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ORIGINAL RESEARCH

Possible Link Between the ABO Blood Group of Bioprosthesis Recipients and Specific Types of Structural Degeneration

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BACKGROUND: Pigs/bovines share common antigens with humans: α-Gal, present in all pigs/bovines close to the human B-antigen; and AH-histo-blood-group antigen, identical to human AH-antigen and present only in some animals. We investigate the possible impact of patients' ABO blood group on bioprosthesis structural valve degeneration (SVD) through calcification/pannus/tears/perforations for patients ≤60 years at implantation.

METHODS AND RESULTS: This was a single-center study (Paris, France) that included all degenerative bioprostheses explanted between 1985 and 1998, mostly porcine bioprostheses (Carpentier-Edwards second/third porcine bioprostheses) and some bovine bioprostheses. For the period 1998 to 2014, only porcine bioprostheses with longevity ≥13 years were included (total follow-up ≥29 years). Except for blood groups, important predictive factors for SVD were prospectively collected (age at implantation/longevity/number/site/sex/SVD types) and analyzed using logistic regression. All variables were available for 500 explanted porcine bioprostheses. By multivariate analyses, the A group was associated with an increased risk of: tears (odds ratio [OR], 1.61; *P*=0.026); pannus (OR, 1.5; *P*=0.054), pannus with tears (OR, 1.73; *P*=0.037), and tendency for lower risk of: calcifications (OR, 0.63; *P*=0.087) or isolated calcification (OR, 0.67; *P*=0.17). A-antigen was associated with lower risk of perforations (OR 0.56; *P*=0.087). B-group patients had an increased risk of: perforations (OR, 1.73; *P*=0.043); having a pannus that was calcified (OR, 3.0, *P*=0.025). B-antigen was associated with a propensity for calcifications in general (OR, 1.34; *P*=0.25).

CONCLUSIONS: Patient's ABO blood group is associated with specific SVD types. We hypothesize that carbohydrate antigens, which may or may not be common to patient and animal bioprosthetic tissue, will determine a patient's specific immunoreactivity with respect to xenograft tissue and thus bioprosthesis outcome in terms of SVD.

Key Words: bovine valve ■ heart valve ■ pannus ■ perforation ■ porcine valve ■ tears ■ xenogenic tissue

More than 200 000 aortic valve replacements are performed each year in the world, and 1 in 5 of those replacements are performed on patients aged 40 to 60.[1] Bioprosthetic valves are often chosen to avoid the risk of thromboembolic events and the need for lifelong anticoagulation. The use of bioprostheses has increased significantly in the past several years with the development of transcatheter aortic valve implantation. The main disadvantage of bioprostheses is their limited durability, which is problematic in young patients.[2] Most failed bioprostheses are subject to structural valve degeneration (SVD) and are most often observed in patients who were under 60 years old at implantation.[3,4] The etiology of bioprosthesis failure seems to be primarily attributable to calcification, which may be the result of an exaggerated immune response,[2] although this has not been fully characterized.[5–7]

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CLINICAL PERSPECTIVE

What Is New?

- We evaluated whether there was a possible correlation between patient ABO blood group and bioprosthesis alteration by calcification, pannus, tears, or perforation, in particular for porcine bioprostheses explanted because of structural valve degeneration.
- Using a multivariate analysis, which included the variable "valve longevity," we found that the B group/B-antigen was correlated with a propensity for calcification and perforation, while the A-group was correlated with an increased risk of tears and pannus but a lower risk of calcification.

What Are the Clinical Implications?

- The ABO blood group of the patient may be an important factor in determining the type of structural valve degeneration.
- We hypothesize that the underlying mechanism may be that shared carbohydrate antigens between the patient and the bioprosthesis determine patient immunoreactivity and influences the type of structural valve degeneration.
- Better compatibility between a patient's ABO blood group and bioprosthetic carbohydrate antigens may be a potential way for improving bioprosthesis outcome.

Nonstandard Abbreviations and Acronyms

BB	bovine bioprosthesis
PB	porcine bioprosthesis
SVD	structural valve degeneration

SVD is a gradual process characterized by fibrocalcific remodeling, thickening, and stiffening of valve leaflets and/or disruption of collagen fibers, leading to leaflet tears or perforations.[8]

Contributing factors to SVD can be divided into 3 groups: (1) patient-related factors, such as age at implantation, valve position, body mass index, and sex; (2) cardiovascular risks and comorbid conditions, such as hypertension, metabolic syndrome, and smoking; and (3) factors related to the valve such as prosthesis size and patient prosthesis mismatch.[9,10] With the exception of calcification,[11] the mechanisms of SVD, such as pannus formation, tears, and perforations, are relatively understudied,[5] as is the possible role of immunogenicity.[10,12]

There is significant evidence in the literature to suggest the possibility of immunogenicity in the SVD process. Humans and pigs share several carbohydrate antigens that are expressed in the bioprostheses even after reticulation,[13] which can trigger an immune response in the recipient. One of these antigens is the "α-Gal,"[14] the major antigen of xenogenic rejection, which is expressed in the extracellular matrix[15,16] and is present in all nonprimate mammals such as pigs or bovines. α-Gal is structurally similar to the human group B-antigen[17,18] and, although the subject of some discussion,[19–21] B-type patients may have a better tolerance toward this and related antigens.[20,22] α-Gal is present in commercially available bioprostheses.[23,24] The titer of α-Gal antibodies increases after bioprosthesis implantation.[24,25] Early degeneration of bioprotheses has been reported in patients with α-Gal allergy.[26]

Another possible immunogenic carbohydrate antigen is the porcine A-antigen of the pig histo-blood group 0 system (ie, locus *EAO* on pig chromosome 6) corresponding to the orthologous site for the A-transferase gene[27,28] and controlling A-antigen expression in the tissue. Unlike the α-Gal, which is expressed by all pigs in all their tissues, expression of the A-antigen is restricted to A-type pigs.[29–32] The porcine A-antigen[28,31,33–35] is identical to the human A-antigen of the ABO blood group[36] and is synthesized by the same enzyme, A-transferase.[27] Human A-antigen and also human H-core have been recently isolated from porcine pericardium[37] and porcine valvular cusps[38] that are used for bioprosthesis synthesis.

The AH histo-blood group system exists in most mammals and also exists in the bovine tissue[27] from which some new generations of bioprostheses are made.

We recently reported a difference in the longevity of porcine or bovine bioprostheses depending on the patient's ABO blood group, with an increased longevity observed in A-group patients that may be attributable to better immunological compatibility with the implanted tissue.[39] An increased survival rate among A-group patients with porcine (PB) or bovine bioprostheses (BB) in the aortic position has been confirmed recently by another group.[40]

Our objective was to further investigate whether there is also a correlation between patient ABO blood and specific types of SVD.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. See also Data S1 for more details.

Ethics Statement

Approval from the local ethics committee was not required because of the retrospective nature of

the study. All patients had previously given written consent for the use of their data for research at the University Hospital.

Patient Cohort

This was a single-center study of patients requiring reoperation for degenerative bioprostheses at Broussais-Hospital/Georges-Pompidou-European-Hospital in Paris. Professor A. Carpentier developed the concept of valvular heart bioprosthesis,[41] and several generations of PB/BB and a large number of cases have undergone initial evaluation studies at this center.[41,42]

Patients' characteristics before 1985 (1975–1980[43–45]/1980–1985[46,47]) and the number of implanted bioprostheses in the mitral/aortic position in this institution have been reported in the literature.[48] Because of a mean longevity of heart valve bioprostheses of 10 years, the period 1985 to 1997 corresponded to Carpentier-Edwards second/third generations that were mostly implanted in the institution between 1975 and 1985.[42,47,48] New generations of Carpentier-Edwards bioprostheses made of xenogenic bovine pericardium[49,50] have been implanted since 1980[46,48] and have almost totally replaced PBs since 1985.

All patients reoperated for a degenerative PB during the 13-year period from January 1985 to December 1997 were eligible for the study and were mainly from our institution. In addition, we included all patients reoperated from January 1998 to January 2014 who had a PB with exceptional longevity (≥13 years). As most PBs were implanted before 1985, we have a total follow-up period of 29 years for most of these patients.

We included patients who had degenerative PBs that required surgical re-replacement due to intrinsic SVD (ie, bioprosthesis SVD). Excluded were bioprosthetic replacements for other causes of valvular dysfunction such as nonstructural valve deterioration,[51] endocarditis, or thrombosis. A standardized classification of SVD in the aortic position has recently been proposed.[9,51,52] The reasons for valve replacement and the type of intrinsic structural valve anomalies (ie, calcification, pannus formation, tears, perforations) were specified by the surgeon at the time of surgery and the data prospectively collected.

Some cases of early failure or intermediate longevity in the new generation of bovine bioprostheses were explanted during this period (1985–1998) and analyzed separately.

Study Variables

The main variables of interest were the interval between valve implantation and replacement (longevity) and the type of SVD. The main risk factor of interest was the patient's blood type (ABO and Rhesus), which was collected retrospectively, but most other known risk factors for SVD, such as age at implantation, sex, and valve position, were prospectively collected at replacement.

Many of the factors that have been associated with SVD[9,52] are linked to a higher risk of calcification.[4,5,9,10,53,54] However, factors associated with SVD due to pannus formation, tears, and perforations have been understudied.[5] None of these factors have been shown to be related to the ABO blood group of patients and thus could not explain the different types of SVD.

Data Collection

A prospective database for bioprosthetic heart valves has been in place since 1985 and has identified several risks for SVD. The type of SVD was reported for 50 of 963 explanted bioprostheses. Patient blood group information was obtained retrospectively from the blood bank (66%) or patients' records (33%). If the information was not available in the blood bank, patients' medical records were consulted for patients with the highest (≥13 years) and lowest longevity (≤7 years).

Since 1998, all PBs with a longevity of ≥13 years have been systematically sent to the laboratory for further analysis. This enabled us to go back to a patient's name and chart.

Since 1985, the new generations of BBs have almost completely replaced PBs, so that for the year 2014, we have at least 29 years of follow-up for PBs.

Statistical Analysis

We obtained frequency distributions for all study variables, for all replaced valves, and for all patients. For the main outcome variable, valve longevity, discrete categories were defined: the approximate lower and upper deciles were isolated (early and late failure), and the remainder was split into 3 classes, resulting in the following 5 longevity categories (years): 0 to 5.9, 6 to 8.9, 9 to 11.9, 12 to 14.9, and 15 to 28. We cross tabulated the 5 levels of the longevity variable with ABO. This analysis suggested that the 3 middle categories were homogeneous, so for simplicity we continued the analysis with a 3-level longevity variable (0–5.9, 6–14.9, 15–28). We then cross tabulated these variables with blood types and other valve and patient characteristics. We did not choose the class of longevity initially, but these classes of longevity appear to be clinically relevant.[3] Categorial data were examined as percentages±SD and compared using the chi-squared test or Fisher's exact test as appropriate.

All variables evaluated in a univariate analysis were entered into the multivariate models. A P value of ≤0.05 was considered statistically significant. A multivariate logistic regression model was used to identify the independent predictors of SVD. In our multivariate analyses, only bioprostheses with known patient ABO data were included.

RESULTS

See expanded results in Data S1.

Patient and Valve Characteristics

A total of 886 patients with BP explants met criteria for inclusion in this analysis. Between 1985 and 1998, 854 PBs were explanted from 641 patients. From 1998 to 2014, 32 additional PBs with high longevity (≥13 years) were removed. Bioprosthesis longevity data were available for 564 of 886 and age at implantation for 559 of 886. Not surprisingly, most patients (89.3%) with failing PBs requiring surgical replacement were ≤60 years of age at implantation. We focus our attention on this group with PBs implanted at ≤60 years of age because it is overrepresented and corresponds to the group of bioprostheses that need major clinical improvements.

