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Thrombocytopaenia after aortic valve replacement with stented, stentless and sutureless bioprostheses

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Abstract

OBJECTIVES: Use of the Freedom SOLO (SOLO) stentless aortic bioprosthesis is associated with a unique, and as yet unexplained, observation of postoperative low platelet count. Potential causes include the valve design, tissue and chemicals used for anticalcification treatment. This study compares the platelet response associated with the SOLO to mechanical, stented, sutureless and stentless valve prostheses.

METHODS: In total, 1587 patients receiving mechanical ATS ($n = 199$), stented Perimount Magna (Perimount, $n = 911$), sutureless 3f Enable ($n = 34$) and Perceval S ($n = 48$), stentless Pericarbon Freedom ($n = 29$) or SOLO ($n = 366$) valves were analysed. The primary end-point was defined as maximum decrease in platelet count expressed as % reduction from baseline within 5 days of aortic valve replacement.

RESULTS: The smallest decrease in platelets was observed for mechanical valves (44 ± 12%), followed by stented Perimount (50 ± 11%) and sutureless 3f Enable (53 ± 12%) bioprostheses. Compared with these valves, significantly greater reductions in platelets of 61 ± 14%, 60 ± 10% and 64 ± 12% were observed for the Pericarbon Freedom, Perceval S and SOLO, respectively. Multivariable linear regression analysis identified combined procedures, female gender, body surface area, date of operation and longer extracorporeal circulation time, but neither age nor valve size, as significant independent predictors for postoperative low platelet count. After adjustment, Sorin valves caused 13% (95% confidence interval 11–14) greater decrease in platelet counts compared with non-Sorin valves, but were associated with lower need for red blood cell [OR 0.56 (CI 0.41–0.75), $P < 0.001$] and platelet [OR 0.49 (CI 0.33–0.74), $P = 0.001$] transfusions.

CONCLUSION: The Pericarbon Freedom, Perceval S and SOLO bioprostheses are associated with a significantly greater decrease in postoperative platelet count compared with non-Sorin valves. Use of these valves is, however, not associated with excess bleeding complications, suggesting a quantitative, rather than qualitative, transient side-effect on platelets. Our results argue for a cause associated with the anticalcification treatment using homocysteic acid.

Keywords: Aortic valvew • Valve surgery • Stentless • Cardiac surgery • Laboratory

INTRODUCTION

Cardiac surgical procedures with use of cardiopulmonary bypass (CPB) are commonly associated with a transient postoperative decrease in the number of platelets. This effect is explained by haemodilution, exposure to artificial surfaces with platelet activation and adhesion, blood loss, hypothermia and mechanical destruction [1]. Furthermore, implantation of aortic valve prostheses into the circulation alters haemodynamics [2] and quickly initiates plasmatic and cellular responses, which are largely governed by the adsorbed proteins that rapidly coat the material surfaces upon blood exposure [3]. Material properties such as surface roughness, charge and wettability (hydrophobic, hydrophilic) determine protein adsorption and subsequent cell interactions at the biomaterial–blood interface which include changes in platelet metabolic biochemistry, shape, as well as receptor-mediated dysfunction and lysis [3]. Any significant deviation from the expected course [i.e. thrombocytopaenia (defined as platelet count $<50 \times 10^9$/l)] raises concerns due to suspected bleeding or serious coagulation disorder [4]. Management of thrombocytopaenia is associated with increased resource utilization for diagnostic tests, change in anticoagulation regimens, platelet transfusions, frequent re-explorations and prolonged lengths of stay (LOS) [5].

