an increasing amount of data have become available, including premarketing clinical results and experience in particular conditions. Overall, excellent performances have been demonstrated in haemodynamic outcomes, safety, and versatility of use. However, several questions remain unanswered, especially regarding the effects of collapsing of the pericardium during the surgical implantation procedure and the long-term durability (the design of this prosthesis closely resembles that of the Freedom Solo stentless prosthesis that was associated with a significant incidence of SVD at 5 years (52).

The research focused on:

1. Analysis of the impact of the “collapsing” in the pericardial tissue (Perceval S) structure through a measurement of potential disruption and loss of elasticity of the collagen.
2. Analysis of Perceval bioprosthesis mode of failure, by reporting Padua pathological experience of Perceval explants and review of the literature.

# 4. MATERIAL AND METHODS

## 4.1 Impact of the "collapsing" in Perceval pericardial tissue

### 4.1.1 Collapsing procedure

The collapsing procedure of Perceval S is performed by a collapsing system composed by a *Dual Collapser* which allows reduction of bioprosthesis diameter, a *base*, which provides support during collapsing phase, and a *Dual Holder and a Smart Clip* which keeps position and releases the valve *in situ (*Figure 23).

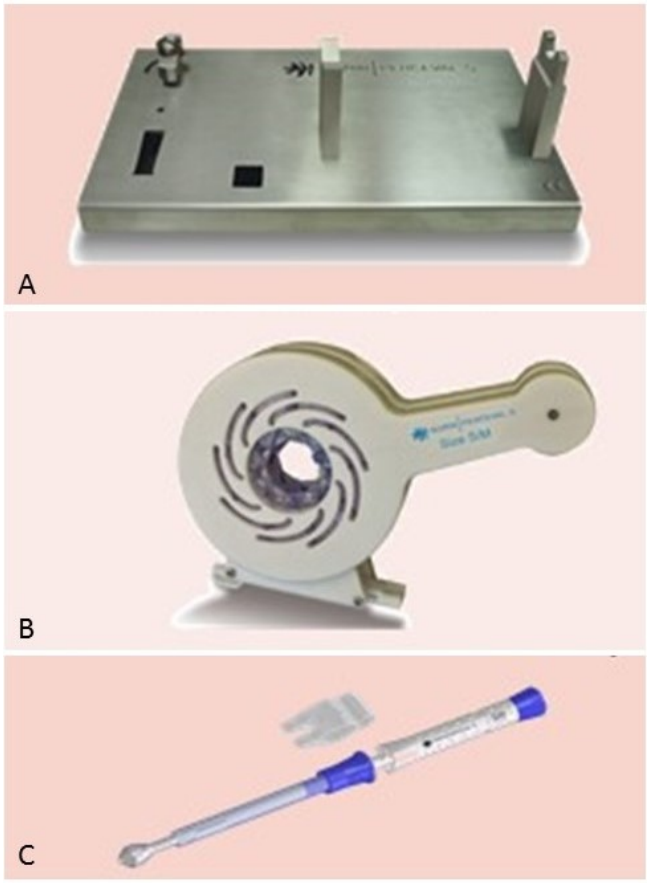



Figure 23. Perceval S collapsing system device: A) Base; B) Dual Collapser; c) Dual holder and Smart clip.

The collapsing system entails radial compression proximally and distally on the anchoring stent without involving the cusps. The valve delivery system is designed to keep only the stent ends collapsed (Figures 24 and 25). During this pre-implantation procedure, the Perceval diameter is approximately 10 mm in size. Ballooning is limited to the distal stent that anchors to the aortic annulus.

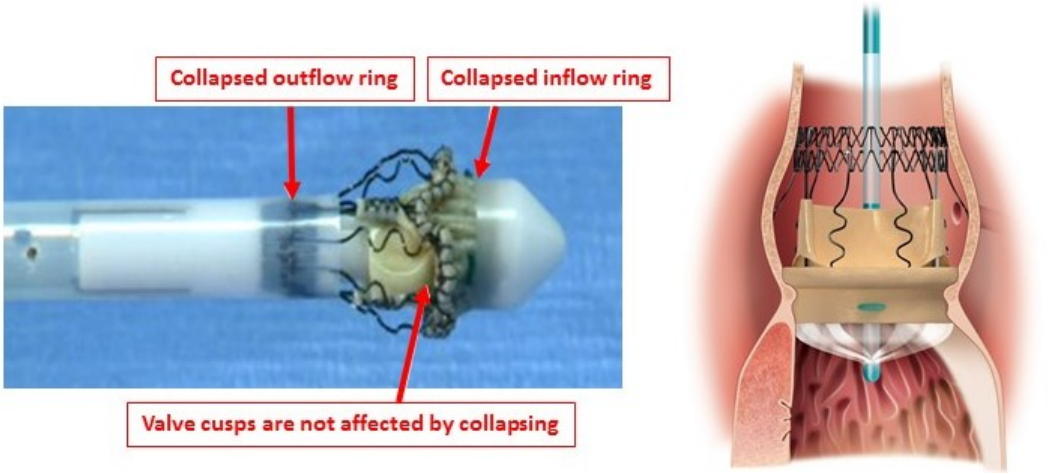



Figure 24. A) Collapsed Perceval S in Dual holder device. B) Perceval S delivered during ballooning procedure.

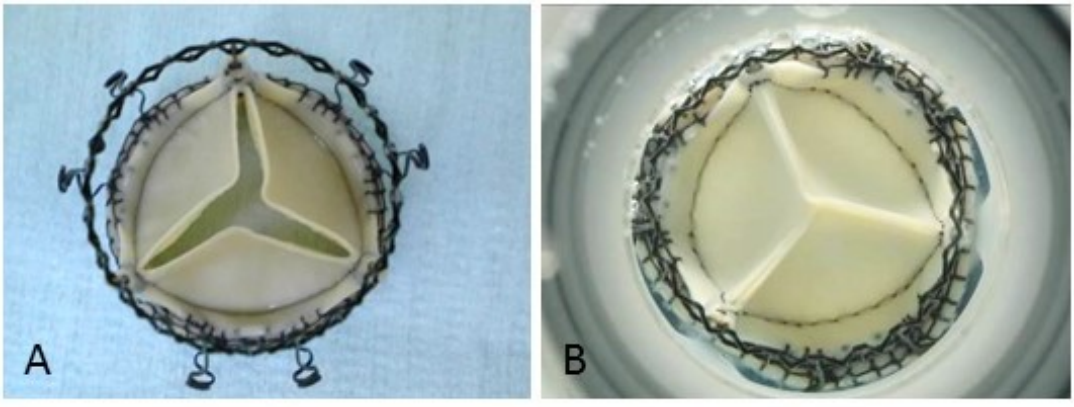



Figure 25. A) Perceval S aortic side. B) Perceval S aortic side as implanted after collapsing.

### 4.1.2 Experimental protocol

Twelve collapsed Perceval sutureless valve prostheses underwent to different collapse times (15, 60 and 180 min duration) followed by ballooning; four uncollapsed Perceval prostheses valves served as control. The cusps were numbered starting from the sewing lines. Samplings from each bioprosthesis were assessed in two cusps (1 and 3) either for histology and scanning electron microscopy (SEM). Cusp number 2 was left intact for further eventual repeating of the investigations.

Sampling was performed in longitudinal and orthogonal direction in order to investigate the collagen fibers in different orientation, as well as in the commissural and stent-implantation region, as shown in Figure 26. Two glutaraldehyde-fixed bovine uncollapsed and unmounted pericardial tissue sheets were used as controls. Gross, histology and scanning electron microscopy (SEM) analysis was performed as well in all.

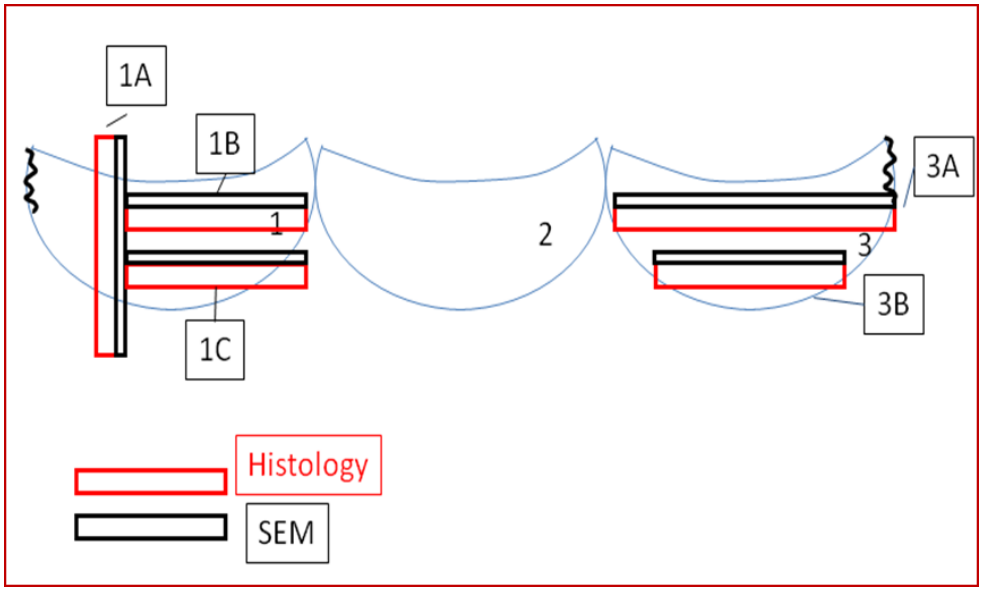



Figure 26. Distribution of the samples harvested in the bioprosthesis under investigation for histology and Scanning Electron Microscopy (SEM). Each cusp is identified (1, 2 and 3). The different sampling regions of the same cusp are then named with a letter (A, B and C).

### 4.1.3 Morphological evaluation procedures

#### 4.1.3.1 Histology

Pericardium samples, after fixation in 4% formaldeyde in phosphate buffer 0.1M pH 7.2, and dehydration in ethanol crescent series, were embedded in paraffin, and 3-4 µm thick sections were stained with hematoxylin–eosin (HE) to detect cells, Azan Mallory, Heidenhein modified for both cells and fibrillar extracellular matrix, Weigert-Van Gieson to evidence collagen and elastin, and Picrosirius red to evidence collagen fibers waviness at light microscopy and under polarized lens; all these sections were observed at the microscope Zeiss Axioplan 2 (Carl Zeiss, Oberkochen, Germany).

#### 4.1.3.2 SEM

SEM was performed on samples adjacent to histology samples. After removal from the 4% formaldehyde in phosphate buffer 0.1M, pH 7.2, the material was over fixed in 2.5 % glutaraldehyde in the same buffer for the material was washed first in normal saline solution and then in distilled water. Subsequently, it was dehydrated in crescent series of alcohol and processed for $CO_2$ critical point drying and gold platinum sputtering. The specimens were observed under scanning electron microscope Philips XL 30 (FEI Company, Eindhoven, NL).

#### 4.1.3.3 Morphometrical Analysis

To assess a potential deformation of collagen after collapsing procedures, collagen fibers wave periodicity was measured (54, 55). All Picrosirius red stained sections were analyzed: five random not overlapping fields per section at light microscope and under polarized lens (Figure 27) were acquired at 200x magnification by using an image analysis system constituted by optical microscope Zeiss Axioplan 2 (Carl Zeiss, Oberkochen, Germany) equipped with the digital photocamera AxioVision, (Carl Zeiss, Oberkochen, Germany) and the morphometrical imaging analyzer software Image PRO-Plus 5.1 (Media Cybernetics, Silver Spring, MA, USA). Collagen fiber periodicity, when clearly defined on the image, was measured, with the modality *measurement/length* of the software previously described, based on the

length of the period of the collagen (Figure 27B). At least 100 measurement values obtained per field were collected in a file excel database and analyzed.