We had a group of 500 explanted PBs for which most variables, especially types of degeneration, were known. This constitutes our study cohort. In this last group, ABO blood group information was missing for 79 bioprostheses.

Demographic data and PB characteristics are shown in Table 1. Types of explanted PBs were as follows: Carpentier-Edwards second, 49.9%; third, 33.8%; first, 2.7%; other PB, 13.6%. Multiple PBs were present in 20.1% of patients. Mean age at implantation was 38.6±12.6 years; the time lapsed before reoperation for SVD for PBs was 9.9±3.4 years (0–28); and the quartiles were 8, 10, and 12 years. Most valves lasted between 6 and 15 years. About 15.9% failed at <7 years, and another 10.6% failed at ≥15 years. In our group of reoperations for failing bioprostheses, many factors known to be predictive for lower longevity, such as mitral site of implantation, younger age, or multiple bioprostheses, were overrepresented compared with the initial population at the time of implantation.[43–48] In our failing group of SVD, there was a high proportion of patients implanted at ≤35 years of age (39.4%).

Table 1. Characteristics of Patients Having a Porcine Bioprosthesis Explanted for SVD and Whose PB Was Implanted at Age ≤60 Years Old (n=500)

Patient Characteristics	All Porcine SVD Bioprostheses (n=500)
Male sex	268 (54.6)
Age at implant, y	
7–20	24 (5.2)
20–30	110 (23.9)
30–40	98 (21.3)
40–50	114 (24.7)
50–60	114 (24.7)
Longevity, y	
0–7	73 (15.9)
7–15	338 (73.5)
15–28	49 (10.6)
Valve replacement	
Mitral	304 (60.9)
Aortic	188 (37.7)
Tricuspid or pulmonary	7 (1.4)
Number of valves replaced	
1	399 (79.9)
2	94 (18.8)
3	6 (1.3)
Blood type	
A	143 (34.2)
B	66 (15.7)
AB	28 (6.7)
O	183 (43.5)
Rhesus +	380 (90.3)
Bioprosthesis type	
Carpentier-Edwards third	161 (33.8)
Carpentier-Edwards second	238 (49.9)
Carpentier-Edwards first	13 (2.7)
Other porcine bioprosthesis	65 (13.6)
Type of SVD	
Pannus	212 (42.4)
Calcification	194 (38.8)
Tears	286 (57.2)
Perforations	52 (10.5)

Values are mean±SD or n (% of n). Patient number by category may be lower than expected because of missing data. SVD indicates structural valve (bioprosthesis) degeneration. .

Risks of Porcine SVD by Pannus

Pannus was present in 42.4% of SVD (95% CI, 38.1%–46.7%) (see Table 2 and Table S1). Using univariate analysis, we identified several risk factors such as longevity (increased risk with greater longevity) (P=0.0036) and bioprosthesis type (less frequent in more recent PBs) (P=0.08). Interestingly, we observed the possible association of an increased risk of pannus in the A group (odds ratio [OR], 1.24; 95% CI, 0.99–1.56; P=0.064). Risk of pannus for the A group for PB with different class of longevity: short longevity (OR 1.15; 95% CI, 0.66–2.01; P=0.61), intermediate longevity (OR, 1.25; 95% CI, 0.96–1.63; P=0.092), and for the group with the highest longevity (OR, 1.06; 95% CI, 0.59–1.89; P=0.85).

Using multivariate analysis, the risks for pannus were found to be associated with longevity (OR, 2.01; 95% CI, 1.81–2.21; P=0.00031) and possibly, although not statistically significant, the A group:(OR, 1.50; 95% CI, 0.99–2.26; P=0.054).

Table 2. Risks for SVD by Pannus for Porcine Bioprostheses Implanted at Age ≤60 years (See Also Table S2)

n=500	SVD Without Pannus n=288 (57.6%)	SVD With Pannus n=212 (42.4%)	Univariate Analysis	
			OR (95% CI)	*P* Value
Male sex	160 (55.9)	108 (52.7)	0.93 (0.75–1.14)	0.47
Age at implantation	*	*	*	0.50
Longevity	*	*	*	0.0036
Valve replaced	*	*	*	0.22
Number of valves	*	*	*	0.31
Blood type				0.22
A	74 (30.3)	69 (39)	1.0 (reference)	
B	38 (15.6)	28 (15.8)	0.8 (0.63–1.20)	
AB	18 (7.4)	10 (5.7)	0.73 (0.45–1.19)	
O	114 (46.7)	69 (39)	0.78 (0.60–1.00)	
Blood A vs others	74 (30.3	69 (38.9)	1.24 (0.99–1.56)	0.064
Blood B vs others	38 (15.6)	28 (15.8)	1.01 (0.74–1.37)	0.95
Blood AB vs others	18 (7.4)	10 (5.6)	0.84 (0.52–1.37)	0.48
Blood O vs others	114 (46.7)	69 (38.9)	0.83 (0.66–1.05)	0.11
Antigen A	91 (37.3)	77 (43.5)	1.16 (0.92–1.45)	0.20
Antigen B	56 (23.0)	38 (21.5)	0.95 (0.72–1.25)	0.72
Rhesus	220 (89.8)	160 (90.9)	1.08 (0.73–1.60)	0.70
Bioprosthesis type	*	*	*	0.08
Pannus			Multivariate Analysis	
			OR (95% CI)	*P* Value
Longevity			2.01 (1.81–2.21)	0.00031
Blood A vs others			1.50 (0.99–2.26)	0.054
Bioprosthesis type			1.08 (0.98–1.17)	0.41

Values are mean±SD or n (% of n). *P* values refers to comparisons between SVD without pannus and SVD with pannus. Statistical analysis by categories (complete analysis is available for Table 1 in supplementary data including all data concerning the global population of 500 explanted bioprostheses for SVD). Patient number by category may be lower than expected because of missing data. OR indicates odds ratio; and SVD, structural valve (bioprosthesis) degeneration.

*Complete file can be found in Supplementary Material.

Risks of Porcine SVD by Calcification

Calcification was present in 38.8% of SVD (95% CI, 34.5%–43.1%) (see Table 3 and Table S2). Using univariate analysis, we identified the classical risk factors such as younger age at implantation (*P*=0.00097), site (*P*=0.0041), and multiple bioprostheses (*P*=0.021). Higher longevity was surprisingly associated with a lower risk of calcification (*P*=0.034). We also identified 2 new additional risk factors for calcification related to ABO blood group: The A group was associated with decreased risk (OR, O.74; 95% CI, 0.56–0.96; *P*=0.024), and B-antigen was possibly associated with an increased risk (OR, 1.28; 95% CI, 0.97–1.67; *P*=0.076).

Using multivariate analysis, and including the A group as a variable, we identified several risk factors for calcification: age at implantation (OR, 0.81; *P*=0.014), valve number (OR, 1.75; *P*=0.039), longevity (OR 0.65; *P*=0.087), and, compared with the B blood group, a tendency for a decreased calcification propensity in A-group patients (OR, 0.67; 95% CI, 0.45–0.89; *P*=0.087), although it was not statistically significant. The same multivariate analysis, with the presence of B-antigen, showed that this latter factor was again associated with an increased risk of calcification (OR, 1.34; 95% CI, 1.09–1.59), although again it was not statistically significant (*P*=0.25).

Risks of Porcine SVD by Tears

Tears were present in 57.2% of SVD (95% CI, 53.4–62.0) (see Table 4 and Table S3). Using univariate analysis, we identified several risk factors for tears such as site of implantation (*P*=0.012), multiple prostheses (*P*=0.024), and, though a weaker relationship, type of bioprosthesis (*P*=0.096). The incidence of tears decreased with longevity (*P*=0.083). The influence of mechanical factors, with repetitive stimulations over time, is known to increase with longevity. Thus, tears did not appear to be directly related to mechanical factors. In addition, we found that the A

Table 3. Risks for SVD by Calcification for Porcine Bioprostheses Implanted at Age ≤60 Years (See Also Table S3)

n=500	SVD Without Calcification n=306 (61.2%)	SVD With Calcification n=194 (38.8%)	Univariate Analysis	
			OR (95% CI)	*P* Value
Male sex	156 (52.2)	112 (58.3)	1.16 (0.93–1.16)	0.18
Age at implantation	*	*	*	0.00097
Longevity	*	*	*	0.034
Valve replaced	*	*	*	0.0041
Number of valves replaced	*	*	*	0.021
Blood type				0.13
A	98 (38.1)	45 (27.4)	1.0 (reference)	
B	35 (13.6)	31 (18.9)	1.47 (1.03–2.11)	
AB	15 (5.8)	13 (7.9)	1.45 (0.48–2.39)	
O	109 (42.4)	74 (45.1)	1.27 (0.95–1.70)	
Blood type A vs others	98 (38.1)	45 (27.4)	0.74 (0.56–0.96)	0.024
Blood type B vs others	35 (13.6)	31 (18.9)	1.25 (0.97–1.64)	0.15
Blood type AB vs others	15 (5.8)	13 (7.9)	1.21 (0.78–1.88)	0.40
Blood type O vs others	109 (42.4)	74 (45.1)	1.07 (0.84–1.36)	0.58
Antigen A	110 (42.8)	58 (35.4)	0.82 (0.64–1.06)	0.13
Antigen B	50 (19.5)	44 (26.8)	1.28 (0.97–1.67)	0.076†
Rhesus	235 (91.1)	145 (89.0)	0.37 (0.59–1.27)	0.47
Bioprosthesis type	*	*	*	0.86

Calcification	Multivariate Analysis	
	OR (95% CI)	*P* Value
Age at implantation	0.81 (0.72–0.89)	0.014
Number of valves	1.75 (1.54–2.02)	0.039
Longevity	0.65 (0.45–0.85)	0.041
Blood type A vs others	0.67 (0.45–0.89)	0.087
Valve replaced	1.18 (0.99–1.37)	0.37
Bioprosthesis type	1.03 (0.94–1.12)	0.74

Values are mean±SD or n (% of n). The total may be below total bioprosthesis number because of missing data. Statistical analysis by categories (complete results can be seen in Table S2). OR indicates odds ratio; and SVD, structural valve (bioprosthesis) degeneration.

*Complete file can be found in Supplementary Material.

†Multivariate analysis comparing the same risk factors but with B-antigen as risk factor belonging to ABO system shown for B-antigen: OR, 1.34; 95% CI, 1.09–1.59; *P*=0.25.

group was globally associated with an increased risk of tears (OR, 1.17; 95% CI, 0.98–1.71; *P*=0.078), especially for bioprostheses with intermediate longevity (OR, 1.30; 95% CI, 1.07–1.57; *P*=0.0091), but not for bioprostheses with short longevity (OR, 1.03; 95% CI, 0.85–1.24; *P*=0.97) or high longevity (OR, 0.77; longevity 0.41–1.45; *P*=0.42).

Using multivariate analysis, only 2 factors were identified as being associated with the risk of tears: PB type (OR, 0.79; *P*=0.014) and A group (OR, 1.61; 95% CI, 1.39–1.83; *P*=0.026), while the number of valves was no longer statistically significant (*P*=0.082).

Risks of Porcine SVD by Perforations

Perforations were the least frequent anomaly associated with SVDs (10.5%; 95% CI, 7.8–13.2) (Table 5 and Table S4). Using univariate analysis did not enable us to identify any classical factor for SVD. The only factors found to be associated with perforations were new factors that we found associated with blood group characteristics. There was a possible association with an increased risk of perforations in the B group (OR, 1.79; 95% CI, 0.95–3.39; *P*=0.072), and the A-antigen was associated with a possible decreased risk (OR, 0.56; 95% CI, 0.30–1.05; *P*=0.071). The association between the presence of A-antigens and the risk of perforation was as follows for the different categories of PB: short longevity (OR, 0.23; 95% CI, 0.04–1.33; *P*=0.19), intermediate longevity (OR, 0.76; 95% CI, 0.36–1.58; *P*=0.46), and high longevity (OR, 1.18; 95% CI, 0.22–6.37; *P*=0.76).