Despite an attractive design and superior haemodynamic performance [6], enthusiasm for the most recent, third-generation Freedom SOLO (SOLO) stentless aortic bioprosthesis has been limited by a unique, and as yet unexplained, observation of more

severely decreased postoperative platelet counts compared with bovine [7–11] and porcine [7] stented prostheses. Unexpectedly, no SOLO-related excess bleeding event or thromboembolism has been reported to date [7, 10, 12]. It has been concluded from a propensity-score-matched study that the mechanism is not patient-related [8], raising the question of impaired SOLO prosthesis biocompatibility. Furthermore, recovery of platelet counts following the nadir argues for a short, non-persistent, valve-dependent effect. We hypothesized that lower counts with SOLO result from platelet lysis caused by temporary, chemistry-induced effects, such as homocysteic acid (HCA), which is used in its unique anticalcification treatment. Although the platelet-lowering effect of SOLO is well documented, other bioprostheses of the same manufacturer, which are produced with identical tissue and chemical treatments, but different in respect to design and implantation technique, have not yet been investigated. We reasoned that the most likely cause for the decrease in platelets could be identified through comparison of prostheses that partially share specific characteristics. Therefore, this study aimed to investigate the platelet response to aortic valve replacement (AVR) with the mechanical ATS bileaflet valve, the sutureless 3f Enable and Perceval S prostheses, the stented Perimount Magna (Perimount), as well as the stentless Pericarbon Freedom and SOLO bioprostheses.

PATIENTS AND METHODS

Patient population and data management

Data of all adult cardiac surgical patients who underwent AVR surgery with CPB between June 2001 and December 2012 in our institution were retrospectively reviewed. Exclusion criteria from analyses were reoperation, active endocarditis, preoperative inflammation, acute myocardial infarction, dialysis, deep hypothermic circulatory arrest (DHCA), positive heparin-induced thrombocytopaenia type 2 (HIT-II) tests, incomplete platelet laboratory data (minimum 5 days) and AVR with use of prostheses other than those selected for analysis. Thus, a total of 1587 patients [mean age 75.4 ± 7.7 years, 594 (37.4%) female] receiving either the mechanical ATS ($n = 199$), the stented Perimount ($n = 911$), the sutureless 3f Enable ($n = 34$), the sutureless Perceval S ($n = 48$), the stentless Pericarbon Freedom ($n = 29$) or the stentless SOLO ($n = 366$) valve met the inclusion criteria and were analysed in this study.

The local ethics committee approved the protocol and patient consent was waived for the retrospective analysis. Patient and perioperative data were retrieved from our institutional database (Dendrite Clinical Systems LTD, Henley-on-Thames, UK). Platelet counts and laboratory results were retrieved from the Opus:L central laboratory database (OSM, Essen, Germany) and were confirmed with clinical data protocols. The primary end-point was defined as maximum decrease in platelet count expressed as % reduction from baseline within 5 days of AVR.

Valve prostheses characteristics and implantation techniques

All AVR procedures were performed under routine general anaesthesia and with median sternotomy, using standard CPB and mild hypothermia (34 °C). After sternotomy and heparin administration (500 IU/kg/body weight, targeted activated clotting time ≥ 400 s), CPB was established and cardioplegic arrest initiated using St Thomas solution in antegrade fashion for myocardial protection.

The mechanical ATS open pivot bileaflet valve (Medtronic Inc., Minneapolis, MN, USA) is fully composed of pyrolytic carbon material, the cuff being designed for use with even size numbers. The Perimount (models 3000TFX and 3300TFX; Edwards Lifesciences, Irvine, CA, USA) stented bovine pericardial bioprosthesis is mounted on an Elgiloy frame. The cusps are fixed with glutaraldehyde (GA; 0.625%) and are treated with surfactant combined with thermal treatment (ThermaFix^{TM}) to retard calcification. Both valves are implanted in supra-annular position using 2-0 mattress pledged sutures.

The stentless Pericarbon Freedom and SOLO valves, as well as the sutureless Perceval S prosthesis (all: Sorin Biomedica, Saluggia, Italy) all share the bovine pericardial tissue and fixation treatment with GA (GA up to 0.5%) with the Perimount valve. Furthermore, all three Sorin valves are identical in using a unique anticalcification treatment with HCA to neutralize aldehyde residues [13, 14]. However, despite identical tissue and treatment, the three valves differ in design and implantation. The Pericarbon Freedom valve is provided with extra tissue, allowing intraoperative tailoring for adaptation to the patient's specific anatomy and the surgeon's technique. The SOLO emerged in May 2004 as a modified version of the Pericarbon Freedom in Europe, and has just recently (June 2014) received FDA approval for use in the USA. The design modification was aimed at allowing sub-coronary, supra-annular implantation with only one suture line, and was achieved by the removal of all extra tissue from the Pericarbon Freedom valve inflow side, and scalloping the outflow side. In short, implantation requires three equidistant, intercommissural sutures placed at the nadir of each sinus that are used for attaching the prosthesis' external strip to the aorta with running sutures that end in extra-aortic fixation at the top of the commissures.