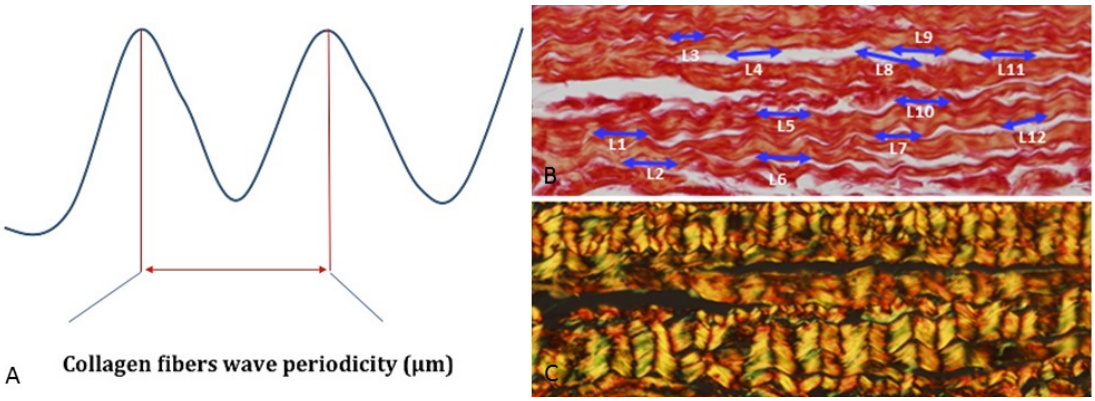



Figure 27. A) Schematic representation of collagen period length measurement. B) Pericardial tissue cusp stained with Picrosirius red; C) the same field of B at polarized light. Some blue double arrows, in only representative number, indicate the collagen fibers wave periodicity linear measurement modality.

### 4.1.4 **Statistical analysis**

For descriptive analysis data are expressed as means±SD. The Student t test was used for comparative analysis of the data among the groups. A p value of less than 0.05 was considered to be statistically significant.

## 4.2 Perceval bioprosthesis mode of degeneration and dysfunction

### 4.2.1 Definitions

***Prosthetic valve performance***

The Valve Academic Research Consortium (VARC) established an independent collaboration between Academic Research organizations and specialty societies (cardiology and cardiac surgery) in the USA and Europe. Two meetings, in San Francisco, California (September 2009) and in Amsterdam, the Netherlands (December 2009), including key physician experts, and representatives from the US Food and Drug Administration (FDA) and device manufacturers, were focused on creating consistent endpoint definitions and consensus recommendations for implementation in TAVI clinical research programs. Important considerations in developing endpoint definitions included (i) respect for the historical legacy of surgical valve guidelines; (ii) identification of pathophysiological mechanisms associated with clinical events; (iii) emphasis on clinical relevance. Consensus criteria were developed for the following endpoints: mortality, myocardial infarction, stroke, bleeding, acute kidney injury, vascular complications, and prosthetic valve performance (56). The sutureless bioprostheses, due to the intermediate position between the TAVI procedures and the AVR procedures, may benefit of the VARC definitions of the prosthetic valve perfomance, excluding obviously the merely transcatheter properties/complications. The clinical presentation of patients with prosthetic valve dysfunction is usually consistent with symptoms and signs of either valvular regurgitation or stenosis. VARC proposes only two criteria to evaluate impaired prosthetic valve performance: (i) prosthetic valve haemodynamics assessed by echocardiography and (ii) associated clinical findings indicating impaired cardiovascular or valvular function (56) (e.g. new or worsening congestive heart failure) (Table 4).

Table 4. Potential failure modes of prosthetic valve dysfunction *(M.B. Leon et al., VARC consensus endpoints after TAVI for high risk AS; European Heart Journal (2011) 32, 205–217).*

| |
|---|
| Aortic stenosis |
| Stent creep |
| Pannus |
| Calcification |
| Support structure deformation (out-of-round configuration), under-expansion, fracture, or trauma (cardio-pulmonary resuscitation, blunt chest trauma) |
| Mal-sizing (prosthesis-patient mismatch) |
| Endocarditis |
| Prosthetic valve thrombosis |
| Native leaflet prolapse impeding prosthetic leaflet motion |
| Aortic regurgitation |
| Pannus |
| Calcification |
| Support structure deformation (out-of-round configuration), recoil, under-expansion, fracture, insufficient radial strength, or trauma (cardio-pulmonary resuscitation, blunt chest trauma) |
| Endocarditis |
| Prosthetic valve thrombosis |
| Malposition (too high, too low) |
| Acute mal-coaptation |
| Leaflet wear, tear/perforation, prolapse, or retraction |
| Suture breakage or disruption |
| Native leaflet prolapse impeding prosthetic leaflet motion |

***Prosthetic aortic stenosis and regurgitation***

The severity of prosthetic aortic valve stenosis is graded as (i) normal, (ii) mild, or (iii) severe and prosthetic aortic valve regurgitation (central or paravalvular) as (i) mild, (ii) moderate, or (iii) severe (56, 57) (Table 5).

Table 5. Prosthetic aortic valve stenosis, regurgitation and mismatch criteria. *(M.B. Leon et al., VARC consensus endpoints after TAVI for high risk AS; European Heart Journal (2011) 32, 205–217, updated to 2013).*

| | Prosthetic aortic valve stenosis* | | |
|---|---|---|---|
| | Normal | Mild stenosis | Moderate/severe stenosis |
| Quantitative parameters (flow-dependent)† | | | |
| Peak velocity (m/s) | <3 m/s | 3-4 m/s | >4 m/s |
| Mean gradient (mm Hg) | <20 mm Hg | 20-40 mm Hg | >40 mm Hg |
| Quantitative parameters (flow-independent) | | | |
| Doppler velocity index‡ | >0.35 | 0.35-0.25 | <0.25 |
| Effective orifice area§ | >1.1 $cm^2$ | 1.1-0.8 $cm^2$ | <0.8 $cm^2$ |
| Effective orifice area‖ | >0.9 $cm^2$ | 0.9-0.6 $cm^2$ | <0.6 $cm^2$ |
| | **Prosthesis–patient mismatch (PPM)** | | |
| | **Insignificant** | **Moderate** | **Severe** |
| Indexed effective orifice area¶ ($cm^2/m^2$) | >0.85 $cm^2/m^2$ | 0.85-0.65 $cm^2/m^2$ | <0.65 $cm^2/m^2$ |
| Indexed effective orifice area# ($cm^2/m^2$) | >0.70 $cm^2/m^2$ | 0.90-0.60 $cm^2/m^2$ | <0.60 $cm^2/m^2$ |
| | **Prosthetic aortic valve regurgitation** | | |
| | **Mild** | **Moderate** | **Severe** |
| Semiquantitative parameters | | | |
| Diastolic flow reversal in the descending aorta—PW | Absent or brief early diastolic | Intermediate | Prominent, holodiastolic |
| Circumferential extent of prosthetic valve paravalvular regurgitation (%)** | <10% | 10%-29% | ≥30% |
| Quantitative parameters‡ | | | |
| Regurgitant volume (mL/beat) | <30 mL | 30-59 mL | ≥60 mL |
| Regurgitant fraction (%) | <30% | 30-49% | ≥50% |
| EROA ($cm^2$) | 0.10 $cm^2$ | 0.10-0.29 $cm^2$ | ≥0.30 $cm^2$ |

*PW*, Pulsed wave; *EROA*, effective regurgitant orifice area. *In conditions of normal or near normal stroke volume (50-70 mL). †These parameters are more affected by flow, including concomitant aortic regurgitation. ‡For LVOT >2.5 cm, significant stenosis criteria is <0.20. §Use in setting of BSA ≥1.6 $cm^2$ (note: dependent on the size of the valve and the size of the native annulus). ‖Use in setting of BSA <1.6 $cm^2$. ¶Use in setting of BMI <30 $kg/cm^2$. #Use in setting of BMI ≥30 $kg/cm^2$. **Not well-validated and may overestimate the severity compared with the quantitative Doppler.

The clinical significance of prosthetic valve dysfunction is further supported by the presence of clinical signs, symptoms, and/or events (e.g. re-hospitalization for worsening symptoms, re-operation or death).

***Prosthetic aortic valve thrombosis and endocarditis***

Valve thrombosis is any thrombus attached to or near an implanted valve that occludes part of the blood flow path, interferes with valve function, or is sufficiently large to warrant treatment. Furthermore valve thrombus, found at autopsy in a patient whose cause of death was not valve related or found at operation for an unrelated indication, should also be reported as valve thrombosis (56, 57).

The diagnosis of prosthetic valve endocarditis is based on one of the following criteria:

- Reoperation with evidence of abscess, paravalvular leak, pus, or vegetation confirmed as secondary to infection by histological or bacteriological studies;
- Autopsy findings of abscess, pus, or vegetation involving a repaired or replaced valve;
- In the absence of reoperation or autopsy, fulfilling the Duke Criteria for endocarditis.

***Prosthetic valve 'associated' complications***

Sutureless aortic valves, similarly to the TAVI valves, may come in close contact with the anterior mitral valve leaflet, the intervalvular fibrosa, the aortic annulus, the ventricular septum, the aortic sinuses and root, the coronary arteries, and the cardiac conduction system. Collectively, these anatomic structures, which are contiguous with the prosthetic aortic valve, are referred to as the aortic valvular complex (Figure 28). As such, prosthetic aortic valve procedures, and in particular sutureless and TAVI, may have untoward effects on any of these structures which may result in important clinical consequences. Therefore, VARC proposes to group these complications as a separate endpoint category (56, 57).

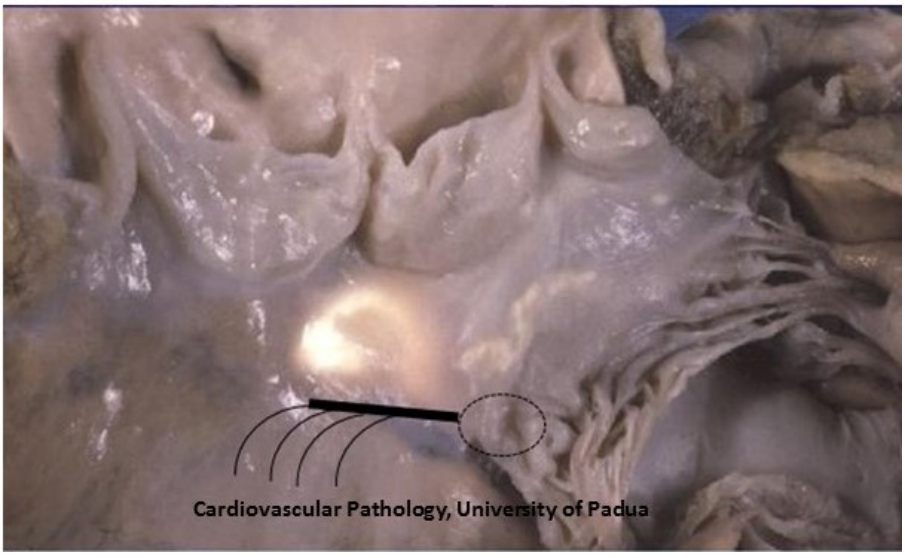



Figure 28. The aortic root and the course His bundle underneath the membranous septum.

### 4.2.2 **Materials**

Thirtythree Perceval bioprostheses inmplanted in humans and explanted in different centers, from July 2007 to January 2017, participating to PILOT TRIAL V10601, PIVOTAL TRAIL V10801, and CAVALIER TRIAL TPS001 were examined at the Padua University, at Cardiovascular Pathology Unit. Demographic data provided, centre of implantation and explantation, size of the bioprosthesis, time in place, cause of dysfunction and explantation were entered in a dedicated database (Table 6).

Table 6. Perceval bioprostheses analyzed at Padua Cardiovascular Pathology Unit.

| Model | Number | Number of valves per Size | Gender | Mean age at implantation (years) | Time in place (months) |
|---|---|---|---|---|---|
| **Perceval (LivaNova, London, UK)** | 33 | 25mm - 8<br>23mm - 19<br>21mm - 6 | 10 male (30%)<br>23 female (70%) | 75.78±7.31<br>(range: 65-90) | 12.51±19.49<br>(range: 0-63) |

The PIVOT TRIAL V10601, PIVOTAL TRAIL V10801, and CAVALIER TRIAL TPS001 were designed to evaluate the safety and effectiveness of the Perceval bioprosthesis as a sutureless option for the treatment of aortic valve stenosis (Table 7).