Separate multivariate analyses, which included all other variables not related to blood type and 1 variable

Table 4. Risks for SVD by Tears for Porcine Bioprostheses Implanted at Age ≤60 Years (See Also Table S4)

n=500	SVD Without Tears n=214 (42.8%)	SVD With Tears n=286 (57.2%)	Univariate Analysis	
			OR (95% CI)	*P* Value
Male sex	109 (53.2)	159 (55.6)	1.04 (0.90–1.21)	0.59
Age at implantation	*	*	*	0.49
Longevity	*	*	*	0.083
Valve replaced	*	*	*	0.012
Number of valves	*	*	*	0.024
Blood type				0.39
A	54 (29.3)	89 (37.6)	1.0 (reference)	
B	32 (17.4)	34 (14.3)	0.83 (0.65–1.07)	
AB	14 (7.6)	14 (5.9)	0.81 (0.57–1.16)	
O	83 (45.1)	100 (42.2)	0.88 (0.73–1.06)	
Blood A vs others	54 (29.3)	89 (37.5)	1.17 (0.98–1.17)	0.078
Blood B vs others	32 (17.3)	34 (14.3)	0.90 (0.71–1.15)	0.39
Blood AB vs others	14 (7.6)	14 (5.9)	0.88 (0.62–1.26)	0.49
Blood O vs others	83 (45.1)	100 (42.3)	0.95 (0.80–1.13)	0.55
Antigen A	68 (37.0)	100 (42.2)	1.08 (0.93–1.10)	0.28
Antigen B	46 (25.0)	48 (20.3)	0.88 (0.72–1.09)	0.25
Rhesus	167 (91.3)	213 (89.5)	0.92 (0.70–1.21)	0.55
Bioprosthesis type	*	*	*	0.096
			Multivariate Analysis	
Tears			OR (95% CI)	*P* Value
Blood A vs others			1.61 (1.39–1.83)	0.026
Bioprosth. type			0.79 (0.69–0.88)	0.014
Number of valves			0.66 [0.43–0.89]	0.082
Longevity			0.84 [0.65–1.03]	0.41
Valve replaced			0.90] [0.72–1.08	0.60

Values are mean±SD or n (% of n). The total may be below total bioprosthesis number because of missing data. Statistical analysis by categories. More complete data are available in Table S4. OR indicates odds ratio; and SVD, structural valve (bioprosthesis) degeneration.

*Complete file can be found in Supplementary Material.

related to blood type, found several risk factors associated with blood type: B group showed an increased risk of perforation (OR, 2.21; 95% CI, 1.83–2.59; *P*=0.043), and in another analysis, there was a decreased risk in the presence of the A-antigen (OR, 0.53; 95% CI, 0.17–0.89; *P*=0.076).

Specific Anomalies Observed With Porcine SVD

In 49.7% of PBs, only 1 type of SVD was present (ie, isolated pannus, 18%; isolated calcifications, 11.2%; isolated tears, 17.8%; isolated-perforations, 2.4%).

In 50.3% of cases, a maximum of 2 anomalies were present (ie, pannus with tears, 15.8%; pannus with calcification, 6.8%; pannus with perforations, 1.2%; calcification with tears, 18%; calcification with perforations, 1.4%; tears with perforations, 5.8%).

Using univariate analysis, most SVD types were statistically highly negatively correlated with each other, except for the presence of perforations with tears (*P*=0.48). Thus, we obtained negative correlations between tears and calcification (OR, 0.70; 95% CI, 0.56–0.88; *P*=0.0016), pannus and tears (OR, 0.43; 95% CI, 0.35–0.52; *P*<0.0000001), pannus and calcification (OR, 0.29, 95% CI, 0.22–0.38; *P*<0.0000001), pannus and perforations (OR, 0.24; 0.13–0.41; *P*=0.00000055); and calcification and perforations (OR, 0.30; 95% CI, 0.17–0.53; 95% CI, *P*=0.000026).

Following numerous univariate analyses to compare the different blood group variables and associations with types of SVD, ABO blood groups were shown to be associated with very few of them: (1) Again there was a possible decreased incidence of isolated calcification in A-group patients (OR, 0.54; 95% CI, 0.28–1.04; *P*=0.064); (2) there was an increased incidence of pannus with tears in the A group (OR, 1.71; 95% CI, 1.08–2.69; *P*=0.021); (3) while pannus is normally more prevalent in the A blood group, there was an increase in the prevalence of pannus with calcification

Table 5. Risks for SVD by Perforations for Porcine Bioprostheses Implanted at Age ≤60 Years (See Also Table S5)

n=497	SVD Without Perforation n=445 (89.5%)	SVD With Perforations n=52 (10.5%)	Univariate Analysis	
			OR (95% CI)	*P* Value
Male sex	237 (54.4	31 (56.4)	1.07 (0.65–1.78)	0.78
Age at implantation	*	*	*	0.36
Longevity	*	*	*	0.71
Valve replac.	*	*	*	0.41
Number of valves	*	*	*	0.68
Blood type				0.13
A	132 (35.0)	11 (25.0)	1.0 (reference)	
B	55 (14.6)	11 (25.0)	2.18 (1.01–4.72)	
AB	27 (7.2)	1 (2.3)	0.47 (0.07–3.21)	
O	162 (43.0)	21 (47.7)	1.50 (0.75–2.99)	
Blood A vs others	132 (35.0)	11 (25.0)	0.65 (0.34–1.23)	0.18
Blood B vs others	55 (14.6)	11 (25)	1.79 (0.95–3.39)	0.072
Blood AB vs others	27 (7.2)	1 (2.8)	0.33 (0.05–1.94)	0.36
Blood O vs other	162 (42.9)	21 (47.7)	1.19 (0.68–2.08)	0.55
Antigen A, n (%)	156 (41.4)	12 (27.3)	0.56 (0.30–1.05)	0.071†
Antigen B	82 (21.8)	12 (27.3)	1.79 (0.41–1.43)	0.41
Rhesus	339 (89.9)	41 (93.2)	1.47 (0.49–4.44)	0.67
Bioprosthesis type	*	*	*	0.21

Perforations	Multivariate Analysis	
	OR (95% CI)	*P* Value
Blood B vs other	2.21 (1.83–2.59)	0.043
Valve replacement	0.70 (0.41–0.99)	0.23
Bioprosthesis type	0.85 (0.70–1.00)	0.30
Age at implantation	0.93 (0.62–1.24)	0.83
Number of valves	1.08 (1.01–1.16)	0.83

Values are mean±SD or n (% of n). The total by factor may be lower than the total of bioprostheses because of missing data. OR indicates odds ratio; and SVD, structural valve (bioprosthesis) degeneration.

*Statistical analysis by categories (complete analysis available in Table S5). Since blood type B was the only significant value following univariate analysis, all the factors were included in the multivariate analyses.

†Multivariate analysis for A blood group antigen and all other risks: for A-antigen: OR, 0.53;95% CI, 0.17–0.89; *P*=0.076.

*Complete file can be found in Supplementary Material.

in patients with the B blood group (OR, 2.42; 95% CI, 1.16–5.05; *P*=0.036) and B-antigen (OR, 2.13; 95% CI, 1.05–4.31; *P*=0.037).

Interestingly, while the A group was associated with pannus in general, when we considered the specific risk of "isolated pannus," the A group was not significantly associated (OR, 1.06; 95% CI, 0.71–1.60; *P*=0.77).

Risk of pannus with tears increased for the A group in bioprostheses with a short longevity (OR, 1.93; 95% CI, 1.18–3.16; *P*=0.0091) but not for those with intermediate longevity (OR, 1.67; 95% CI, 0.50–5.50; *P*=0.64) or the highest longevity (OR, 0.58; 95% CI, 0.16–2.05; *P*=0.60).

By conducting separate multivariate analyses that included all classical risk factors for SVD (such as age at implantation, male sex, site, number of valves, bioprosthesis types, longevity), and one type of specific SVD and one variable related to blood type, the A group was found to be significantly associated with pannus with tears (OR, 1.73; 95% CI, 1.48–1.98; *P*=0.037) but not with the group of isolated calcification (OR, 0.65; 95% CI, 0.26–1.04; *P*=0.17). For SVDs through pannus with calcification, multivariate analysis revealed the B group to be the only factor statistically associated (OR, 3.0; 95% CI, 2.54–3.46; *P*=0.025), while a possible association was revealed for B-antigen (OR, 2.24; 95% CI, 1.81–2.67; *P*=0.077).

Potential Link Between ABO Blood Group and Bovine SVD

During the study period, some of the new generations of BB with short or intermediate longevity were also explanted (n=82), and 62 of these that were implanted at age ≤60 years were considered for subsequent analysis.

Using univariate analysis, and considering only BB and all types of SVDs, no blood group variables were found to be associated in a statistically significant manner.

By associating the different PBs (n=500) with the 62 BBs, an important finding was that for the first time, when using univariate and multivariate analysis and including type of BB versus PB, the AB group was revealed to be significantly associated with specific SVD types such as isolated calcification (OR, 2.74; 95% CI, 2.30–3.18; *P*=0.035) or the presence of pannus or tears (OR, 0.037; 95% CI, 0.07–0.67; *P*=0.03) (see also Tables S5 and S6).

A summary of the main results is presented in Figures 1 and 2.

DISCUSSION

In this study, we have demonstrated that the ABO blood group of a patient influences the type of SVD. We hypothesized that carbohydrate antigens shared between the bioprostheses and a patient's ABO blood group may determine the patient's specific immunoreactivity against bioprostheses and subsequent SVD types. Using the same cohort, we recently reported observing an increased longevity of bioprostheses in A-group patients.[39]

In the present study, we have further demonstrated that, after stratification for bioprosthesis longevity, the type of SVD is associated with a patient's blood group. Using multivariate analysis, it was found that A-group patients have an increased risk of developing bioprostheses with tears (OR, 1.61; *P*=0.026), pannus (OR, 1.5; *P*=0.054) or biprostheses with pannus and tears (OR, 1.73; *P*=0.037) and lower risks of calcification (OR, 0.63; *P*=0.087), isolated calcification (OR, 0.67; *P*=0.17), and perforations (OR, 0.56; *P*=0.087). On the other hand, B-group patients have an increased risk of calcifications (OR, 1.34; *P*=0.25), the development of pannus that is also calcified, (OR, 3.0; *P*=0.025), and perforations (OR, 1.73; *P*=0.043) (see also Figures 1 and 2).

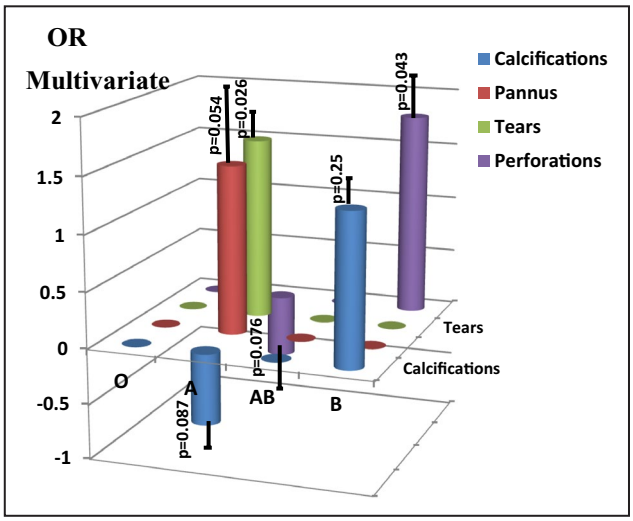

Figure 1. Forest plot showing the most important factors obtained using several separate multivariate analyses to compare the different blood groups, 0/A/AB/B, each to one another and a specific type of porcine bioprosthesis degeneration (SVD) (ie, calcifications, pannus, tears, or perforations).
The odds ratio (OR) obtained for the different multivariate analyses are reported on the vertical axis. The *P* values for each OR as well as the SD of OR are also reported.