Sutureless valves are not surgically sutured into the implant site, instead, the compressed stent-mounted valve is positioned in a valve delivery system and then deployed. The sutureless Perceval S prosthesis is mounted within a super-elastic expandable alloy nitinol (nickel and titanium) frame. An inserted balloon models the Perceval S with dilatation under pressure (4 atm for 30 s), followed by flushing with warm saline at 37 °C to optimize final sealing. The 3f Enable (Medtronic Inc.) is assembled from three equal sections of equine pericardial tissue, pretreated with GA. The leaflets form a tubular structure with commissural tabs that are reinforced with polyester material. The device is folded and positioned like the Perceval S. Its shape and size return to the preset dimensions upon deployment of the self-expanding nitinol frame and the outward radial forces keep the valve fixed in place.

Postoperative anticoagulation

If no bleeding occurred on the day of surgery, patients received intravenous unfractionated heparin (partial thromboplastin time: 60 s). Heparin was replaced with subcutaneous weight-adapted enoxaparin on the second or third postoperative day. Usually, after the fourth postoperative day and following mobilization of the patient, antiplatelet therapy with 100 mg acetylsalicylate daily

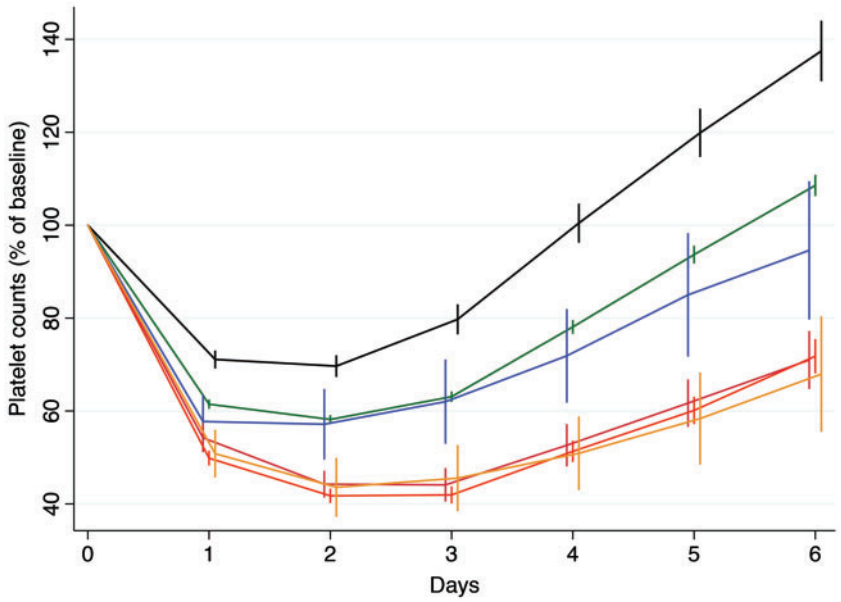

Figure 1: Changes in platelet counts normalized with respect to the baseline counts, as means and confidence intervals. Data show the mean values on each day for groups, whereas the end-point was defined as maximum decrease in platelet count expressed as % reduction from baseline within 5 days of AVR for individual patients. AVR: aortic valve replacement.

was instituted. Only mechanical valves were anticoagulated with warfarin as standard protocol.