Table 7. Trials characteristics and number of valve bioprostheses explanted from each trial population.

| Trial | Pilot | Pivotal | Cavalier |
|---|---|---|---|
| **Enrolment status** | Completed<br>2007 – 2008 | Completed<br>2009 - 2010 | Completed<br>2010 - 2013 |
| **Patients and centres** | 30 pts<br>3 EU centres | 150 pts<br>9 EU centres | 658 pts<br>26 EU centres |
| **Age (inclusion criteria)** | ≥ 75 years | ≥ 75 years | ≥ 65 years |

| | | | |
|---|---|---|---|
| **Follow-up** | 5 years completed | Up to 5 years ongoing | Up to 5 years ongoing |
| **Endpoints** | Feasibility, Safety 30 days | Safety, Effectiveness 3-6 months | Safety, Effectiveness 12 months |
| **Bioprostheses size** | S-M | S-M | S-M-L |
| **Surgical Approach** | Median sternotomy | Median and Mini sternotomy | Median and Mini sternotomy |
| **Number of prostheses examined at Padua Pathology Core Lab** | 1/33 (3%) | 17/33 (51.5%) | 15/33 (45.5%) |

### 4.2.3 Bioprostheses evaluation procedures

#### 4.2.3.1 Gross examination and radiography

Each Perceval bioprosthesis was grossly analyzed and submitted to radiography with the XPERT 80 cabinet x-ray system for pathological anatomy (Kubtec Medical Imaging, Stratford, CT, USA). The presence of calcium deposits was quantified on the basis of a 0–4 score as follows (1): 0 = absent; 1 = focal, pinpoint, <1 mm of diameter; 2 = focal, >1 mm of diameter or pinpoint multiple; 3 = multiple >1 mm of diameter; 4 = massive deposition. (58).

#### 4.2.3.2 Histology

Bioprosthetic samples underwent histological analysis. The histology preparation procedures are the same as reported in section 1 methods. Gram stain was employed to detect bacteria, while Von Kossa stains was used to detect of $Ca^{2+}$ deposits.

#### 4.2.3.3 Morphometrical analysis

To assess a potential reduction of the effective orifice area (EOA) due to fibrous tissue overgrowth, the ratio between the EOA area and the total area of the bioprosthesis considering the inner stent diameter on ventricular side were

measured (Figure 29). The ratio was expressed in percentage. The measurements were performed with the same image analysis system reported in section 1 methods. The modality *measurement/area* was chosen.

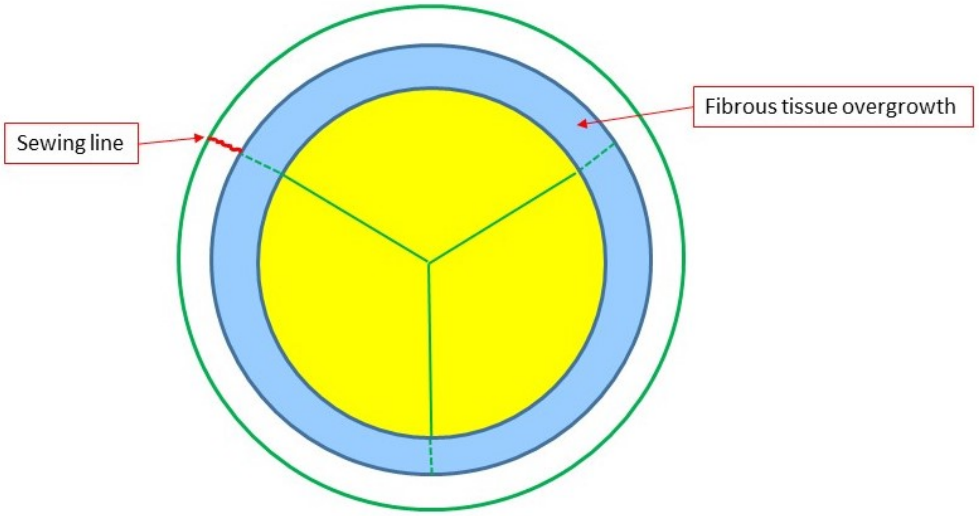



Figure 29. Schematic representation of Perceval aortic bioprosthesis on ventricular side. In yellow is represented the EOA, while in blue the fibrous tissue overgrowth area. For the calculation of the EOA reduction was considered the ratio yellow area/blu+yellow areax100.

### 4.2.4 **Statistical analysis**

For descriptive analysis, categoric data are expressed as absolute and relative frequencies, and continuous data are expressed as means±SD. Cumulative survival and freedom from events were estimated using the Kaplan-Meier method, with 95% confidence intervals (CIs). The Student t test was used for comparative analysis of haemodynamic data. Cox regression was performed to identify independent predictors for SVD. A p value of less than 0.05 was considered to be statistically significant. Wald statistics was used to identify the association between time in place and the valve orifice reduction.

# 5. RESULTS

## 5.1 Impact of the "collapsing" in Perceval pericardial tissue

Perceval bioprosthetic valves, after valve collapse and deployment, revealed no macroscopic evidence of traumatic injury to the pericardial cusps. There were optimal pericardial cusp coaptation in the absence of tears, perforations or folding in all (Figure 30). Prosthetic frame showed a preserved shape without distortion.

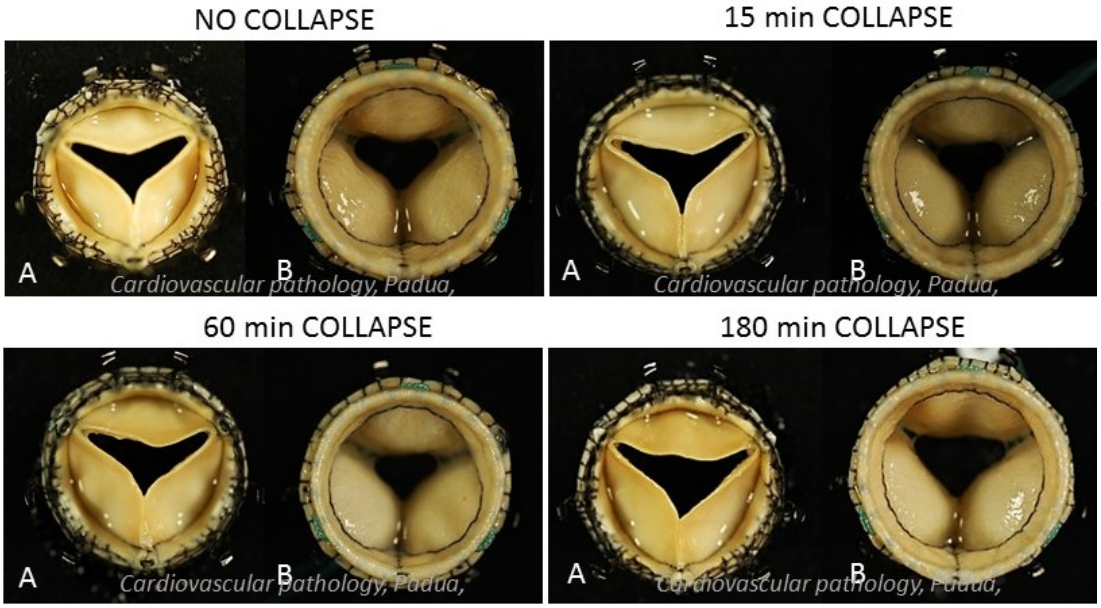



Figure 30. Gross images of Perceval S bioprostheses before collapse and after collapsing and ballooning procedures for 15, 60 and 180 minutes. A) aortic view; B) ventricular view.

### Microscopic evaluation

Ultrastructural SEM analysis showed optimal preservation of the pericardial tissue. At the same magnification, tridimensional orientation of collagen bundles was

maintained in all bioprosthesis valves. No distortion also in depth of collagen fibers we detected (Figures 31 A-D). Histology slides show an intact preservation of the collagen bundles with no disruptions, interruptions neither fragmentations (Figures 31 E-P). The collagen periodicity waviness of the fibrosa collagen fibers was unaltered when compared to controls (Figures 31 I-T). The surface microstructure, in particular collagen bundles, did not show any changes owing to collapsing in bioprostheses cusps study groups (Figure 32).

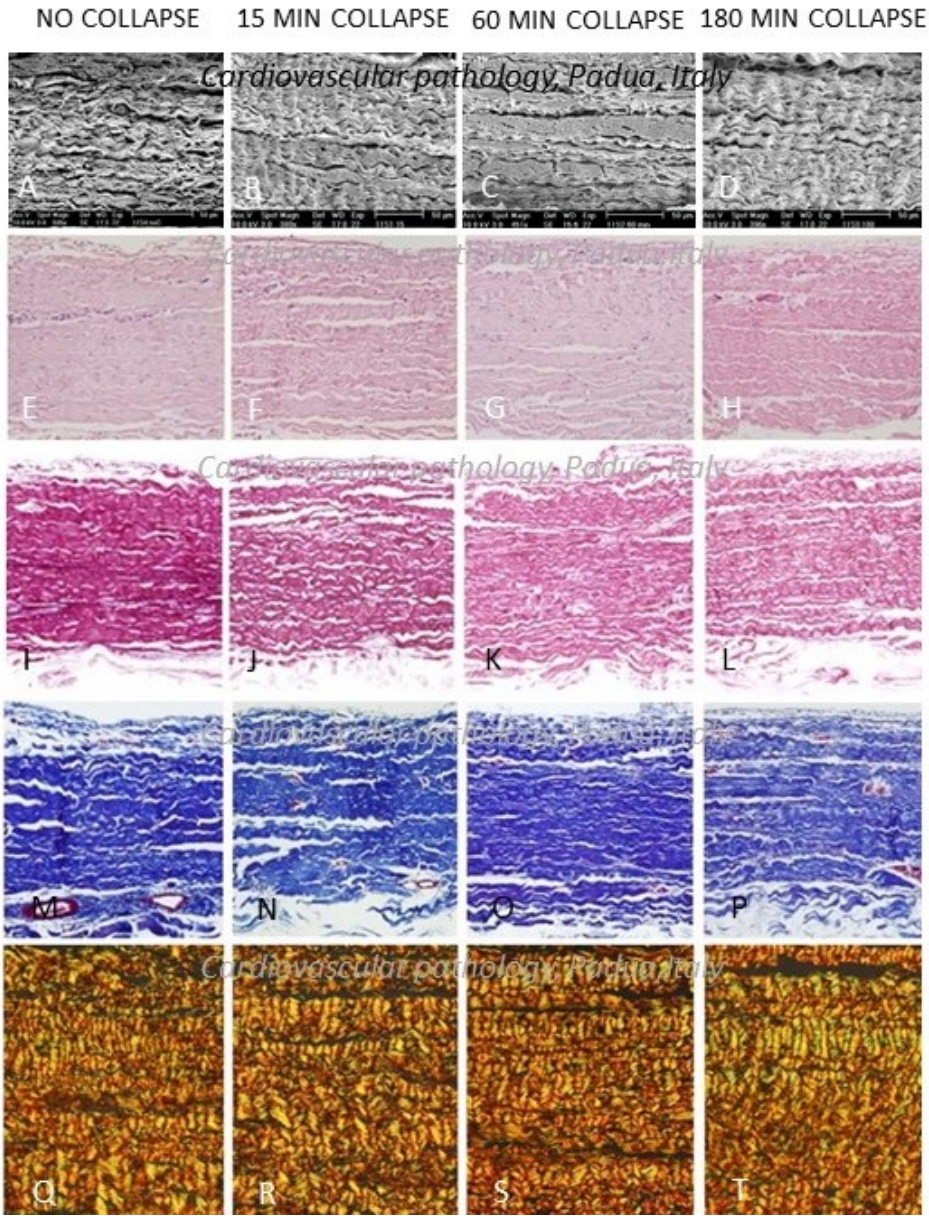



Figure 31. Collagen fibers waviness of pericardial cusps in control and at different times of collapse. A-D) SEM images; E-H) HE, I-L) Elastic Van Gieson; M-P) Azan

Mallory Heidenhein modified and Sirius Red stain at polarized light (Q-T). E-T) 125x, original magnification.