The origin of bioprosthesis degeneration is still a subject of debate, as is the cellular and molecular mechanism of failure. The relationship between ABO blood group and type of bioprosthesis alteration has never been demonstrated.

Potential Link Between Blood Group and SVD by Calcification

Calcification is responsible for up to 70% of bioprosthesis failures through increased stiffness or tears.[55] Mechanical stress is a key factor in the initial development and aggravation of any type of calcification.[56] Thus, a close correlation between areas of high mechanical stress and calcification has been established.[57] Mechanical stress has been reported to cause abnormal calcification[58] by inducing fiber disruption and separation and then by opening small cavities[59] that are predisposed to initial local lipid accumulations before secondary calcifications.

Our results support the earlier association suggested between several risk factors and SVD calcification, including younger age, longevity of bioprosthesis, aortic site, and number of valves. We also identified new risk factors related to a patient's ABO blood group. A-group patients had a lower risk of calcification, while the presence of B-antigen/B group was associated with an increased risk. The same trend was observed with respect to isolated calcification and the incidence of pannus, with more frequent calcification occurring in the 2 latter groups. Thus, patients with A blood group may have a better overall immune compatibility with xenogenic tissue and therefore be less susceptible to calcification than B-group/B-antigen patients.

Calcification has long been considered a passive mechanism linked to glutaraldehyde reticulation,[60] but more recent studies have shown that calcification is an active process mediated by the infiltration of lipids and an unspecific inflammatory cellular response,[10,12,54] a more specific immune response,[12] or the dysregulation of infiltrating extracardiac cells that promotes local calcification.[54,61–64]

It has been shown that infiltrating cells (ie, macrophages/Lymphocytes T/Lymphocytes B) are predominant in the fibrosa layer, where calcification begins and

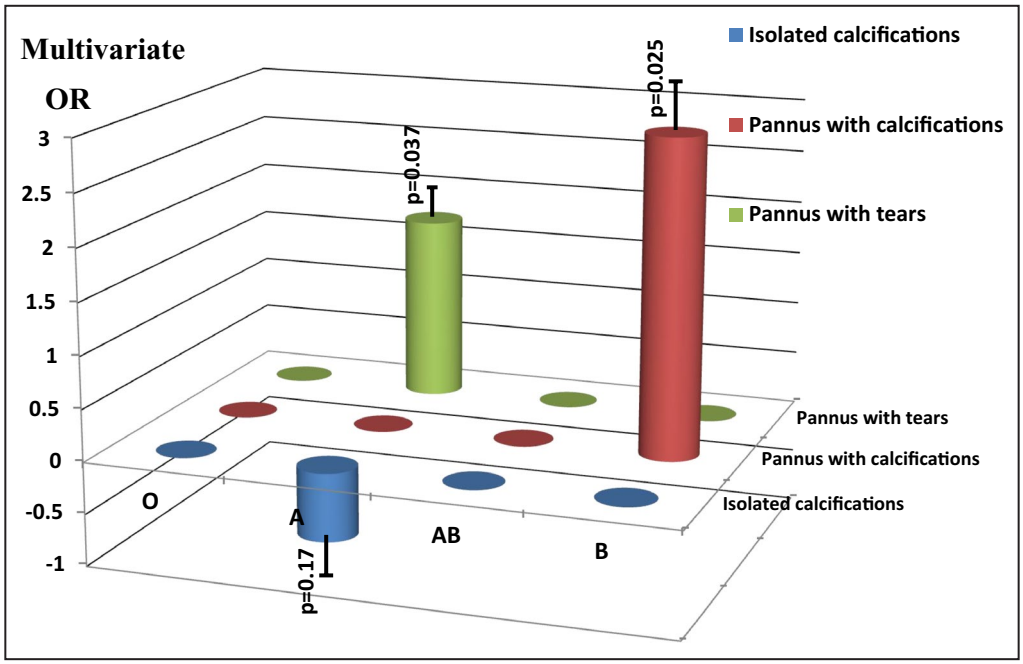

Figure 2. Forest plot showing the most important factors obtained for several separate multivariate analyses comparing the different blood groups, 0/A/AB/B, each to one another and a specific type of porcine bioprosthesis degeneration (SVD) (ie, isolated pannus, isolated calcifications, isolated tears, isolated perforations, pannus with tears, pannus with calcification, pannus with perforations, calcification with tears, calcification with perforations, tears with perforations).

Only 3 of these (ie, isolated calcifications, pannus with calcifications, pannus with tears) appear to be significantly associated, following univariate analysis, with a specific blood group. The odds ratio (OR) obtained for the separate multivariate analyses are reported on the vertical axis. The *P* values for each OR as well as the SD of OR are also reported.

lipids accumulate.[10] Macrophages at this level produce metalloproteinase, which contributes to local SVD.[54] Apart from inflammatory cells, presenting dendritic cells have also been observed in the bioprostheses and may prolong the inflammatory/immune response.[65]

It appears that glutaraldehyde decreases, but not entirely eliminates, xenogenic tissue antegenicity.[24,66–68]

One of the main factors affecting xenogenic tissue rejection is the binding of antibodies on xenograft antigen and subsequent activation of the complement by IgG monoclonal antibodies or destruction by natural killer cells.[69]

Studies of animals have demonstrated that a carbohydrate antigen (ie, α-Gal) is the target of host IgM/IgG antibodies entering the valve matrix, leading to macrophage deposition on the valve surface, followed by collagen breakdown and finally calcification.[24] This clearly indicates that the immune system plays a key role in the initiation of calcification.[24]

The carbohydrate α-Gal,[14] the major antigen of xenogenic rejection, is present in mammals such as pigs and bovines, and persists after chemical reticulation.[13] The presence of α-Gal associated with the nonacid fraction of sphingolipids on fresh unreticulated porcine valvular leaflet[38] has also been recently reported in both porcine[37] and bovine[37] pericardium. The α-Gal is structurally almost identical to the human B-antigen,[17,18] and B-type patients may have a better tolerance to this and related antigens.[20,22] Besides α-Gal, several other main carbohydrate antigens such as *N*-glycolylneuramic acid and the Forssman antigen have been identified as the major antigens of xenograft tissue recognition.[21,70] The latter two antigens have also been shown to be present in pig and bovine pericardium and also associated with the same sphingolipids fraction.[37] All these antigens, and especially α-Gal, have structural similarities with the B-antigen but not the A-antigen.[71]

Recently, several approaches have been developed to reduce the immunoreactivity of bioprostheses by controlling the expression of carbohydrate antigens, especially α-Gal. Tissues from α-galactosyltransferase knockout pigs are still immunogenic,[72,73] with increased reactivity against minor antigens derived from the same Gal framework[18,72–74] such as *N*-glycolylneuramic acid [72] but also surprisingly A/H-antigens.[75,76] *GBGT1* gene encodes Forssman glycolipid synthetase, a glycozyl synthetase that produces Forssman antigen. Most humans

are Forssman antigen negative. Recently, it has been shown that A-transferase inhibits the production of Forssman antigen by direct inhibition of the enzyme involved in its biosynthesis.[77,78]

Another carbohydrate antigen has been shown to be present on fresh pig heart valve cusps[38] and pericardium[37] and to be associated with the same sphingolipid fraction,[37] and thus, as with α-Gal, should persist after fixation by glutaraldehyde/ethanol: the AH-antigen. The synthesis of A-antigen involves the A-transferase enzyme. The carbohydrate human A-antigen has been shown to be present in some pig cardiac tissue[29,31,79] and, in some animals, to be even associated with human H-substance type 2.[37] As in the case of the human heart,[36,80] the pig A-antigen has been expressed in the same locations, such as the endocardium, the endothelial cells of myocardium, and the mesothelial cells and capillaries of the cardiac epicardium.[79] There are 4 genotypes for the "pig O-histo-group system" coding for A-histo group antigenicity: AA/AO/OO/--.[28,30–32] Unlike humans, pigs can also add the A-terminal antigen to other substances, but not necessarily the H-substance, and thus A+ pigs may have the A+H+ or A+H– phenotypes.[29,32] *FUT1*, which is coding for the Fucozyl transferase involved in H-core synthesis, is a widespread gene in mammals, including pigs,[27,35] with a high homology to the human *FUT1* gene.[35] H-substance[29,76]/*FUT1*[31] are present in some pig cardiac tissue.

Potential Link Between Blood Group and SVD by Pannus

In contrast to calcification and perforations, pannus formation represents tissue growth with collagen production, not destruction, and thus to some extent is part of the normal healing reaction after prosthetic implantation.[5] An impaired balance between proliferative/apoptotic cells has been shown to be present during SVD.[63]

Fibroblasts/myofibroblasts and endothelial cells compromise the initial host tissue reaction (pannus) probably resulting from small amounts of thrombus and inflammatory cells from initial surgical injury.[5] The exact origin of these cells is still under discussion.[61,65,81] α-smooth muscle actin–positive cells have been observed in the specific location of fibrosa[63] associated with the secondary production of collagen.[63] Extension of the pannus onto the adjacent portion of the cusps is an exuberant reaction leading to a thickening of the cusps, thus increasing their stiffness and affecting their possibility to open fully. This ultimately results in stenosis and possibly incompetence when the collagen matures and the cusp retracts. Thickening of the cusps changes their stress aggregation point, which moves away from the commissure to the cusp central area, resulting in tears at the junctional area.

It has been reported that the main determinants for pannus are valve longevity and location (ie, the mitral position).[82] We have also recently reported that A blood group patients have an increased longevity[39] in their bioprostheses, and this may favor the development of pannus over time. The effect of the A blood group was also observed to persist in multivariate analyses that included PB longevity, indicating that other pathophysiological mechanisms may also be involved.

A-group patients did not have an increased risk of isolated pannus, but only the risk of pannus associated with tears.

Potential Link Between Blood Group and SVD by Tears

Tears can develop secondary anarchic calcification that provokes cusp tears through mechanical traction and secondary valvular cusp incompetence[5,56] or secondary collagen degradation leading to tissue fragility and a greater likelihood of tears.[56] The most common sites for calcium deposits are 2 regions of high stress (ie, cusp commissural/basal areas),[5,83] and calcification is present a long time before the apparition of tears in these areas.[5,84] The site for collagen alteration is different, being located in the central fibrosa,[5] an area in which delayed tears and perforations mainly develop.[56]

Our univariate analysis revealed that the risks for tears were increased with mitral site, multiple PBs, older generations of PBs, longevity (ie, with a decreased risk of tears for higher longevity) and the novel variable, blood group A. As previously reported,[83] we observed that mitral site valves are more prone to tears because of increased mechanical high closing pressure.[5] Design modifications of new bioprostheses[85] can influence the amount of pressure experienced by the different parts of PBs and thus the risk of tears.[5] Blood group A was associated with an increased risk of tears. However, blood group A patients also had a lower propensity for calcifications, so that the hypercalcification mechanism cannot explain the greater prevalence of tears.