Laboratory assays

Postoperative daily platelet counts are mandatory in our patient management protocol. Collection tubes containing K_3-EDTA (1.6 mg EDTA/ml blood; S-Monovettes™, Sarstedt, Germany) as anticoagulant were used, and filled according to the markings.[AQ5] All samples were stored at room temperature and taken to the laboratory for analysis within 30 min. Platelets were counted with the automated routine haematological analyser Sysmex XE 2100 (Sysmex, Norderstedt, Germany). Further routine laboratory tests were performed when appropriate and according to standard protocol. Blood analyses were pursued with 3.1% Na_3-citrate collection (S-Monovettes™) and blood smears if EDTA-pseudothrombocytopaenia was suspected.

Statistical analyses

Continuous variables were summarized using mean and standard deviation, categorical variables as number and %. Preoperative platelet count values were converted to 100% (baseline) and all postoperative changes were calculated as changes relative to baseline. Patients without available platelet count within 2 days before surgery were excluded from the analysis. We linearly interpolated measurements in cases of missing values, at most three sequential values, and eliminated when more than three sequential values were missing or when extrapolation would have been needed to construct the values of the first five postoperative days. The end-point (maximum decrease in platelet count) was normally distributed and required no transformation for linear regression modelling. All variables potentially influencing on platelet decreases were included in the multivariate model to adjust for confounding effects: age, gender, diabetes, body surface area (BSA), preoperative renal insufficiency, hypertension, aortic insufficiency, aortic stenosis, perfusion time, isolated AVR, date of operation as a binary variable (before or after median inclusion time) and type of valve. We calculated a linear regression with the primary end-point (maximal decrease in platelet count) as the dependent variable. As a sensitivity analysis, we repeated this linear regression including only patients who did not receive platelet transfusions. As a second sensitivity analysis, we excluded all patients who received a mechanical valve. We also calculated logistic regressions with the same independent variables as well as the need for red blood cell (RBC) or platelet transfusions as dependent variables. All *P*-values and confidence intervals are two-sided. The analyses were performed using Stata 12 (College Station, TX, USA).

RESULTS

Platelets

Baseline characteristics varied slightly between groups as expected, due to valve-specific patient selection (Table 1). Postoperative decreases in platelet counts relative to baseline were observed in all patients, with lowest platelet counts on the first postoperative day (Fig. 1). The smallest decrease (defined end-point) was seen with the mechanical valve (44 ± 12%), followed by the stented Perimount (50 ± 11%), and the sutureless 3f Enable (53 ± 12%) bioprostheses. Importantly, all three Sorin valves showed significantly greater decreases in platelet counts, namely 61 ± 14% for the Pericarbon Freedom, 60 ± 10% for the Perceval S and 64 ± 12% for the SOLO, compared with non-Sorin valves (all: $P < 0.001$). Daily mean values for the various valves are shown in Fig. 1. After reaching the nadir, platelet count increased for all types of valves over the course of 9 days, suggesting parallel recoveries. At discharge (Day 9), platelets had increased to 164%, 141%, 100%, 93%, 116% and 100% of baseline values for

Table 1: Baseline characteristics, *P*-values relate to a comparison of the biological valves