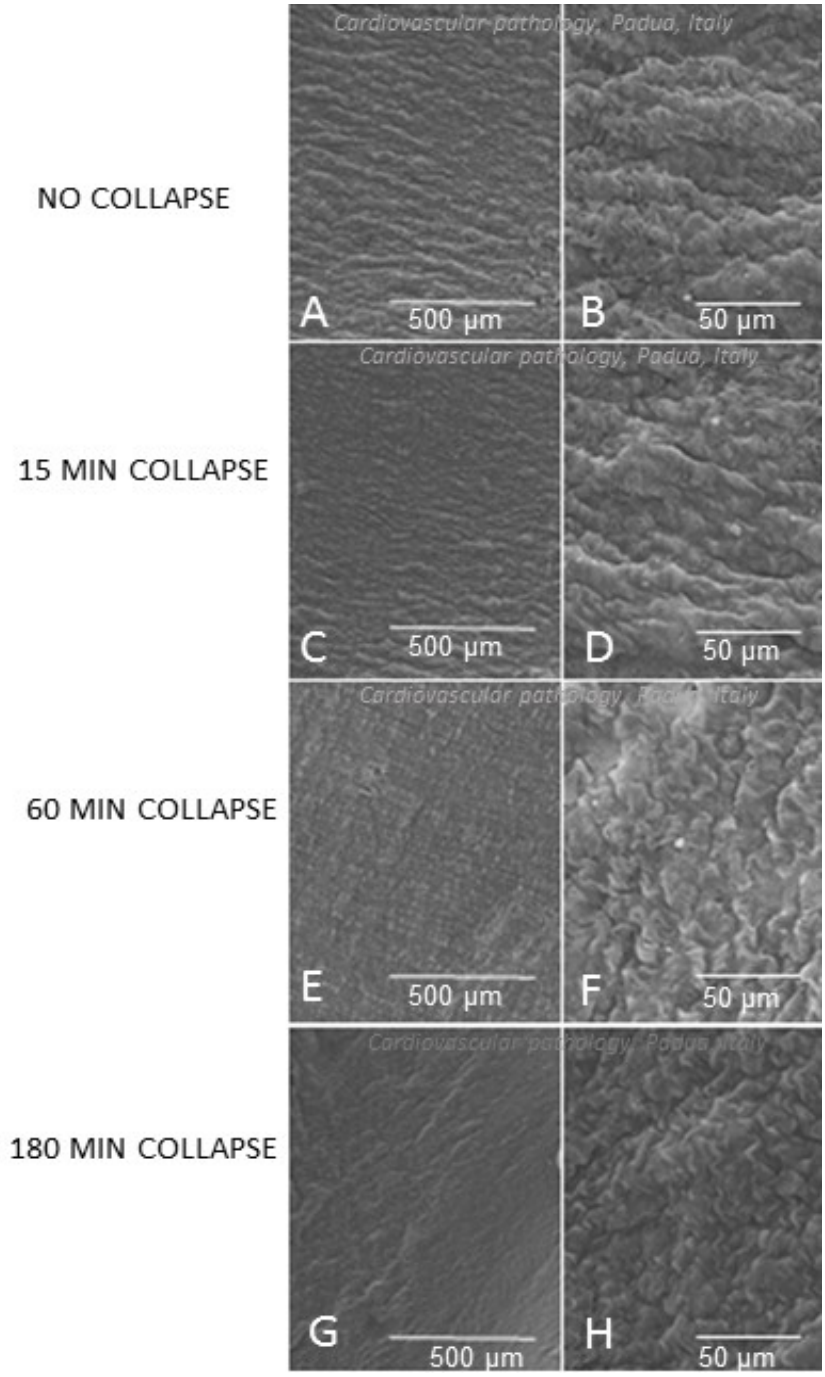
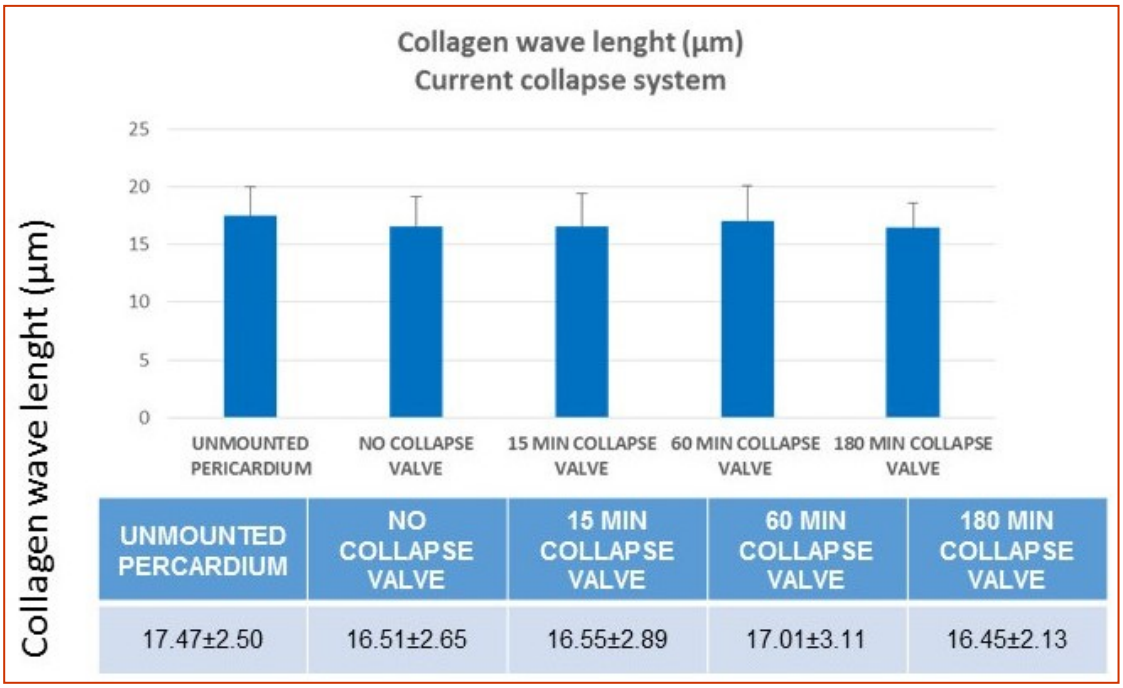



Figure 32. SEM images of the cuspal surfaces of uncollapsed valves at low (A) and at higher magnification (B) as well as at different times of collapse at low (C, E, F) and at higher magnification (D, F, H). Note microstructure cuspal surface preservation, especially collagen bundles, with no injury owing to collapsing. A, C, E, G)50x original magnification; B, D, F, H)500x original magnification.

## Morphometrical analysis

Fibrosa collagen wave length periodicity measurements data did not reveal any statistically significant differences among the study groups (15 min collapse: 16.55±2.89 µm; 60 min collapse: 17.01±3.11 µm; 180 min collapse: 16.45±2.13 µm) and the uncollapsed controls (16.51±2.65 µm) and the unmounted pericardium (17.47±2.50 µm) (P=NS) (Table 8).

Table 8. Histogram displaying collagen wave length at different collapsing time in comparison with controls (uncollapsed bioprosthesis valves and unmounted pericardium). All values are expressed as mean±S.D. No differerences were observed among the groups.




| UNMOUNTED PERCARDIUM | NO COLLAPSE VALVE | 15 MIN COLLAPSE VALVE | 60 MIN COLLAPSE VALVE | 180 MIN COLLAPSE VALVE |
|---|---|---|---|---|
| 17.47±2.50 | 16.51±2.65 | 16.55±2.89 | 17.01±3.11 | 16.45±2.13 |

## 5.2 Perceval bioprosthesis mode of failure and dysfunction

Thirtythree bioprostheses were examined after explantation at Padua Pathology Core Lab one by one by expert cardiovascular pathologists, in collaboration with the author, who is a cardiac surgeon. Description of all retrieved specimens, individually, with TIP, patient age, trial of origin, predominant cause of failure and pathological findings are presented in Table 9. The incidence of explantation was of 3% (1/30pts) in the Pilot trial, 11.3% (17/150 pts) in the Pivotal trial and 2.3% (15/658 pts) in the Cavalier trial. Of the 33 Perceval bioprostheses analyzed, nine (27.3%) were explanted at less than one month of time in place (TIP). When compared the two groups of bioprostheses (TIP 0 months versus TIP ≥ 1 month), the patients of the second group were younger, treated with larger bioprostheses, with predominant stenosis as cause of explantation (Table 10). On the other hand, the valve bioprostheses with less than one month of TIP were predominantly explanted for incompetence mostly due to PVL, although the declaired failure of the surgical implant was recorded in seven cases due to learning curve.

Main cause of failure was endocarditis diagnosed in 36% of all bioprostheses, calcific dystrophy in 12%, fibrous pannus overgrowth in 12% and PVL in 12% (Table 11). In total fibrous tissue overgrowth (on the valve and on the stent) was 61%, with and incidence of almost 83% in the bioprostheses with TIP more than one month (Table 10).

The fibrous tissue was in 20% the sole pathology of the valve, in 50% associated with endocarditis, in 20% associated to calcific dystrophy (Figure 33) and 10% associated to PVL (Figure 34).

Table 9. Description of all retrieved specimens.

Continuation of Table 9.