Potential Link Between Blood Group and SVD by Perforations

In our study, perforations were a rare complication, accounting for around 10.5% of the cases examined, which is within the range reported in the literature (10%–15%).[86] Perforations lead to leaflet incompetency and valve regurgitation. Cuspal perforations have been

associated with commissural sutures in areas of intense high stress[86] but also independently of the process of calcification in areas of collagen breakdown and disorganization in the fibrosa.[56,59] Using separate multivariate analyses, we found that B-group patients have an increased risk (OR, 1.73; *P*=0.043), while A-group patients have a possible decreased risk (OR, 0.56; *P*=0.076).

Identifying underlying mechanisms may need further investigation, but such mechanisms may be linked to the tissue being more tolerant in the presence of A-antigen and thus less prone to collagen alteration.

Potential Link Between Carbohydrate Antigens, Including Blood Group and Bovine SVD

PBs and BBs, although from different species and tissue types (ie, native cusp versus pericardial tissue), may ultimately be subject to somewhat similar modes of failure by calcification and stenosis.[5] Bovine pericardial valves have a greater propensity to develop stenosis as a mode of failure[4] attributable to excessive calcification, which is both more diffuse and deeper in the tissue and can result in tears provoked by stress.[86] Although the true impact of ABO blood type needs further assessment in a larger study, our preliminary study of BBs with short/intermediate longevity showed that blood group may also impact bovine SVD. This is in line with our earlier report showing that that there is a lower rate of early failure of BBs in A-group recipients.[39]

In some bovines, as for some pigs, human A/H-type 2 antigen has been shown to be present in saliva, gut epithelium, the urinary tract, and respiratory tract cells.[87,88] Bovine A-transferase has also been isolated and cloned[31,89] and has a high level of homology with humans. *FUT1* is a common gene in mammals,[35] including bovines (ie, *Bos taurus*) and is present in bovine hearts.[35,90]

α-Gal carbohydrate and *N*-glycolylneuramic acid have been shown to be present and again associated with sphingolipids in the bovine pericardium.[37] However, there was no H-core or A-antigen detected in the small number of animals investigated.[37]

Study Limitations

The main limitation was that the tissue antigens borne by the bioprosthesis were unknown, unlike the patient's blood group. Therefore, we could not directly demonstrate that biocompatibility was associated with specific types of bioprosthesis structural alteration. Another limitation was that we had access only to patients whose bioprostheses had failed, thus requiring replacement, and not to the full cohort of patients who initially received a bioprosthesis. For this reason, we were not able to compute risks of failure or to construct Kaplan–Meier time-to-failure curves.

At the same time, meta-analysis of prospective studies has shown a very low rate of SVD.[53] Following 2758 patients with Carpentier-Edwards bioprostheses in the aortic position over a period of 20 years, Bourguignon et al[3] reported only 123 patients requiring reoperation during this time. This has to be compared with the 426 patients requiring reoperation in the present study. Large-size populations as in our study will allow for an equal distribution of confounding factors. The low frequency of bioprosthetic valve degeneration could also make it more difficult to properly evaluate all the clinical parameters that have been reported as possible influences on bioprosthetic valve degeneration in prospective studies.

Today, we are able to access data from more than 29 years of observations. This interval exceeds the mean longevity of bioprostheses, especially if these were implanted before patients reached 60 years old, with 90% of SVDs occurring after 25 years.[3,4,91,92] Thus, we may expect to have covered all the bioprostheses that failed, but we may have "missed" bioprostheses of patients who died before their bioprostheses required replacement. However, we expect to have a full spectrum of bioprothesis anomalies regarding longevity.

Another limitation of the study is that we do not have data on all the parameters that have recently been identified as influencing SVD. However, none of these factors were found to be correlated with the ABO blood group.

Some studies have shown a possible association between longevity and the ABO blood group.[93] In Japan, the B-antigen has been shown to be possibly associated with higher longevity.[94] On the other hand, the A-antigen has been shown to be associated with higher cardiac mortality, including ischemic events.[95,96] A-group patients have a higher level of circulating von Willebrand factor/factor VIII[97,98] and possibly higher circulating cholesterol levels.[99] Lipids have been shown to positively influence the risk of calcification[10,12] but could not explain the decreased risk of calcification we reported in patients with blood type A. The A-antigen has also recently been found to be associated with an increased risk of mechanical cardiac aortic valve thrombosis.[100]

In a more recent study, Lehmann et al[40] confirmed an improved survival rate for PBs or BBs in A blood group patients and a decreased survival rate for B and AB patients. In this study, Lehmann et al followed 4274 patients with 1521 PBs and 2753 BBs implanted in the aortic position for >10 years. Kaplan–Meier survival analysis has shown a better survival in A-group patients as compared with B- or AB-group patients and for both types of bioprostheses (BBs or PBs) and no influence of Rhesus. Cox regression showed that blood group B (hazard ratio,

5.87; *P*=0.015), like group AB, is an independent risk factor regarding mortality after aortic bioprosthesis replacement.[40] This difference between ABO blood groups was not observed for patients implanted with a mechanical valve in the same position (n=1500). Valve-related mortality was not reported, nor was the presence (or absence) of SVD or the type of SVD.

Clinical Considerations

The design of new bioprostheses that are more resistant to hosts' immune responses would be a major improvement in valvular heart surgery.[101] Thus, this may well improve the best possible long-term cure for patients. We,[39] like others,[40] have recently reported how the ABO blood group influences PB/BB longevity. Our results here further indicate how the ABO group of the patient may also be associated with porcine and bovine types of SVD.

Multivariate analysis showed that the ABO group was not only a new additional risk factor, but also one of the most predictive for calcification/tears/pannus/perforations, suggesting that it may play a key role in the immune processes in all SVD types. Moreover, we may be underestimating its positive influence for several reasons. First, we did not have information about the AH phenotype of the pig. Since the prevalence of A-allele in pig varies from 0.15 to 0.67,[30,33,102] only a fraction of A-group patients could randomly receive the corresponding A bioprosthesis. Since we were measuring this effect on the basis of a purely arbitrary adequate allocation between pig AH tissue and human A, we would probably be able to multiply the beneficial effect on SVD by a factor of 2 to 5 if we deliberately matched patients and prosthesis phenotypes.

CONCLUSIONS

To our knowledge, this study is the first to report a possible correlation between patient ABO blood group and SVD types. We hypothesize that there is an underlying mechanism that shares carbohydrate antigens between the patient and the bioprosthesis and that this may determine patient immunoreactivity with regard to the tissue and subsequent SVD. These results further emphasize the potentially important role of immunoreactivity in specific types of SVD.

Greater compatibility between a patient's ABO blood group and bioprosthesis tissue carbohydrate antigens may help to provide new generations of bioprostheses that are less prone to calcification and that provide a safer and more durable outcome for patients of a younger age.

ARTICLE INFORMATION

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None.

Supplementary Materials

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Ethics Statement

The ethics committee was not involved on account of the retrospective nature of the study. All patients gave consent for the use of their data for research at the University Hospital.

Patient Cohort

Single-centre study of patients requiring reoperation for degenerative bioprostheses at Broussais-Hospital/Georges Pompidou European Hospital in Paris, (Chair: Professor Alain Carpentier). Professor A. Carpentier has developed the concept of valvular heart bioprostheses [41] and overseen the development of several generations of porcine and bovine bioprostheses. Moreover, a large number of cases have undergone initial evaluation studies at this center [41, 42].

Patients' characteristics before 1985 (1975-1980 [43-45] and 1980-1985 [46, 47]) and a number of implanted bioprostheses in the mitral or aortic position in the Broussais Hospital have already been reported in the literature [48]. Due to a mean longevity of heart valve bioprostheses of 10 years, the period 1985-1997 corresponded to CE-2nd/3rd generation bioprostheses that were implanted at the institution mainly between 1975 and 1985. Unlike the 2nd-CE generation of bioprostheses made from one pig, the 3rd-CE was manufactured using the cusps of two pigs with a view to improving the hemodynamics [43 48,104]. New generations of CE-bioprostheses made from xenogenic bovine pericardium [49, 50,] have been implanted since 1980 [48,46] and have almost totally replaced porcine bioprostheses since 1985. Chemical fixation by glutaraldehyde is still the main

reticulating reagent. Patients' clinical conditions were not taken into account for the choice between a porcine or bovine bioprosthesis and, after 1985, almost all patients received the new generation of bovine bioprostheses.

All patients reoperated for degenerative porcine bioprostheses during the 13-year period from January 1985 to December1997 were eligible for the study and mainly comprised patients from our institution.

In addition, we included all patients reoperated on from January 1998 to January 2014 who had a porcine bioprosthesis with exceptional longevity (≥ 13 years). Since most porcine bioprostheses were implanted before 1985, we have a total follow-up period of 29 years for most of them.

Criteria for inclusion: a degenerative porcine bioprosthesis that needs replacement during this period because of bioprosthetic valve dysfunction due to intrinsic structural valve deterioration (i.e. bioprosthesis degeneration (SVD)). Excluded are bioprosthetic replacements for other causes of valvular dysfunction such as nonstructural valve deterioration (i.e. any abnormality not intrinsic to the valve itself such as para-prosthetic regurgitation, malposition) [51], endocarditis or thrombosis. The cause of valve replacement and the type of intrinsic structural valve anomalies were prospectively specified by the surgeon at the time of bioprosthesis replacement, with observations on the presence of calcification, pannus, tears or perforations. A standardized classification of bioprosthesis valve degeneration in the aortic position has been recently proposed and excludes bioprosthesis thrombosis [9, 51, 52]. In a recent prospective study evaluating 25 years of Carpentier-Edwards bovine bioprostheses in the aortic position, the causes for valvular replacement were revealed to be: SVD (73%), endocarditis (15%), nonstructural dysfunction (11%), and thrombosis (exceptional) [3].

Additional recent factors that lead to accelerated bioprosthetic valve dysfunction, such as patient prosthesis mismatch or the small size of some prostheses, were not specifically investigated. In the case of multiple bioprosthesis implants, only degenerative bioprostheses were considered. Some cases of early failure or intermediate longevity in the new generation of bovine bioprostheses were explanted during this period (1985-1998) and analysed separately.

Study variables

The main outcome variable was the interval between valve implantation and explantation (longevity) and the type of SVD. The main risk factor of interest was the patient's blood type (ABO and rhesus) and was retrospectively collected. Other known classical risk factors for structural degeneration of bioprostheses, and for which data were prospectively collected at replacement, were as follows: patient's age at the moment of implantation, sex, valve location and number of bioprostheses implanted initially. Some additional risk factors in bioprosthesis degeneration, especially in the aortic position, have also been reported recently and include factors that increase hemodynamic stress (larger body surface area, small prosthesis size, prosthesis-patient mismatch, left ventricle hypertrophy) and cardiovascular risk factors such as smoking, hypertension, metabolic syndrome, diabetes mellitus, dyslipidemia [9] [52]. Chronic dialyses and hyperparathyroidism have also been shown to be associated with early structural valve degeneration, although patients presenting these characteristics are rare in this study.

The only type of bioprosthesis degeneration that has been well studied is that caused by calcification[4, 5, 9, 10, 53, 54]; while the roles of pannus, tears or perforations are mostly unknown[5]. None of these factors have been shown to be related to patient ABO blood group and could

therefore not explain the different levels of bioprosthesis degeneration between the different ABO blood groups.

Data collection

A prospective database for bioprosthetic heart valves has been developed since 1985. Thus, from 1985 to 1998, data was collected prospectively and valve information recorded in the operating room. Data on the type of bioprosthesis alteration was prospectively reported by the surgeon in the operating room, so that we now have information for the main criteria (pannus/calcification/tears/perforation) for most degenerative bioprostheses (913 out of 963). Other additional information collected included factors contributing to bioprosthesis replacement (e.g. thrombosis, endocarditis, non-dysfunction valve), date of implantation, longevity, site of implantation and number, origin (bovine or porcine) and type of bioprosthesis. Information on the patient's blood group was obtained from the blood bank (2/3 of cases) and patients' records (1/3 of cases). If the information was not available in the blood bank, patients' medical records were consulted for those patients with the highest ($\geq$ 13 years) and lowest longevity ($\leq$ 7 years).