	Perimount Magna	3f Enable	ATS	Perceval S	Freedom SOLO	Pericarbon Freedom	*P*-value
n	911	34	199	48	366	29	
Age, years	72.1 ± 8.9	77.8 ± 4.3	51.1 ± 10.0	76.2 ± 5.2	74.0 ± 7.4	69.6 ± 8.8	<0.001
Female gender, *n* (%)	308 (33.8%)	14 (41.2%)	45 (22.6%)	30 (62.5%)	182 (49.7%)	15 (51.7%)	<0.001
BSA, m^2	1.9 ± 0.2	1.8 ± 0.2	2.0 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	0.013
Ejection fraction, %	57.0 ± 12.5	56.7 ± 14.8	58.2 ± 11.0	63.8 ± 9.8	56.6 ± 10.9	63.1 ± 16.1	<0.001
Additive EuroScore	6.9 ± 2.6	7.5 ± 2.1	4.5 ± 1.9	6.8 ± 1.4	7.2 ± 2.5	6.1 ± 2.5	0.367
Diabetes	190 (20.9%)	9 (26.5%)	17 (8.5%)	7 (14.6%)	110 (30.1%)	6 (20.7%)	0.005
Hypertension	668 (73.3%)	24 (70.6%)	95 (47.7%)	33 (68.8%)	300 (82.0%)	18 (62.1%)	0.005
COPD	129 (14.2%)	4 (11.8%)	18 (9.0%)	6 (12.5%)	64 (17.5%)	6 (20.7%)	0.207
Preop. sinus rhythm	771 (84.6%)	24 (70.6%)	175 (87.9%)	38 (79.2%)	268 (73.2%)	23 (79.3%)	<0.001
Preop. renal impairment	82 (9.0%)	6 (17.6%)	4 (2.0%)	3 (6.3%)	57 (15.6%)	4 (13.8%)	0.005
Preop. platelet count ($\times 10^9$/l)	222.1 ± 68.1	220.6 ± 63.5	234.1 ± 81.9	245.8 ± 76.6	239.8 ± 78.5	241.1 ± 54.5	<0.001
Valve size							<0.001
19 (18)[a]	27 (3.0%)	1 (2.9%)	5 (2.5%)	0 (0.0%)	6 (1.6%)	1 (3.4%)	
21 (20)[a]	202 (22.2%)	8 (23.5%)	23 (11.6%)	12 (25.0%)	94 (25.7%)	2 (6.9%)	
23 (22)[a]	313 (34.4%)	14 (41.2%)	51 (25.6%)	23 (47.9%)	121 (33.1%)	6 (20.7%)	
25 (24)[a]	239 (26.2%)	6 (17.6%)	61 (30.7%)	13 (27.1%)	108 (29.5%)	10 (34.5%)	
27 (26)[a]	89 (9.8%)	5 (14.7%)	37 (18.6%)	0 (0.0%)	37 (10.1%)	4 (13.8%)	
29 (28)[a]	15 (1.6%)	0 (0.0%)	10 (5.0%)	0 (0.0%)	0 (0.0%)	6 (20.7%)	
31 (30)[a]	1 (0.1%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
ECC time, min	91.4 ± 35.6	65.4 ± 24.2	91.8 ± 33.3	50.4 ± 13.7	101.6 ± 36.3	137.5 ± 32.3	<0.001
Cross-clamp time, min	66.5 ± 28.8	44.1 ± 20.0	68.2 ± 24.1	33.7 ± 9.8	72.8 ± 25.5	107.3 ± 25.3	<0.001
Aortic stenosis	624 (68.5%)	30 (88.2%)	83 (41.7%)	41 (85.4%)	309 (84.4%)	23 (79.3%)	<0.001
Aortic regurgitation	107 (11.7%)	0 (0.0%)	66 (33.2%)	0 (0.0%)	11 (3.0%)	3 (10.3%)	<0.001
Combined pathology	140 (15.4%)	4 (11.8%)	36 (18.1%)	6 (12.5%)	35 (9.6%)	3 (10.3%)	0.081
Isolated AVR procedure	327 (35.9%)	24 (70.6%)	66 (33.2%)	33 (68.8%)	208 (56.8%)	11 (37.9%)	<0.001
Concomitant procedure, CABG	416 (45.7%)	8 (23.5%)	40 (20.1%)	15 (31.3%)	129 (35.2%)	16 (55.2%)	<0.001
Concomitant procedure, MVR	64 (7.0%)	0 (0.0%)	20 (10.1%)	0 (0.0%)	15 (4.1%)	2 (6.9%)	<0.001
Concomitant procedure, other[b]	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	15 (4.1%)	0 (0.0%)	<0.001
In-hospital mortality, *n* (%)	46 (2.7%)	1 (2.1%)	10 (2.0%)	2 (4.3%)	17 (4.0%)	2 (5.5%)	0.453
Minimal platelet count[c] ($\times 10^9$/l)	109.6 ± 37.3	100.4 ± 32.0	128.3 ± 46.9	97.6 ± 37.2	82.7 ± 32.1	90.8 ± 35.2	<0.001
RBC transfusion, *n* (%)	498 (54.7%)	21 (61.8%)	63 (31.7%)	28 (58.3%)	193 (52.7%)	16 (55.2%)	0.830
Platelet transfusion, *n* (%)	133 (14.6%)	3 (8.8%)	8 (4.0%)	2 (4.2%)	31 (8.5%)	2 (6.9%)	0.009
LOS in ICU/IMC, days	2 [1; 3]	2 [1; 5]	1 [1; 2]	1 [1; 2]	2 [2; 4]	2 [1; 2]	0.106
Total LOS, days	10 [8; 13]	11 [8; 14]	9 [8; 11]	10 [9; 13]	10 [9; 13]	9 [8; 11]	0.939