| # Bp | Gender | Age At Impl. (Years) | Size (mm) | Time In Place (Months) | Causes of Failure | Predominant Pathological Findings cause Of Dysfunction | Valve Orifice Reduction (%) | Study | Centre |
|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 75 | 23 | 1 (13 DAYS) | Autopsy - UNKONWN | NONE | 0 | PIVOT TRIAL V10601 | Hannover (D) |
| 2 | F | 75 | 23 | 0 (3 DAYS) | INCOMPETENCE | PARAVALVULAR LEAK | 0 | PIVOTAL TRAIL V10801 | Paris (F) |
| 3 | M | 75 | 23 | 3 | INCOMPETENCE | ACUTE ENDOCARDITIS PARAVALVULAR LEAK | 0 | PIVOTAL TRAIL V10801 | Leuven (B) |
| 4 | F | 79 | 21 | 1 (7 DAYS) | INCOMPETENCE | PARAVALVULAR LEAK | 0 | PIVOTAL TRAIL V10801 | Paris (F) |
| 5 | F | 72 | 23 | 1 | STENO-INCOMPETENCE | ACURE INFECTIVE ENDOCARDITIS PARAVALVULAR LEAK (pannus in valve+pannus in nitinol) | 0 | PIVOTAL TRAIL V10801 | Hannover (D) |
| 6 | F | 86 | 23 | 0 | INCOMPETENCE | PARAVALVULAR LEAK | | PIVOTAL TRAIL V10801 | Paris (F) |
| 7 | F | 75 | 23 | 10 | STENOSIS | ACUTE ENDOCARDITIS (pannus in valve + pannus in nitinol) | 39,64 | PIVOTAL TRAIL V10801 | Essen (D) |
| 8 | F | 71 | 25 | 0 | INCOMPETENCE - TECHNICALLY FAILED SURGICAL IMPLANTATION | NONE | 0 | PIVOTAL TRAIL V10801 | Bad Oeynhausen (D) |
| 9 | F | 90 | 21 | 0 | INCOMPETENCE | CUSP RETRACTION | 0 | PIVOTAL TRAIL V10801 | Lille (F) |
| 10 | F | 81 | 23 | 0 | INCOMPETENCE – TECHNICALLY FAILED SURGICAL IMPLANT | PARAVALVULAR LEAK | 0 | PIVOTAL TRAIL V10801 | Leuven (B) |
| 11 | F | 83 | 23 | 0 | INCOMPETENCE | PARAVALVULAR LEAK, | 0 | PIVOTAL TRAIL V10801 | Paris (F) |
| 12 | F | 83 | 21 | 0 | INCOMPETENCE | CUSP RETRACTION | 0 | PIVOTAL TRAIL V10801 | Paris (F) |
| 13 | M | 68 | 25 | 0 | INCOMPETENCE – TECHNICALLY FAILED SURGICAL IMPLANTATION | NONE | 0 | PIVOTAL TRAIL V10801 | Paris (F) |
| 14 | F | 77 | 21 | 0 | INCOMPETENCE | CUSP RETRACTION | 0 | PIVOTAL TRAIL V10801 | Bad Oeynhausen (D) |
| 15 | F | 79 | 23 | 0 | OTHER - CARDIOGENIC SHOCK AT IMPLANT | NONE | 0 | PIVOTAL TRAIL V10801 | Paris (F) |
| 16 | M | 78 | 23 | 6 | ENDOCARDITES | VEGETATIVE INFECTIVE ENDOCARDITIS (pannus in valve) | 11,49 | PIVOTAL TRAIL V10801 | Hannover (D) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 17 | F | 82 | 23 | 2 | STENOSIS | VEGETATIVE INFECTIVE ENDOCARDITIS (pannus in valve +pannus in nitinol) | 29,33 | CAVALIER TRIAL TPS001 | Aalst (B) |
| 18 | F | 84 | 21 | 19 | STENOSIS | FIBROUS PANNUS (pannus in valve +pannus in nitinol) | 24,66 | PIVOTAL TRIAL V10801 | Paris (F) |
| 19 | F | 74 | 25 | 4 | OTHER - FISTULA aorta -right ventricle | HEALED SUBACUTE ENDOCARDITIS (pannus in nitinol) | 0 | CAVALIER TRIAL TPS001 | Braunschweig (D) |
| 20 | M | 81 | 25 | 1 | Autopsy - UNKNOWN | SUBACUTE ENDOCARDITIS | 0 | CAVALIER TRIAL TPS001 | Bern (CH) |
| 21 | M | 80 | 23 | 2 | STENOSIS | INFECTIVE VEGETATIVE ENDOCARDITIS (pannus in nitinol) | 0 | PIVOTAL TRIAL V10801 | Amsterdam (NL) |
| 22 | M | 65 | 23 | 1 | INCOMPETENCE | PARAVALVULAR LEAK (pannus in nitinol) | 0 | CAVALIER TRIAL TPS001 | Paris (F) |
| 23 | F | 75 | 23 | 24 | STENOSIS | FIBROUS PANNUS (pannus in valve+pannus in nitinol) | 13,20 | CAVALIER TRIAL TPS001 | Zabrze (PL) |
| 24 | M | 70 | 25 | 39 | STENOSIS | ACUTE INFECTIVE ENDOCARDITIS (pannus in nitinol) | 30,60 | CAVALIER TRIAL TPS001 | Bochum (D) |
| 25 | M | 77 | 25 | 40 | STENOSIS | SUBACUTE VEGETATIVE ENDOCARDITIS (pannus in valve) | 20,03 | CAVALIER TRIAL TPS001 | Bochum (D) |
| 26 | F | 76 | 25 | 13 | STENOSIS | FIBROUS PANNUS (pannus in valve+pannus in nitinol) | 13,35 | CAVALIER TRIAL TPS001 | Bad Oeynhausen (D) |
| 27 | M | 67 | 23 | 41 | STENOSIS | FIBROUS PANNUS (thrombus organization) (pannus in valve+pannus in nitinol) | 24,37 | CAVALIER TRIAL TPS001 | Bern (CH) |
| 28 | F | 79 | 23 | 53 | STENOSIS | CALCIFIC DISTROPHY FIBROUS PANNUS (pannus in valve+pannus in nitinol) | 20,73 | CAVALIER TRIAL TPS001 | Paris (F) |
| 29 | F | 75 | 23 | 63 | INCOMPETENCE | SUBACUTE INFECTIVE ENDOCARDITIS (pannus in valve+pannus in nitinol) | 14,08 | CAVALIER TRIAL TPS001 | Bern (CH) |
| 30 | F | 49 | 23 | 6 | STENOSIS | VEGETATIVE INFECTIVE ENDOCARDITIS (pannus in valve+ pannus in nitinol) | 40,30 | CAVALIER TRIAL TPS001 | Virginia (USA) |
| 31 | M | 70 | 25 | 58 | STENOSIS | CALCIFIC DISTROPHY FIBROUS PANNUS OVERGROWTH (pannus in valve+pannus in nitinol) | 27,29 | CAVALIER TRIAL TPS001 | Hannover (D) |
| 32 | F | 75 | 21 | 59 | STENOSIS | CALCIFIC DISTROPHY FIBROUS PANNUS OVERGROWTH (pannus in valve + pannus in nitinol) | 46,95 | CAVALIER TRIAL TPS001 | Hannover (D) |
| 33 | F | 75 | 23 | 63 | STENOSIS | CALCIFIC DISTROPHY FIBROUS PANNUS OVERGROWTH (pannus in valve+pannus in nitinol) | 22,12 | CAVALIER TRIAL TPS001 | Hannover (D) |

Table 10. Demographic data, predominant causes of failure that led to explantation and detailed pathological findings.

| **Time in place** | **0 months (n=9)** | **≥ 1 month (n=24)** | **Combined (n=33)** | **P value** |
|---|---|---|---|---|
| **Gender (male)** | 11% (1) | 38% (9) | 30% (10) | 0.14 |
| **Age at implantation (years)** | 77/81/83 | 73.5/75/78.25 | 74/75/80 | 0.036 |
| **Size (mm)** | 22/23/23 | 23/23/25 | 23/23/25 | <0.001 |
| **Cause of failure that led to explantation** | | | | 0.003 |
| **Stenosis** | 0 | 58% (14) | 42% (14) | |
| **Incompetence** | 56% (5) | 21% (5) | 30% (10) | |
| **Steno-incompetence** | 0 | 4% (1) | 3% (1) | |
| **Other** | 0 | 12% (3) | 9% (3) | |
| **Unknown** | 11% (1) | 4% (1) | 6% (2) | |
| **Declaired failed surgical implantation** | 33% (3) | 0 | 9% (3) | |
| **Pathological Findings** | | | | |
| **Calcific dystrophy** | 0 | 17% (4) | 12% (4) | 0.19 |
| **Infective endocarditis** | 0 | 50% (12) | 36% (12) | 0.008 |
| **Fibrous tissue overgrowth** | 0 | 83% (20) | 61% (20) | <0.001 |
| **Tears (secundary to endocarditis)** | 0 | 4% (1) | 3% (1) | 0.53 |
| **Thrombus** | 0 | 4% (1) | 3% (1) | 0.53 |
| **Perforation** | 0 | 0 | 0 | NA |
| **Not preserved coaptation** | 44% (4) | 8% (2) | 18% (6) | 0.017 |
| **Stent deformation or rupture** | 0 | 0 | 0 | NA |

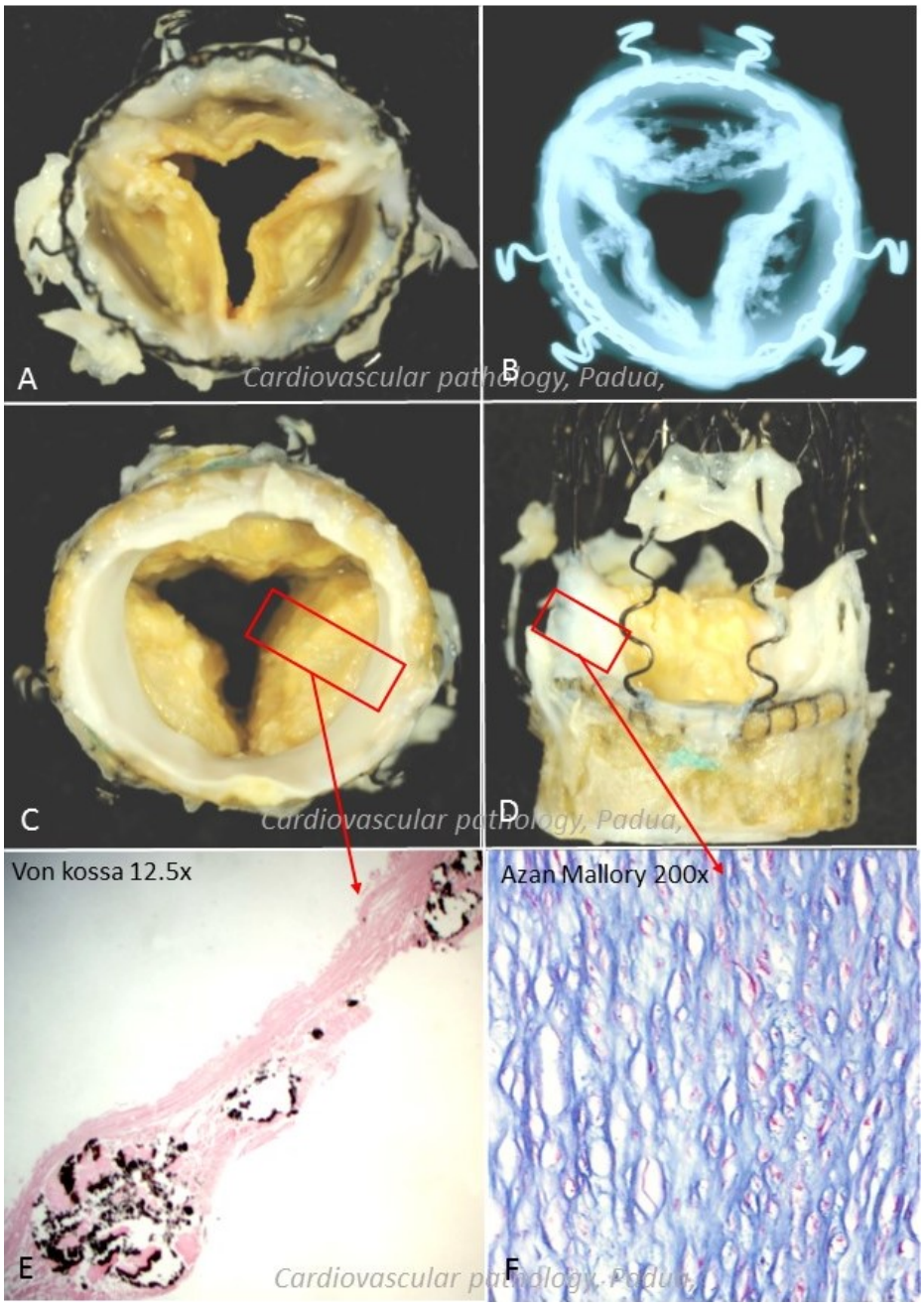



Figure 33. Explant of Perceval S 23 mm in size, in female, 79 yrs at implant, time in place: 53 months, dysfunction: stenosis. SVD: calcific dystrophy and fibrous pannus A) Aortic view; B) X-ray: score 4; C) ventricular view (note EOA reduction due to fibrous pannus); D) lateral view. Fibrous pannus is present at commissural struts, partially obstructing the space in between nitinol network; E) histology of a cusp with intrinsic nodular calcific deposits corresponding to the sampling of C (red square); F) fibrous pannus histology of correspondent sampling of D (red square). E) Von Kossa, 12.5x original magnification; F) Azan Mallory Heidenhein modified, 200x original magnification.

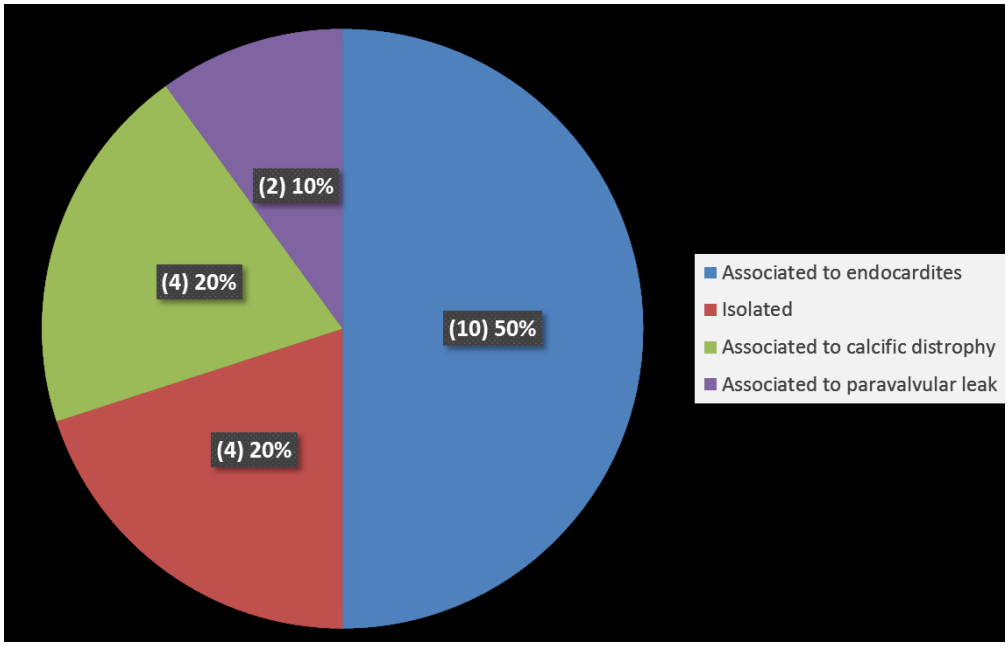



Figure 34. Distribution of concomitant pathological entities with the fibrous tissue overgrowth. The fibrous pannus overgrowth was identified in 20 specimens; it was the sole pathological process involving the bioprosthesis in 20%, while in the remnant 80% it was associated to other processes of dysfunction (50% endocarditis, 20% calcific dystrophy, 10% paravalvular leak).