After 1998, all porcine bioprostheses with a longevity of $\geq$ 13 years were systematically sent to the laboratory for further analysis. This enabled us to refer back to a patient's name and chart.

Since 1985 the new generations of bioprostheses made from bovine tissue have replaced porcine prostheses, so that for the year 2014 we have at least 29 years of follow-up for porcine bioprostheses.

Statistical analysis

We obtained frequency distributions for all study variables, for all replaced valves, and for all patients. For the main outcome variable, i.e. valve longevity, discrete categories were defined: the approximate lower and upper deciles were isolated (early and late failure), and the remainder was split into 3 classes, resulting in the following 5 longevity categories (years): 0-5.9, 6-8.9, 9-11.9, 12-14.9 and 15-28. We cross-tabulated the 5-levels of longevity variable with ABO and rhesus blood types. This analysis suggested that the three middle categories were homogenous, so for simplicity we continued the analysis with a 3-level longevity variable (0-5.9, 6-14.9, 15-28). We cross-tabulated this variable with blood types and other valve and patient characteristics. We did not choose the class of longevity initially, but these classes of longevity appear to be clinically relevant[3].

Categorial data were examined as percentage ± SD and compared by χ^2 test or Fisher's exact test as appropriate. All variables used in the univariate analysis were entered into the multivariate models. A *p* value of ≤ 0.05 was considered statistically significant. A multivariate logistic regression model was used to identify the independent predictors of structural bioprosthesis degeneration. Only bioprosthesis patients with known ABO blood group were included in the multivariate analyses.

The analyses were conducted using SPSS-version18 or the statistical software system SEM (Silex Development, Mirefleurs, France).

Supplemental Results

Patient and valve characteristics

Between 1985 and 1998, 854 porcine bioprostheses were explanted from 641 patients. From 1998 to 2014, 32 additional porcine bioprostheses with longevity $\geq$ 13 years were removed (total 886). The blood group was retrospectively found for 736 out of 886 patients and longevity information was available for 564 out of 886, while the important 'age at implantation' factor was available for 559 of the cohort.

Not surprisingly, most patients (89.3%) in this group with porcine bioprostheses needing surgical replacement were $\leq$ 60yrs at implantation. We focus our attention on this group with porcine bioprostheses implanted before the age of 60 because it is over-represented and corresponds to the group of bioprosthesis patients that needs major clinical improvements. Most of the patients with bioprostheses implanted at 60 years or before will need to be re-operated after a period of 25 years for degenerative bioprosthesis[4]. SVD data collected was available for 29 years so that we assume we have an unbiased full spectrum of bioprosthesis degenerative type anomalies for several periods of longevity.

We have a group of 500 porcine bioprostheses that were implanted before the patients were 60 years old and for which most variables, especially the type of alteration, were known. This group thus constitutes our study cohort. However, in this cohort, information on the ABO blood group was missing for 79 bioprosthesis patients.

During the period 1987-1998, we also explanted 82 bovine bioprostheses with intermediate and short longevity and 62 BP implanted when patients were $\leq$ 60yrs.

Demographic data and porcine valve characteristics are shown in Table 1. Types of explanted porcine bioprostheses were as follows: CE-2nd 49.9%, 3rd 33.8%, CE-1st 2.7%, other porcine bioprostheses 13.6%. 20.1% of patients had had more than one porcine bioprosthesis. Mean age at implantation was 38.6±12.6 years, the time lapsed before reoperation for degenerative porcine bioprosthesis was 9.9 years ±3.4 [0-28] and the quartiles were 8/10/12 years. Most valves lasted between 6 and 15 years. About 15.6% failed before 7 years and another 10.6% failed after ≥15years. In our group of patients re-operated for failing bioprostheses, many factors known to be predictive for lower longevity, such as mitral site of implantation (60.9%), younger age or multiple bioprostheses (20.1%), were over-represented compared with the initial population at the time of implantation [43-45] [46, 47] [48]. In this failing group, the number of young patients ≤ 35 years old at implantation (39.4%) was very high.

The prevalence of ABO in this small cohort was: A: 34.2%, 95% CI +/- 4.5; B: 15.7%, 95% CI +/- 3.5; AB: 6.7%, 95% CI, +/- 2.4; O: 43.5%, 95% CI +/- 4.7; and Rhesus (-): 9.7%, 95% CI +/- 2.8. Prevalence of ABO blood group in France or in Caucasian populations is around 45% for Group A, 43% for Group O, 9 % for Group B, 3% for Group AB [105]. Thus the distribution by blood group matched the expected prevalence for blood group O, the incidence was higher for blood groups B and AB, and slightly lower for blood group A.

Risks of porcine SVD by pannus

Pannus were present in 42.4% of SVD (CI 95%, [38.1%-46.7%]) (see Table 2 and Table S1). Using univariate analysis, we identified several risk factors such as increased risk with longevity (p=0.0036) and bioprosthesis type (less frequent in more recent PB)(p=0.08). Interestingly, we

observed a possible association indicating an increased risk of pannus in A-group: OR 1.24 [0.99-1.56] (p=0.064)(p=*ns*). Risk of pannus for A-group for PBs with different classes of longevity was as follows: short longevity: OR 1.15 [0.66-2.01] (p=0.61), intermediate longevity: OR 1.25 [0.96-1.63] (p=0.092) and, for the group with the longest longevity: OR 1.06 [0.59-1.89] (p=0.85).

Using Multivariate analysis, the risks for pannus were found to be associated with: longevity: OR 2.01 [1.81-2.21] (p=0.00031) and possibly, although not statistically significant, A-group: OR 1.50 [0.99-2.26] (p=0.054)(p=*ns*).

Risks of porcine SVD by calcification

Calcification was present in 38.8% of SVDs (CI 95%, [34.5-43.1%]) (see table 3 and Table S2). Using univariate analysis, we identified the classical risk factors such as younger age at implantation (p=0.00097), site (p=0.0041), and multiple bioprostheses (p=0.021). Higher longevity was surprisingly associated with a lower risk of calcification (p=0.034). We also identified two new additional risk factors for calcification related to ABO blood group: the A-group factor was associated with a decreased risk: OR O.74 [0.56-0.96] (p=0.024), while the B-antigen showed a possible association with an increased risk: OR 1.28 [0.97-1.67] (p=0.076)(p=*ns*).

Using multivariate analysis, and including A-group as a variable, we identified several risks for calcification: age at implantation: OR 0.81 (p=0.014), valve number: OR 1.75 (p=0.039), longevity: OR 0.65 (p=0.087) and, compared with B blood group patients, there was a decreased calcification propensity in A-group patients: OR 0.67 [0.45-0.89] (p=0.087), although it was not statistically significant. The same multivariate analysis, with the presence of B-antigen, showed that this last factor was again associated with an increased risk of calcification: OR 1.34 [1.09-1.59], although it was not statistically significant (p=0.25).

Risks of porcine SVD by tears

Tears were present in 57.2% of SVDs (CI 95%, [53.4-62.0]) (see Table 4 and Table S3). Using univariate analysis, we identified several risks of tears such as: site of implantation (p=0.012), multiple prostheses (p=0.024), and tendency for types of bioprosthesis (p=0.096). The incidence of tears decreased with longevity (p=0.083). The influence of mechanical factors, with repetitive stimulations, is known to increase with longevity. Thus, tears did not appear to be the sole variable directly related to mechanical factors. In addition, we found that A-group was globally associated with an increased risk of tears: A-group: OR 1.17 [0.98-1.71] (p=0.078), especially for bioprostheses with intermediate longevity: OR 1.30 [1.07-1.57] (p=0.0091), but not for bioprostheses with a short longevity: OR 1.03 [0.85-1.24] (p=0.97)($p=ns$) or a long longevity: OR 0.77 [0.41-1.45] (p=0.42)($p=ns$).

Using multivariate analysis, only two factors were identified as being associated with the risk of tears: PB types: OR 0.79 (p=0.014) and A-group: OR 1.61 [1.39-1.83] (p=0.026) while the number of valves was no longer statistically significant (p=0.082).

Risks of porcine SVD through perforations

Perforations were the least frequent anomaly in relation to SVDs (10.5%, CI 95%, [7.8-13.2]) (Table 5 and Table S4). Using univariate analysis did not enable us to identify any classical factor for SVD. The only factors that we found to be associated with perforations were new factors that we found associated with blood group characteristics. There was a possible association with an increased risk of perforations in B-group: OR 1.79 [0.95-3.39] (p=0.072)($p=ns$) and A-antigen was associated with a possible decreased risk: OR 0.56 [0.30-1.05] (p=0.071)($p=ns$). The association of the presence of A-antigen and the risk of perforation was as follows for the different classes of

PB: short longevity: OR 0.23 [0.04-1.33] p=0.19)(p=*ns*), intermediate longevity: OR 0.76 [0.36-1.58] (p=0.46), and high longevity: OR 1.18 [0.22-6.37] (p=0.76)(p=*ns*).

Separate multivariate analyses, which included all other variables not related to blood type and one variable that was related to blood type, found several risk factors associated with blood type: B-group with an increased risk: OR 2.21 [1.83-2.59] (p=0.043) and, for another analysis, a decreased risk in the presence of the A-antigen: OR 0.53 [0.17-0.89] (p=0.076)(p=*ns*).

Development of new scoring system for porcine SVD

We developed two new indicators: the first described the presence of calcification or perforations and was positive in 48.9% of the patients, while the second described the presence of tears or pannus and was positive in 84.2% of the patients (Table S6).

Using univariate analysis, the calcification or perforations factor was found to be associated with site (p=0.0052), age at implantation (p=0.017), and number of valves (p=0.092) and was also significantly associated with some ABO variables: blood type (i.e. general distribution) (p=0.021), blood type B: OR 1.36 [1.06-1.75] (p=0.016), blood type A: OR 0.75 [0.60-0.94] (p=0.012), A antigen: OR 0.79 [0.64-0.97] (p=0.028), and B antigen: OR 1.27 [1.01-1.60] (p=0.039). Following multivariate analysis, in addition to longevity, the site and blood type B were significantly associated with calcifications or perforations: OR 1.78 [1.51-2.05] (p=0.035). The same multivariate analysis was also conducted for B antigen: OR 1.6 [1.36-1.84] (p=0.041), for blood group A: OR 0.72 [0.46-0.88] (p=0.067), and A antigen: OR 0.72 [0.52-0.92] (p=0.12)(p=*ns*).

The risk factors for the presence of tears or pannus, as determined by univariate analysis, were: valve replaced (p=0.0000061), bioprosthesis type (p=0.002) and blood type A: OR 1.11 [1.02-1.22] (p=0.014) (Table 7). Following multivariate analysis, only two factors were shown to

be associated with the presence of pannus or tears: valve replacement: OR 0.37 [0.14-0.97] (p=0.000022) and blood group A: OR 2.03 [1.71-2.35] (p=0.021) (Table 7).

Risks for specific porcine SVD

In our study, two anomalies were present during SVD in 50.3% of cases.

The bioprosthesis presented only one type of SVD in 49.7% of cases as follows: isolated pannus 18%, isolated calcification 11.2%, isolated tears 17.8%, isolated perforations 2.4%.

In 50.3% of cases, two anomalies were associated with each other: pannus with tears 15.8%, pannus with calcification 6.8%, pannus with perforation 1.2%, calcification with tears 18%, calcification with perforation 1.4%, tears with perforation 5.8%.