The fibrous tissue grew in 70% of the cases on valve and in nitinol stent, climbing on the struts and obstructing the space in between (Figure 35); in 20% of the cases involved the sole nitinol stent, while only in 10% involved only the valve orifice and the cusp (Figure 36).

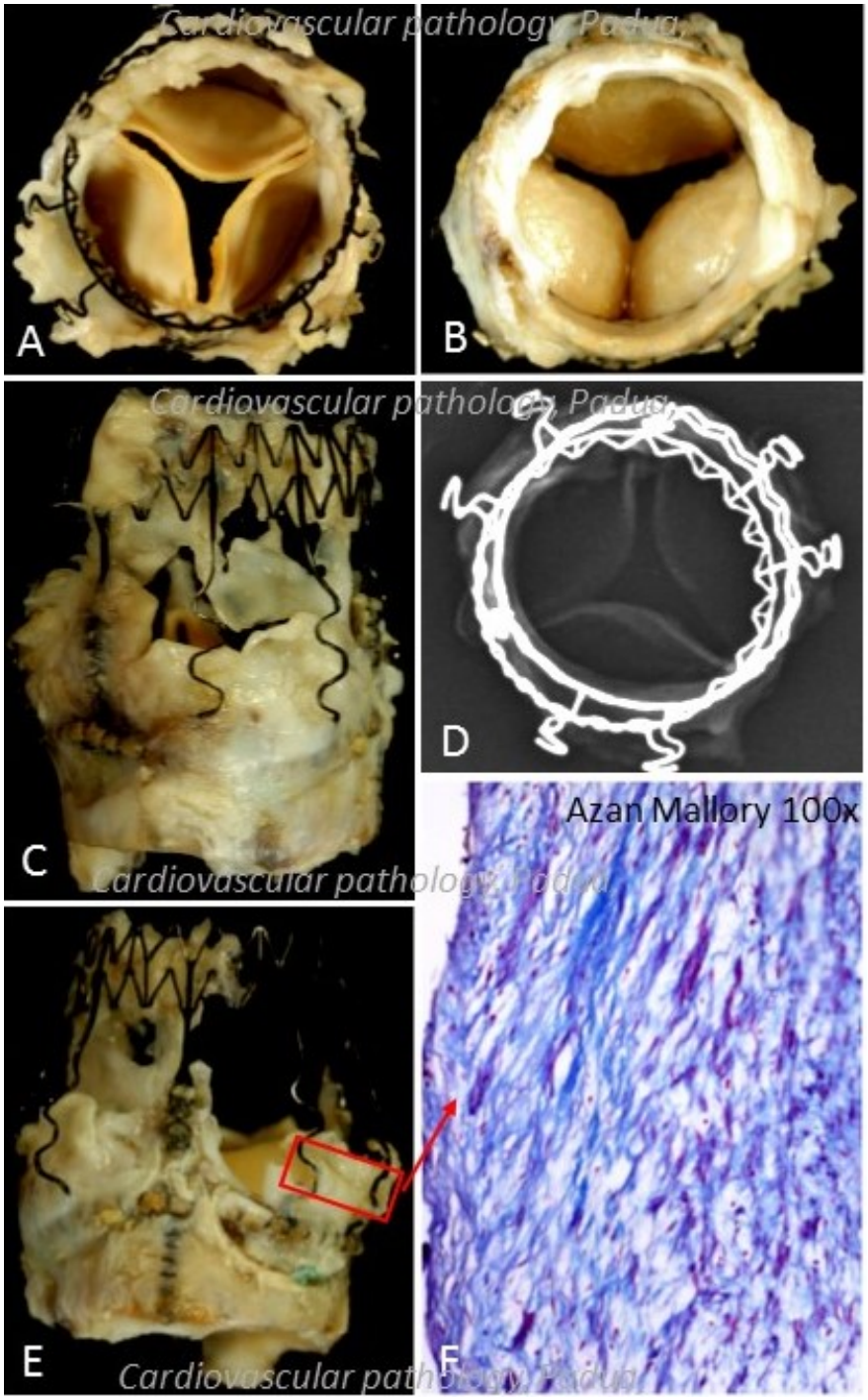



Figure 35. Perceval S explant, 23 mm in size, in female, 75 yrs at implant, time in place: 24 months, dysfunction: stenosis. SVD: fibrous pannus. A) Aortic view; B) Ventricular view with fibrous pannus all around the circumference of the annulus; C) lateral view. Fibrous pannus is widely distributed on the nitinol network of the stent, remarkably obstructing the space in between; D) X-ray: score 0; E) another lateral view; F) fibrous pannus histology of correspondent sampling of E (red square). Azan Mallory Heidenhein modified, 100x original magnification.

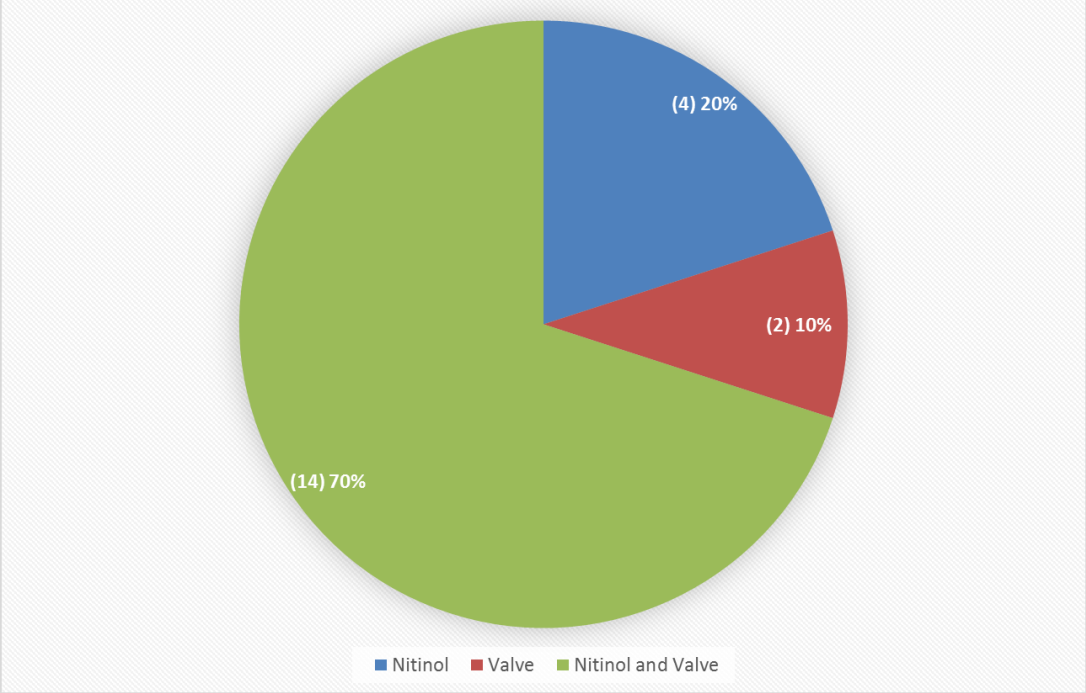



Figure 36. Fibrous tissue overgrowth distribution on valve component.

Table 11. Main pathological findings, and correspondent mean time in place of Perceval sutureless bioprosthesis involved by the different pathological processes. Except for the implant failure and the paravalvular leak, which led to immediate or at very short time at explant, the other structural valve dysfunctions appear after some time from the implant: the time range in the case of endocarditis is wide, ranging from one up to 63 months; instead the calcific dystrophy and the fibrous pannus overgrowth develop later in time.

| **Perceval Main Pathological Findings** | | |
|---|---|---|
| | **Nr** | **Mean Time In Place, Range (Mos)** |
| Acute Infective Endocarditis | 9 (27.3%) | 15.2±21.3 (Range: 1-63) |
| Subacute-Healed Endocarditis | 3 (9.1%) | 15±21.7 (Range: 1-40) |
| Fibrous Pannus Overgrowth | 4 (12.1%) | 24.2±12 (Range: 13-41) |
| Paravalvular Leak | 4 (12.1%) | 0.2±0.5 (Range: 0-1) |
| Calcific Dystrophy | 4 (12.1%) | 58.2±4.1 (Range: 53-63) |
| Unknown | 2 (6.1%) | 0 |
| Implant Failure (Learning Curve) | 7 (21.1%) | 0 |

The fibrous tissue overgrowth was severe in 12 out of the 20 affected bioprostheses (Figures 35 and 36), with a median reduction of the EOA of almost 14% (25.2±10.7 %) (Table 12).

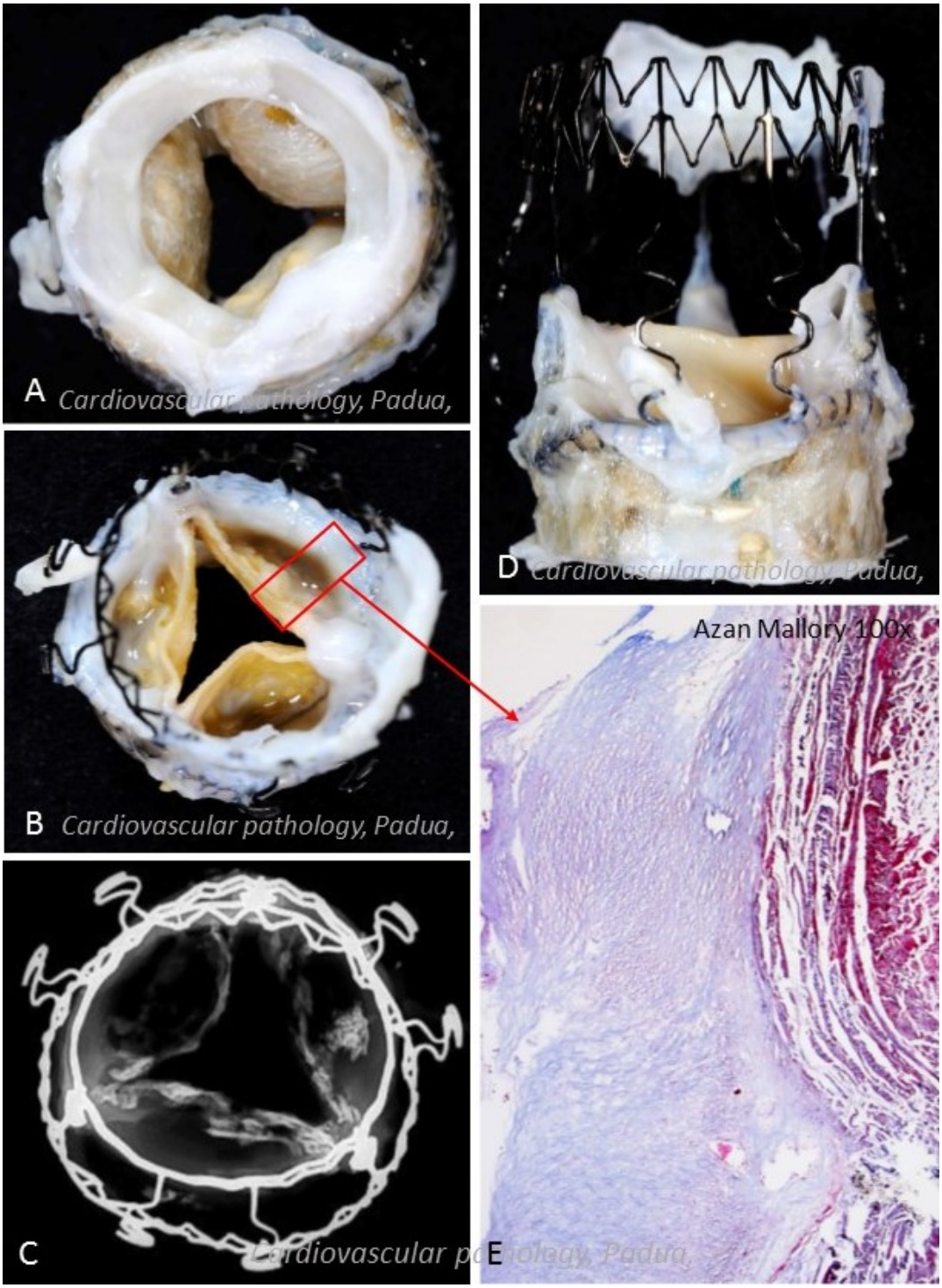



Figure 37. Perceval explant, 21 mm in size, in female, 75 yrs at implant, time in place: 59 months, dysfunction: stenosis. SVD: calcific dystrophy, fibrous pannus

overgrowth. A) Ventricular view (note the EOA reduction due to the severe fibrous pannus (47%); B) Aortic view. C) X-ray: score 4; D) lateral view; E) fibrous pannus histology of correspondent sampling of B (red square). Azan Mallory Heidenhein modified, 200x original magnification.