While most pannus/calcification/tear/perforation factors were statistically highly negatively associated with each other, one pair was not associated: perforations with tears (p=0.48).

Furthermore, weaker correlations were revealed between tears/calcification: OR 0.70 [0.56-0.88] (p=0.0016), pannus/tears: OR 0.43 [0.35-0.52] p<0.0000001, pannus/calcification: OR 0.29 [0.22-0.38] (p<0,0000001), pannus/perforations: OR 0.24 [0.13-0.41] (p=0.00000055), and calcification/perforation: OR 0.30 [0.17-0.53] (p=0.000026).

Following numerous univariate analyses to compare the different blood group variables and types of SVD associations, ABO blood groups were shown to be associated with very few of them: again there was a possible decreased incidence of isolated calcification in A-group patients: OR 0.54 [0.28-1.04] (p=0.064)(p=ns); an increased incidence of pannus with tears in A-group patients: OR 1.71 [1.08-2.69] (p=0.021); and, while the pannus is normally more prevalent in A blood group, there was an increase in the prevalence of the pannus with calcification in B-group: OR 2.42 [1.16-5.05] (p=0.036) and B-antigen: OR 2.13 [1.05-4.31] (p=0.037).

Interestingly, while the A-group was associated with pannus in general, when we considered the specific risk of "isolated pannus", the A-group was not significantly associated: OR 1.06 [0.71-1.60] (p=0.77).

Risk of pannus with tears increased for A-group in bioprostheses with short longevity: OR 1.93 [1.18-3.16] (p=0.0091), but not for those with intermediate longevity: OR 1.67 [0.50-5.50] (p=0.64) or the high longevity: OR 0.58 [0.16-2.05] (p=0.60).

By conducting separate multivariate analyses, which included all classical risk factors for SVD (i.e. such as age at implantation, male sex, site, number of valves, bioprosthesis types, longevity), one type of specific SVD and one variable related to blood type, the A-group variable was found to be significantly associated with pannus with tears: OR 1.73 [1.48-1.98] (p=0.037) but not with the group of isolated calcification: OR 0.65 [0.26-1.04] (p=0.17). For SVD by pannus with calcification, multivariate analysis revealed B-group to be the only factor statistically associated: OR 3.0 [2.54-3.46] (p=0.025), while the B-antigen showed a weaker association: OR 2.24 [1.81-2.67] (p=0.077).

Potential link between ABO blood group and bovine SVD

During the study period, some new generations of bovine bioprostheses with short (<7 years) or intermediate longevity (7-15 yrs) were explanted (n=82) and analyzed separately. Only the 62 bovine bioprostheses implanted before the patient was 60 years old were considered. In this small group of degenerative bovine bioprostheses, multiple univariate analyses comparing any type of SVD and any type of ABO blood group variables, found there were no significant statistical associations.

By associating the different porcine bioprostheses (n=500; age of implantation $\leq$60yrs) with the 62 new-generation bioprostheses (also implanted $\leq$60yrs), it appeared that, for the first time using

univariate analysis, the rare AB blood group was significantly associated with certain types of SVD. Considering the AB blood group, we did not find such a correlation for porcine bioprostheses alone. There was a significant association for the AB blood group for increases in the presence of isolated calcification: OR 2.10 [1.14-3.86] (p=0.017) (Table S5) and a decrease in the presence of pannus or tears: OR 0.87 [0.75-1.00] (p=0.044).

By conducting multivariate analyses, which included all the risk factors and the type of porcine or bovine bioprosthesis, for isolated calcification, the following associations were revealed: for AB-group: OR 2.74 [2.30-3.18] (p=0.035), and BB versus PB: OR 1.97 [2.30-3.18] (p=0.28) (Table S6). Multivariate analysis also revealed the following associations for risks of pannus or tears: AB-group: OR 0.37 [0.07-0.67] (p=0.03), BB versus PB: OR 0.53 [0.28-0.78] (p=0.28).

A summary of the main results are presented in Figures 1 and 2.

Table S1. Risks for SVD by pannus for porcine bioprostheses implanted at age ≤60 years.

	All Porcine Bioprostheses n=500	**SVD without Pannus n=288 (57.6%)**	**SVD with Pannus n=212 (42.4%)**	**Univariate Analysis**	
				OR (95%CI)	***P-value***
Male sex	268 (54.6%)	160 (55.9%)	108 (52.7%)	0.93 [0.75-1.14]	0.47
Age at implantation					0.50
[7-20]	24 (5.2%)	18 (6.6%)	6 (3.2%)	1.0 (reference)	
]20-30]	110 (23.9%)	64 (23.5%)	46 (24.5%)	1.67 [0.87-3.23]	
]30-40]	98 (21.3%)	56 (20.6%)	42 (22.3%)	1.71 [0.89-3.31]	
]40-50]	114 (24.7%)	64 (23.5%)	50 (26.6%)	1.75 [0.92-3.34]	
]50-60]	114 (24.7%)	70 (25.7)	44 (23.4%)	1.54 [0.79-3.03]	
Longevity					0.0036
[0-7[	73 (15.9%)	55 (20.3%)	18 (9.6%)	1.0 (reference)	
[7-15[	338 (73.5%)	194 (71.3%)	144 (76.6%)	1.73 [1.19-2.52]	
[15-28]	49 (10.6%)	23 (8.4%)	26 (13.8%)	2.15 [1.35-3.44]	
Valve replaced, n (%)					0.22
Mitral	304 (60.9%)	167 (58.0%)	137 (64.9%)	1.0 (reference)	
Aortic	188 (37.7%)	115 (39.9%)	73 (34.6%)	1.11 [0.95-1.30]	
Tricuspid or pulmonary	7 (1.4%)	6 (2%)	1 (0.5%)	1.56 [0.91-2.67]	
Number of valves replaced, n (%)					0.31
1	399 (79,9%)	224 (77.8%)	175 (82.9)	1.0 (reference)	
2	94 (18,8%)	59 (20.5%)	35 (16.6%)	1.12 [0.93-1.35]	
3	6 (1.3%)	5 (1.7%)	1 (0.5%)	1.48 [0.83-2.65]	
Blood type, n (%)					0.22
A	143 (34.2%)	74 (30.3%)	69 (39%)	1.0 (reference)	
B	66 (15.7%)	38 (15.6%)	28 (15.8%)	0.8 [0.63-1.20]	
AB	28 (6.7%)	18 (7.4%)	10 (5.7%)	0.73 [0.45-1.19]	
O	183 (43.5%)	114 (46.7%)	69 (39%)	0.78 [0.60-1.00]	
Blood type A (versus other)	143 (34.0%)	74 (30.3%)	69 (38.9%)	1.24 [0.99-1.56]	0.064
Blood type B (versus other)	66 (15.7%)	38 (15.6%)	28 (15.8%)	1.01 [0.74-1.37]	0.95
Blood type AB (versus other)	28 (6.7%)	18 (7.4%)	10 (5.6%)	0.84 [0.52-1.37]	0.48
Blood type O (versus other)	183 (43.5%)	114 (46.7%)	69 (38.9%)	0.83 [0.66-1.05]	0.11
Antigen A, n (%)				1.16 [0.92-1.45]	0.20

Positive (A or AB)	168 (39.9%)	91 (37.3%)	77 (43.5%)		
Negative (B or O)	253 (60.1%)	153 (62.7%)	100 (56.5%)		
Antigen B, n (%)				0.95 [0.72-1.25]	0.72
Positive (B or AB)	94 (22.3%)	56 (23.0%)	38 (21.5%)		
Negative (A or O)	327 (	118 (77.1%)	149 (84.2%)		
Rhesus, n (%)				1.08 [0.73-1.60]	0.70
Positive	380 (90.3%)	220 (89.8%)	160 (90.9%)		
Negative	41 (9.7%)	25 (10.2%)	16 (9.1%)		
Bioprosthesis type					0.08
CE-3rd	161 (33.8%)	104 (37.4%)	57 (28.7%)	1.0 (reference)	
CE-2nd	238 (49.9%)	128 (46.0%)	110 (55.3%)	1.31 [1.02-1.66]	
CE-1st	13 (2.7%)	10 (3.6%)	3 (1.5%)	0.65 [0.26-1.66]	
Other porcine biopr.	65 (13.6%)	36 (13.0%)	29 (14,6%)	1.26 [0.89-1.79]	
Pannus				**Multivariate Analysis**	
				OR (95%CI)	***P-value***
Longevity				2.01 [1.81-2.21]	0.00031
Blood type A (versus other)				1.50 [0.99-2.26]	0.054
Bioprosthesis type				1.08 [0.98-1.17]	0.41

Values are mean +/-SD or n (% of n). p values refer to comparisons between SVD without pannus and SVD with pannus.

SVD: structural bioprosthesis degeneration

CE-1st /CE-2nd /CE-1^{3rd}: Carpentier Edwards First/Second/Third generation of porcine bioprostheses

Patient numbers by category may be lower than expected due to missing data.

Table S2. Risks for SVD by calcification for porcine bioprostheses implanted at age ≤60 years.

n=500	**SVD without calcification n=306 (61.2%)**	**SVD with calcification n=194 (38.8%)**	**Univariate Analysis**	
			OR (95%CI)	***P-Value***
Male sex	156 (52.2%)	112 (58.3%)	1.16 [0.93-1.16]	0.18
Age at implantation				0.00097
[7-20]	11 (4.0%)	13 (7.1%)	1.0 (reference)	
]20-30]	58 (20.9%)	52 (28.4%)	0.87 [0.56-1.35]	
]30-40]	54 (19.5%)	44 (24.0%)	0.83 [0.53-1.30]	
]40-50]	74 (26.7%)	40 (21.9%)	0.65 [0.40-1.05]	
]50-60]	80 (28.9%)	34 (18.6%)	0.55 [0.33-0.92]	
Longevity				0.034
[0-7[	38 (13.2%)	41 (21.7%)	1.0 (reference)	
[7-15[	214 (74.8%)	132 (69.8%)	0.74 [0.56-0.96]	
[15-28]	35 (12.0%)	16 (8.5%)	0.60 [0.39-0.93]	
Valves replaced, n (%)				0.0041
Mitral	199 (65.2%)	105 (54.1%)	1.0 (reference)	
Aortic	105 (34.4%)	83 (42.8%)	1.25 [0.96-1.63]	
Tricuspid or pulmonary	1 (0.3%)	6 (3.1%)	2.48 [0.31-4.69]	
Number of valves replaced, n (%)				0.021
1	253 (83.0%)	146 (75.3%)	1.0 (reference)	
2	51 (16.7%)	43 (22.2%)	1.25 [0.96-1.63]	
3	1 (0.3%)	5 (2.6%)	2.28 [1.15-4.52]	
Blood type, n (%)				0.13
A	98 (38.1%)	45 (27.4%)	1.0 (reference)	
B	35 (13.6%)	31 (18.9%)	1.47 [1.03-2.11]	
AB	15 (5.8%)	13 (7.9%)	1.45 [0.48-2.39]	
O	109 (42.4%)	74 (45.1%)	1.27 [0.95-1.70]	
Blood type A (versus other)	98 (38.1%)	45 (27.4%)	0.74 [0.56-0.96]	0.024
Blood type B (versus other)	35 (13.6%)	31 (18.9%)	1.25 [0.97-1.64]	0.15
Blood type AB (versus other)	15 (5.8%)	13 (7.9%)	1.21 [0.78-1.88]	0.40
Blood type O (versus other)	109 (42.4%)	74 (45.1%)	1.07 [0.84-1.36]	0.58
Antigen A, n (%)			0.82 [0.64-1.06]	0.13
Positive (A or AB)	110 (42.8%)	58 (35.4%)		
Negative (B or O)	147 (57.2%)	106 (64.6%)		

Antigen B, n (%)			1.28 [0.97-1.67]	0.076 **+**
Positive (B or AB)	50 (19.5%)	44 (26.8%)		
Negative (A or O)	207 (80.5%)	120 (73.2%)		
Rhesus, n (%)			0.37 [0.59-1.27]	0.47
Positive	235 (91.1%)	145 (89.0%)		
Negative	23 (8.9%)	18 (11.0%)		
Bioprosthesis type				0.86
CE-3rd	101 (34.7%)	60 (32.3%)	1.0 (reference)	
CE-2nd	142 (48.8%)	96 (51.6%)	1.08 [0.84-1.39]	
CE-1st	7 (2.4%)	6 (3.2%)	1.24 [0.64-2.40]	
Other porcine bio.	41 (14.1%)	24 (12,9%)	0.99 [0.68-1.44]	
Calcification			**Multivariate Analysis**	
			OR (95%CI)	***P-value***
Age at implantation			0.81 [0.72-0.89]	0.014
Number of valves			1.75 [1.54-2.02]	0.039
Longevity			0.65 [0.45-0.85]	0.041
Blood type A (versus other)			0.67 [0.45-0.89]	0.087
Valves replaced			1.18 [0.99-1.37]	0.37
Bioprosthesis type			1.03 [0.94-1.12]	0.74

The total may be below total number of bioprotheses due to missing data.