Table 12. Fibrous tissue characteristics. The presence of fibrous tissue overgrowth was described as severe in 20 bioprosthesis. As shown in figure 34, only in 4 valves it was the sole cause of structural valve dysfunction, while the other 16 specimens were affected by other principal pathological processes.

| **Fibrous tissue overgrowth** | **Total N=20** |
|---|---|
| **Severe** | 60% (12) |
| **EOA ratio (%)** | 0/14.1/25.97 |

Relation between TIP and the reduction of the valve EOA was detected with the Wald statistics (p 0.01): important linear progression of the reduction of the area was identified till the 20$^{th}$ month of implantation. Statistically significant progression of the reduction keeps on in time (p 0.04), but higher number of bioprostheses is necessary to identify the entity of progression (Figure 38). Furthermore, by excluding the bioprostheses with TIP less than one month (9 bioprostheses), we compared the remaining bioprostheses (24), by dividing them into two groups in accordance to the fibrous tissue overgrowth (bioprostheses without fibrous tissue versus bioprostheses with fibrous tissue) (Table 13).

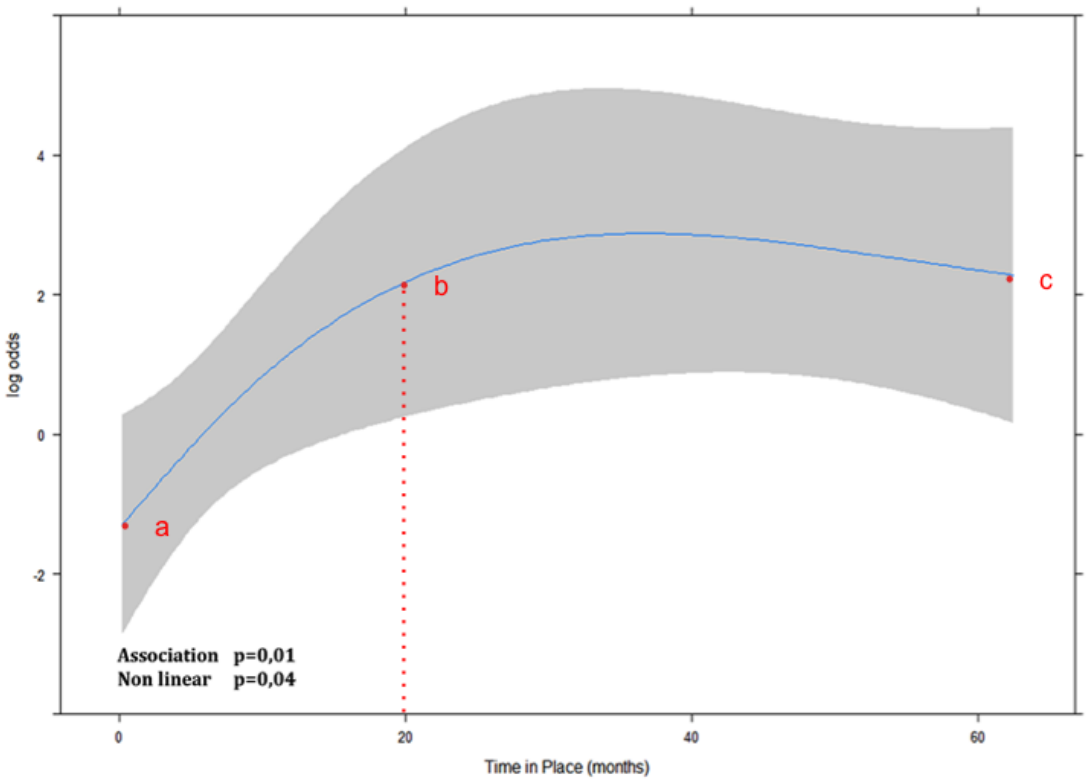



Figure 38. Association between time in place and valve orifice reduction rate (%) [OR 0.78 (0.4-3.47) CI 95%]. The blue line shows the relation of the rate of valve orifice reduction during time. Linear progression of the reduction of the area was identified till the 20$^{th}$ month from implantation (a to b section of the line, p=0.01). Additionally, statistically significant progression of the reduction during time was demostrated (a to c section of the line, p 0.04).

Longer TIP was recorded in the bioprostheses that presented fibrous tissue overgrowth (p 0.004), with stenosis as predominant cause of explantation (0.032). In fact, when present, the valve orifice was reduced significantly (p 0.009) (Table 13).

Four patients showed pure calcific distrophy at gross examination. The mean time in place was 58.25±4.11 months, with a range 53 to 63 months. At X-ray examination, seven bioprostheses showed some grade of calcification, as shown in Table 14. “Intrinsic” calcification occurred within the pericardial cusps, while “extrinsic” occurred on thrombus, infective vegetation or fibrous tissue overgrowth.

Table 13. Characteristics of the bioprosthesis with TIP more than one month (24 bps), in accordance to the presence of the fibrous tissue. Data are presented as percentage or first, median and third interquartile.

| | **No fibrous tissue % (N=4)** | **Fibrous tissue % (N=20)** | **P value** |
|---|---|---|---|
| **Gender (female)** | 25% (1) | 40 (8) | 0,57 |
| **Age at implant (yrs)** | 75/75/76 | 71.50/75.00/78.25 | 0,76 |
| **Size (mm)** | 22.5/23.0/23.0 | 23.0/23.0/25.0 | 0,18 |
| **Time in place (mos)** | 0.19/ 0.33/ 1.07 | 3.5/16/44 | 0,004 |
| **EOA reduction (%)** | 0/0/0 | 12.35/20.73/28.31 | 0,009 |
| **Predominant cause of dysfunction:** | | | 0,032 |
| **Stenosis** | 0 | 70 (14) | |
| **Incompetence** | 75 (3) | 10 (2) | |
| **Steno-incompetence** | 0 | 5 (1) | |

Table 14. Grade of calcification detected at X-ray.

| **X-Ray calcification** | **In N=7 bioprostheses** |
|---|---|
| **1** | 0 |
| **2** | 0 |
| **3** | 28.5% (2) |
| **4** | 71.5% (5) |

# 6.DISCUSSION

## 6.1 Impact of the "collapsing" in Perceval pericardial tissue

The PERCEVAL S valve is a bovine pericardium bioprosthesis assembled on a self-expanding Nitinol stent and designed for sutureless implantation.

The major difference between sutureless Perceval valve and traditional surgical valve replacement bioprostheses, except for the absence of sutures, is the "collapsing" process. In the operating room, the valve is prepared, collapsed, and placed in a dedicated delivery system. The valve may remain collapsed for some time (15 minutes as suggested by the manufacturer), but the time may differ depending on the complexity of the procedure and the learning curve of the surgeon. Because of the nature of the preparation process and the potential for increased collapsing time, it is important to evaluate the detrimental effects this process would have on the tissue cusps, even if the company claims that the collapsing system entails radial compression proximally and distally on the anchoring stent without involving the cusps.

During TAVI, the "crimping" of the bioprosthesis to minimize the size while reaching the final set in the aortic root followed by ballooning, can alter the pericardium structure with fragmentations and disruptions of the collagen fibers (53, 53). In either natural or processed pericardial tissue cusp, collagen fibers reinforce the tissue and provide structural integrity to bear enormous loads related to cyclic pressure changes. It is hard to predict at the present whether these traumatic lesions will have an impact on prosthesis durability. Nevertheless, materials science has taught that anytime a structure, such as a heart valve cusp, is altered and exposed to repeated high stress, it will experience increased fatigue that may lead

to premature failure (69, 70), with possible increase in the incidence of prosthetic valve endocarditis, thrombosis and dystrophic calcification (71).

Finally, valve function may be impaired as glutaraldehyde-fixed xenograft tissue has a great deal of elasticity and pliability, and the damage induced by crimping may take away some of this pliability, impairing cusp function (71).

In Perceval bioprosthesis “collapsing” and not “crimping” procedure is performed. The latter entails a minor radial compression and diameter valve reduction without involving the valve cusps, apparently protecting collagen fibers from injury. The mechanical response of the leaflet tissue greatly depends on collagen fiber concentration, characteristics, orientation, and above all, integrity. So far pericardium integrity was evaluated with descriptive morphological techniques (histology, transmission and scanning electron microscopy) (53, 54) and some authors performed also a morphometric evaluation (69).

In this study, collapsing tests at different times showed absence of lacerations, disruption of the tissue and collagen wave period length measurements comparable with controls. Tests of potential alteration of the tissue after crimping and delivery simulation considering the degree of fragmentation of collagen fibers are reported in literature for TAVI (52, 69, 71), whereas no study on the measurement of potential deformation of collagen structure was performed so far in sutureless valves. Loss of collagen waviness or “crimp” (i.e. straightening) can be the first microscopic pericardium architectural ECM deformation that can lead to frying or fracture. The study, for the first time, tried to define a “deformation grade” before the final collagen fracture consequence.

We demonstrate that collagen tissue natural waviness of the pericardium is maintained, after collapsing and ballooning during the Perceval sutureless bioprostheses implantation.

Sutureless valve therapies have been a breakthrough in the treatment of valvular heart disease, particularly in the patients at intermediate risk who require shorter ischemia times, who are at high risk for prostheses-patient mismatch and the ones who require faster recovery. These patients are considered the “gray zone” of indications to intervention between the transcatheter procedures and the traditional surgical aortic valve replacement (38-40).

Currently different clinical trials have demostrated feasibility, safeness of the procedure and excellent haemodynamics of these devices (23, 33-35). These valves in combination with a minimally invasive approach might be the appropriate treatment option even for high-risk operable patients and a valid alternative to transcatheter aortic valve implantations (32). We need more data to confirm the hypothesis, however we believe that the future will be without suture! Consequently, there is a pressing need to determine whether they have comparable durability to a surgical sutured bioprosthetic valve.

By examining the absence of the effects of collapsing on tissue cusps, we have illustrated an important aspect when choosing a therapeutic option for patients and surgeons. We have shown for the first time that collapsing, differently from TAVI crimping, does not cause structural damage to pericardial tissue cusps.

## 6.2 Perceval bioprosthesis mode of failure and Dysfunction

Patients with high surgical risk, advanced age, or those judged inoperable are typically good candidates for the TAVI procedure, whereas the appropriateness of this approach in younger patients with a lower risk profile is still under debate. In fact, recently published European guidelines (72) recommend TAVI in intermediate risk patients (class Ib, level of evidence B), but an age less than 75 years still favors conventional surgery and suggests that the choice of the intervention must take into account the characteristics of the patients as well as advantages and disadvantages of every valve substitute. Therefore, the crucial aspects in considering the choice of best therapeutic approach and most suitable aortic substitute, especially in patients with intermediate- to low-risk, are: (I) evaluation of the haemodynamic performance (PVL and transvalvular gradients); (II) valve durability; (III) rate of pacemaker implantation; (IV) patients' quality of life and (V) costeffectiveness analysis.

Regarding valve durability, the intermediate risk patient profile often entails a younger patient age and longer life expectancy, which therefore makes prosthesis

durability a major concern. Durability in TAVI is related to the biological nature of the valve cusps and to pre-procedural steps, such as valve crimping and intravalvular balloon inflation, which is essential for a transcatheter delivery. Several reports have been recently published, showing specific lesions (transverse fractures and longitudinal cleavages) on pericardial cusps, especially in balloon-expandable valves (52-54), phenomena that can potentially lead to valve deterioration. Unfortunately, long term durability data after TAVI are still not available in literature and just a few reports present results in patients with 5-year follow-up (73) that, for bioprosthesis, are not sufficient. Therefore, longer follow-up is needed to reach time points when valve related adverse events are more likely to occur.