+ multivariate analyses comparing the same risk factors but with B antigen as a risk factor and no another ABO type variable show that for B antigen OR 1.34 [1.09-1.59] (*p*=0.25).

Table S3. Risks for SVD by tears for porcine bioprostheses implanted at age ≤60 years.

n=500	**SVD without tears n=214 (42.8%)**	**SVD with tears n=286 (57.2%)**	**Univariate Analysis**	
			OR (95%CI)	***P-value***
Male sex	109 (53.2%)	159 (55.6%)	1.04 [0.90-1.21]	0.59
Age at implantation				0.49
[7-20]	10 (5.2%)	14 (5.2%)	1.0 (reference)	
]20-30]	50 (26.2%)	60 (22.3%)	0.94 [0.63-1.38]	
]30-40]	42 (22.0%)	56 (20.8%)	0.98 [0.67-1.44]	
]40-50]	41 (21.5%)	73 (27.1%)	1.10 [0.78-1.55]	
]50-60]	48 (25.1%)	66 (24.5%)	0.99 [0.68-1.44]	
Longevity				0.083
[0-7[	24 (12.6%)	49 (18.3%)	1.0 (reference)	
[7-15[	141 (73.9%)	197 (73.2%)	0.86 [0.73-1.06]	
[15-28]	26 (13.5%)	23 (8.5%)	0.70 [0.51-0.96]	
Valves replaced, n (%)				0.012
Mitral	118 (55.4%)	186 (65.0%)	1.0 (reference)	
Aortic	89 (41.8%)	99 (34.6%)	0.86 [0.73-1.01]	
Tricuspid or pulmonary	6 (2.8%)	1 (0.4%)	0.23 [0.07-0.73]	
Number of valves replaced, n (%)				0.024
1	247 (77.4%)	395 (84.8)	1.0 (reference)	
2	69 (21.6%)	66 (14.2%)	0.79 [0.67-0.94]	
3	3 (0.9%)	5 (1.1%)	1.02 [0.59-1.76]	
Blood type, n (%)				0.39
A	54 (29.3%)	89 (37.6%)	1.0 (reference)	
B	32 (17.4%)	34 (14.3%)	0.83 [0.65-1.07]	
AB	14 (7.6%)	14 (5.9%)	0.81 [0.57-1.16]	
O	83 (45.1%)	100 (42.2%)	0.88 [0.73-1.06]	
Blood A vs other	54 (29.3%)	89 (37.5%)	1.17 [0.98-1.17]	0.078
Blood B vs other	32 (17.3%)	34 (14.3%)	0.90 [0.71-1.15]	0.39
Blood AB vs other	14 (7.6%)	14 (5.9%)	0.88 [0.62-1.26]	0.49
Blood O vs other	83 (45.1%)	100 (42.3%)	0.95 [0.80-1.13]	0.55
Antigen A, n (%)			1.08 [0.93-1.10]	0.28
Positive (A or AB)	68 (37.0%)	100 (42.2%)		
Negative (B or O)	116 (63.0%)	137 (57.8%)		
Antigen B, n (%)			0.88 [0.72-1.09]	0.25
Positive (B or AB)	46 (25.0%)	48 (20.3%)		
Negative (A or O)	138 (75.0%)	189 (79.7%)		

Rhesus, n (%)			0.92 [0.70-1.21]	0.55
Positive	167 (91.3%)	213 (89.5%)		
Negative	16 (8.7%)	25 (10.5%)		
Bioprosthesis type				0.096
CE-3rd	59 (29.4%)	102 (37.0%)	1.0 (reference)	
CE-2nd	104 (51.7%)	134 (48.6%)	1.19 [0.93-1.52]	
CE-1st	9 (4.5%)	4 (1.5%)	1.89 [1.10-3.24]	
Other porcine bio.	29 (14.4%)	36 (13,0%)	1.22 [0.86-1.72]	
Tears			**Multivariate Analysis**	
			OR (95%CI)	***P-value***
Blood A vs other			1.61 [1.39-1.83]	0.026
Bioprosthesis type			0.79 [0.69-0.88]	0.014
Number of valves			0.66 [0.43-0.89]	0.082
Longevity			0.84 [0.65-1.03]	0.41
Valves replaced			0.90] [0.72-1.08	0.60

The total may be below total number of bioprostheses due to missing data.

Table S4. Risks for SVD by perforations for porcine bioprostheses implanted at age ≤60 years.

n=497	SVD without perforations n=445 (89,5%)	SVD with perforations n=52 (10.5%)	Univariate Analysis	
			OR (95%CI)	*P-value*
Male sex	237 (54.4%)	31 (56.4%)	1.07 [0.65-1.78]	0.78
Age at implantation				0.36
[7-20]	20 (4.7%)	4 (7.8%)	1.0 (reference)	
]20-30]	104 (24.5%)	10 (19.6%)	0.53 [0.18-1.55]	
]30-40]	89 (20.9%)	12 (23.5%)	0.71 [0.25-2.04]	
]40-50]	106 (24.9%)	14 (27.5%)	0.70 [0.25-1.97]	
]50-60]	106 (24.9%)	11 (21.6%)	0.56 [0.19-1.64]	
Longevity				0.71
[0-7[	67 (16.3%)	6 (12.0%)	1.0 (reference)	
[7-15[	299 (72.9%)	39 (78.0%)	1.40 [0.63-3.15]	
[15-28]	44 (10.8%)	5 (10.0%)	1.24 [0.40-3.84]	
Valve replac., n (%)				0.41
Mitral	275 (61.9%)	31 (56.4%)	1.0 (reference)	
Aortic	162 (36.5%)	24 (43.6%)	1.27 [0.73-2.10]	
Tricusp. or pulm.	7 (1.6%)	0 (0.0%)	0.00	
Number of valves replaced, n (%)				0.68
1	354 (79.7%)	45 (81.8)	1.0 (reference)	
2	84 (18.9%)	10 (18.2%)	0.94 [0.49-1.80]	
3	6 (1.4%)	0 (0.0%)	0.00	
Blood type, n (%)				0.13
A	132 (35.0%)	11 (25.0%)	1.0 (reference)	
B	55 (14.6%)	11 (25.0%)	2.18 [1.01-4.72]	
AB	27 (7.2%)	1 (2.3%)	0.47 [0.07-3.21]	
O	162 (43.0%)	21 (47.7%)	1.50 [0.75-2.99]	
Blood A vs other	132 (35.0%)	11 (25.0%)	0.65 [0.34-1.23]	0.18
Blood B vs other	55 (14.6%)	11 (25%)	1.79 [0.95-3.39]	0.072
Blood AB vs other	27 (7.2%)	1 (2.8%)	0.33 [0.05-1.94]	0.36
Blood O vs other	162 (42.9%)	21 (47.7%)	1.19 [0.68-2.08]	0.55
Antigen A, n (%)			0.56 [0.30-1.05]	0.071 +
Positive (A or AB)	156 (41.4%)	12 (27.3%)		
Negative (B or O)	221 (58.6%)	32 (72.7%)		
Antigen B, n (%)			1.79 [0.41-1.43]	0.41
Positive (B or AB)	82 (21.8%)	12 (27.3%)		
Negative (A or O)	295 (78.3%)	32 (72.7%)		
Rhesus, n (%)			1.47 [0.49-4.44]	0.67
Positive	339 (89.9%)	41 (93.2%)		
Negative	38 (10.1%)	3 (6.8%)		

Bioprosthesis type				0.21
CE-3rd	141(33.2%)	20 (38.5%)	1.0 (reference)	
CE-2nd	218 (51.3%)	20 (38.5%)	0.68 [0.38-1.21]	
CE-1rst	10 (2.4%)	3 (5.8%)	1.86 [0.61-5.65]	
Other porcine bio.	56 (13.2%)	9 (17,3%)	1.11 [0.53-2.32]	
Perforations			**Multivariate analysis**	
			OR (95%CI)	***P-value***
Blood B vs other			2.21 [1.83-2.59]	0.043
Valve replac.			0.70 [0.41-0.99]	0.23
Bioprosthesis type			0.85 [0.70-1.00]	0.30
Age at implantation			0.93 [0.62-1.24]	0.83
Number of valves			1.08 [1.01-1.16]	0.83

The total by factor may be lower than total number of bioprostheses because of missing data.

Since blood type B was the only significant value using univariate analysis, all the factors were included for multivariate analysis.

+ Additional Multivariate analysis for blood group A antigen and all other risks, except variables related to ABO blood group, demonstrates that SVD by perforation will decrease in presence of A antigen: OR 0.53 [0.17-0.89] (p=0.076)

Table S5. Multivariate analysis for involvement of AB blood group in porcine and bovine SVD by calcifications (age at implantation ≤60yrs).

Bioprostheses n=562 Porcine n=500 + Bovine n=62	**Risk of SVD isolated calcification 11% [8.4-13.6]**	
Variables	**Multivariate Analysis**	
	OR (95%CI)	***P-value***
Valves replaced	1.92 [1.66-2.14]	0.0084
Blood group AB vs others	2.74 [2.30-3.18]	0.035
Age at implantation	0.82 [0.70-0.94]	0.10
Number of valves	1.49 [1.21-1.77]	0.17
Bovine vs Porcine	1.97 [1.69-2.59]	0.28
Longevity	0.80 [0.53-1.07]	0.43
Male sex	0.83 [0.54-1.12]	0.55
Bioprosthesis type	0.92 [0.78-1.06]	0.58
Rhesus positive	1.00 [0.53-1.47]	1.00

Table S6. Multivariate analysis for involvement of AB blood group in porcine and bovine SVD by pannus or tears (age at implantation ≤60yrs).

Bioprostheses n=562 **Porcine n=500** **+ Bovine n=62**	**Risk of SVD pannus or tears** **84.4% [81.4-87.4]**	
Variables	**Multivariate Analysis**	
	OR (95%CI)	***P-value***
Number of valves	0.52 [0.27-0.77]	0.014
Blood group AB vs other	0.37 [0.07-0.67]	0.03
Valves replaced	1.54 [1.32-1.76]	0.05
Bioprosthesis type	1.18 [1.06-1.3]	0.17
Bovine vs Porcine	0.53 [0.28-0.78]	0.28
Age at implantation	1.08 [0.98-1.18]	0.47
Male sex	0.93 [0.55-1.07]	0.81
Rhesus positive	0.90 [0.49-1.31]	0.82
Longevity	1.02 [0.78-1.26]	0.91