The sutureless bioprosthesis are currently one of the more appealing substitutes for surgeons, who can take advantage of their simplified implantation technique whilst maintaining auxiliary cardiopulmonary bypass and cardioplegic arrest, with increasing frequency of implantation in low risk patients. So understanding the durability and the mode of failure of those bioprostheses becomes crucial for therapy.

The performance of this bioprostheses depends on (I) the stent impact on cusps, (II) the durability of the biologic tissue of the cusps due to their treatments, and (III) the interaction between the two components, assessable by pathologic examination of the specimens explanted.

Actually, Perceval is one of the most widely implanted sutureless bioprosthesis valve in the European centers. It has a similar design to the LivaNova SOLO stentless bioprostheses. As previously demonstrated, the collagen tissue natural waviness is maintained after collapsing in the Perceval sutureless bioprostheses.

For what concerns the durability of the biological tissue, the review of Wollersheim LW. et al (59) reports a 0.5% reoperation rate per patient-year for the Freedom SOLO measured during a mean follow-up of 22 months. In comparison, the reoperation rate for stentless aortic bioprostheses is 1.4% to 2.2% after 5 years and 10% after 10 years (59). On the contrary, Repossini et al, in their recent study showed, at 10 years of follow-up, freedom from SVD and freedom from reoperation due to SVD of 90.8% and 91.9%, respectively (51). Christ T. and colleagues

presented similar data, with overall freedom from valve reintervention due to SVD at 5 and 10 years of 97.8 ± 2.2% and 82.9 ± 7.5%, respectively (60). So, long-term durability remains controversial for LivaNova Solo stentless aortic bioprostheses. Anyhow, Jelle et al. (74) in their recent study on 625 patients at medium-term of follow-up stated that correct sizing and perfectly symmetrical implantation may play an important role in long-term durability of the SOLO stentless bioprosthesis, considering an asymmetrical implantation and oversizing as the responsible cause of the early SVD. Stress on the cusps is in part a cause of tissue degeneration. Decades have been spent perfecting the incorporation of tissue into the valve stent housing and using strut material with "spring" properties to reduce the tissue load during cusp closure. In the Perceval bioprostheses, the same tissue of the Solo Livanova bioprostheses is mounted into self-expanding sutureless aortic bioprostheses, eliminating the bias of asymmetric implantation and stress cusp due to of oversizing.

*Perceval bioprostheses Structural Valve Deterioration:*

Bouhout et al. (61) reported a case of early SVD of a Perceval prosthesis. A 54-year-old man with symptomatic AS underwent SAVR with a size extra large Perceval. The postoperative course was uneventful, and the patient was discharged on 4$^{th}$ postoperative day. Two years later, the patient presented with a reoccurrence of symptoms and echocardiography showed immobile cusps and a mean aortic gradient of 84 mm Hg. A redo SAVR was performed. Intraoperative examination revealed stiffened cusps with no tear and no thrombus; however, the prosthesis was tightly embedded. Removal of the prosthesis required 52 minutes, followed by implantation of a mechanical prosthesis. Macroscopic examination of the explanted Perceval S prosthesis revealed severe calcifications on both sides of all cusps. X-rays of the prosthesis also showed large intrinsic calcifications in all cusps. Histologic study revealed thick free edges due to fibrous pannus and large intrinsic calcifications (61).

Votsch et al. (62) reported valvular thrombosis of the noncoronary cusp of a medium Perceval valve several months after implantation. It was suspected that high-dose cortisol therapy after implantation might have contributed to the valve thrombosis.

In large single-arm studies examining outcomes of the Perceval, no SVD has yet been reported at 30 days (658 patients) (63), 13.4 ± 11.6 months (143 patients) (64), and 10 ± 20 months (208 patients) (65). In the largest cohort of patients studied to date for haemodynamics and clinics, combining results of 3 European trials with a total of more than 700 patients and follow-up up to 5 years, no SVD has been reported (23).

*Perceval bioprostheses nitinol stent:*

Fleissner and collegues (66) described two cases that identified a so far unreported problem that may occur in patients receiving these novel devices. Despite uneventful implantation, careful valve inspection and intraoperative transesophageal echocardiography (TOE) control, delayed stent distortion within the first days after surgery was detected and resulted in paravalvular leak, which increased transvalvular gradients.

They estimated an incidence of delayed stent distortion 1–2% of all patients treated with this valve type (66). The authors' hypothesis was that the relative oversizing was the reason for this complication. Under non-beating heart conditions during valve implantation, the flexible stent of the sutureless valve seems to be able to maintain a correct position within the aortic annulus even if oversized. With additional forces acting on the stent during the postoperative course under beating heart conditions, the oversized stent might bend inward in a delayed fashion during postoperative course. Both relative under- and oversizing may result in paravalvular leak and poor valvular performance. They suggested additional native decalcification as a feasible option to implant the larger sized valve, if necessary. Additionally, they conclude by considering the implantation of a different valve model, if sizing is unclear (66).

*Implications of the Present Study:*

Thirtythree bioprostheses were examined after explant at Padua Pathology Core Lab in almost 10 years. The bioprostheses derived from three different multi European center trials: Pilot, Pivotal and Cavalier trial that were designed to investigate feasibility, safety and effectiveness of the device.

Low incidence of explant was observed, 2.3% in the Cavalier trial, 3% in the Pilot trial and 11.3% in the Pivotal trial. High incidence of female patients was recorded

in the explanted series (70%). This should be related to the indications to implantation of the Perceval bioprostheses given by the manufacturer that indicates mainly females, as smaller annulus' dimensions characterize this population generally.

In our Perceval bioprostheses series, seven were explanted at short time after intervention, probably due to surgical technique failure. Stent deformation and tissue valve lesion, as tear, perforation or other, were not identified. Explantation due to PVL with severe regurgitation occurred in 4 patients, maybe due to insufficient decalcification of the aortic annulus or to uncorrect sizing. The knowledge of the temporal distribution of the relative surgical procedures, during the learning curve of the different centers, might have been helpful to better explain this phenomenon. The same observation should be done for the failed surgical procedures.

Endocarditis was diagnosed in 12 bioprostheses, with gram-positive bacteria, at histology examination in acute phase. The high incidence of endocarditis diagnosed (36% of the bioprostheses examined; 50% of the bioprostheses explanted at more than one month after implantatation) (67).

SVD due to calcific dystrophy was identified in 4 bioprostheses with severe grade of calcification at mid term of TIP (53-63 months of range). Despite the optimal haemodynamics reported of the Perceval bioprostheses, the time of SVD was shorter when compared to the excellent results reported in literature for other aortic bioprostheses, as Carpentier Edwards Perimount.

Of particular interest was the prosthetic valve pannus formation. Fibrous tissue overgrowth occurred in most of the cases but it was the main cause of dysfunction only in 4 bioprostheses. The fibrous tissue caused stenosis, by progressively reducing the effective orifice area during time, with linear progression of the reduction of the area till the 20$^{th}$ month of implantation. The further progression of the reduction was demonstrated, but higher number of specimens is necessary to identify the entity of progression.

The exact etiology of pannus formation is not known. Multifactors are involved in its formation. Basically, pannus represents a bioreaction to prosthetic material associated with coexisting factors such as surgical technique, thrombus organization

from inadequate anticoagulation, and wall shear stress [75,76]. All types of available prosthetic valves can be affected by pannus formation. The interesting fact observed in our series is that a high number of specimens (20/33 explanted) were affected by fibrous pannus, although it was the sole pathology only in 4 cases. Therefore it is a phenomenon that, in this type of bioprosthesis, accompanies endocarditis, calcific dystrophy, or others, with development also at short TIP.

Recent study by Teshima and colleagues [75] found that patients with prosthetic valve dysfunction secondary to pannus are associated with significant increase in the level of transforming growth factor beta. This cytokine is essential for regulation of cell growth, differentiation, and matrix production. Thus, the increase production of these cytokines is implicated in the formation of pannus by inducing exaggerated healing, fibrosis and scar tissue formation. Pannus usually originates in the neointima of the periannular tissue [77]. Histologically, it is mainly formed of collagen fibrous tissue accompanied by endothelial cells, chronic inflammatory cell infiltration and myofibroblasts [77]. Therefore the fibrous pannus overgrowth seems like a reparative phenomenon that is triggered once the Perceval prostheses is implanted, perhaps due to its design with high structure.

In most of the bioprosthesIs the formation of pannus arises from the left ventricular side of the valve. In rare cases they appear to grow from the aortic side [78]. In our series of bioprostheses analyzed, the fibrous tissue arose not only by the ventricular side, but also within the aortic valve, involving also the nitinol structure, with climbing phenomenon on struts and cage sinuses. This may suggest that the height of the structure can be the responsible of continuous fluttering that triggers the phenomenon of pannus formation. Pannus arising from the aortic aspect of prosthetic valve usually causes obstruction with immobility of cusps [78].

The effect of pannus formation on haemodynamics depends on the extent and site of fibrous tissue. Pannus arising from the left ventricular aspect may extend to the orifice and hinges of the prosthetic valve causing restriction of inflow orifice [79]. Severe stenosis may occur due to obstruction and narrowing of left ventricular outflow tract by a circumscribed pannus without hindering cusp motion [79]. In our series of bioprostheses examined, it was the sole responsible of stenosis in only 4

cases; anyway, when present, it caused restriction of the orifice area of different grade.

The formation of pannus can also lead to regurgitation caused by impairment of diastolic motion of the prosthetic cusps [80], as noticed in 3 bioprostheses of our series.

In light of the above observations, the phenomenon of the climbing of the fibrous pannus on the stent could be similar to TAVI, with the necessity to investigate in further studies the potential for coronary obstruction.

Instead, regarding the comparison with surgical bioprostheses, the pannus overgrowth is reported in all the valves, even of the latest generation. Obviously, this is an observational study of only Perceval explants, and not comparison with other bioprostheses, so further studies are required for comparison.

# 7.CONCLUSIONS

Currently AVR using sutured biological valves is the conventional treatment of choice in elderly patients with severe aortic valve disease. This surgical approach has shown excellent outcomes and haemodynamic performance, and a very high rate of freedom from SVD for up to 15 years (68). Consequently, the introduction of a new generation of prostheses is a challenge. Nevertheless, cardiac surgery is changing. Heart surgeons are moving toward less-invasive approaches, with the aim of reducing surgical trauma and myocardial damage. In this setting, sutureless technology represents the implant evolution.

The ideal prosthetic valve should be easy to implant, have an excellent hemodynamic performance without intrinsic thrombogenicity, and long-term durability associated with a low risk of SVD. Sutureless aortic valves have demonstrated many of these characteristics, however data regarding long-term durability are not available yet.

Available evidence suggests that the use of sutureless valves is associated with decreased operative and ventilation time, intensive care unit and hospital stay, as well as fewer postoperative complications. However, the actual scientific evidence does not address some important issues: no data were reported on the learning curve and the impact of malposition on development SVD; only few case reports or small case series are reported on modalities of failure of sutureless bioprostheses.

In this pathological series of Perceval explants, we identified few cases of SVD, several cases of endocarditis, and overgrowth of fibrous tissue as the predominant pathology of this bioprostheses. The pannus involves not only the valve from the ventricular side, but also climbs the nitinol stent from the aortic side. Consequently, the height of the nitinol structure and the consequent fluttering as possible trigger of the phenomenon should be considered.

Despite the occurence of SVD, due to calcific dystrophy as early as in 5-6 years of time in place in a few explants, when compared with the sutured bioprostheses'evidence in literature, particular attention should be paid to the anticalcification process of the Perceval bioprostheses pericardial tissue and implementation of new antimineralization strategies.

# 8. LIMITATIONS OF THE STUDY

*"Impact of collapsing" findings*: We limited the investigation to the histology ad SEM because they are the best combination analysis for two-three dimensional view of collagen fibers and the best way to measure the collagen period length by morphometrical analysis at low magnification.

Moreover, SEM is a tested ultrastructural technique to reveal the potential architectural alteration of the collagen fibers [54, 81].

Investigation with transmission electron microscope (TEM) at high magnification

can increase the sensitivity of morphological analysis in assessing potential clinically damage.

*"Perceval bioprosthesis mode of failure" findings:* Incomplete patient clinical data led to an often difficult correlation between pathological processes observed and Perceval bioprosthesis function impairment.

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