AVR: aortic valve replacement; BSA: body surface area; CABG: coronary artery bypass grafting; COPD: chronic obstructive pulmonary disease; ECC: extracorporeal circulation; ICU: intensive care unit; IMC: intermediate care; LOS: length of stay; MVR: mitral valve repair (replacement).

[a]Persistent foramen ovale (PFO), ablation, tricuspid annuloplasty.

[b]End-point defined as maximum decrease in platelet count (% reduction from baseline within 5 days of AVR).

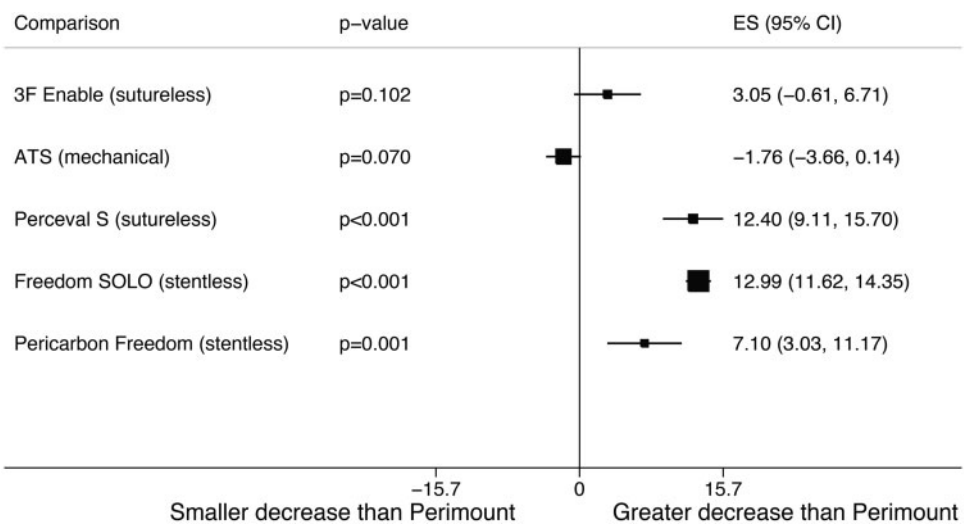

Figure 2: Differences in platelet count adjusted for the confounding factors mentioned in the Patients and Methods section, compared with Perimount Magna. Forrest plot.

the ATS, Perimount, 3f Enable, Perceval S, Pericarbon Freedom and SOLO valves, respectively.

Multivariable linear regression analysis was used to identify combined procedures [coefficient 2.03 (0.82–3.24), $P = 0.001$], female gender [coefficient 2.01 (0.85–3.36), $P = 0.001$], age [coefficient 0.08 (0.02–0.14), $P = 0.010$], BSA [coefficient 13.10 (10.16–16.05) $P < 0.001$], date of surgery before median [coefficient 4.03 (2.98–5.08), $P < 0.001$] and longer extracorporeal circulation (ECC) time [coefficient 2.87 (1.98–3.76) per hour, $P < 0.001$], but not diabetes ($P = 0.43$), renal dysfunction ($P = 0.13$), hypertension ($P = 0.68$), preoperative rhythm ($P = 0.09$), nor valve size ($P = 0.37$), as significant independent predictors for postoperative low platelet count. Furthermore, preoperative valve pathology had no significant effect on postoperative platelet response; i.e. aortic stenosis ($P = 0.26$), regurgitation ($P = 0.12$), or combined pathology ($P = 0.62$). Valve-specific differences in postoperative platelet counts remained unchanged after adjustment for all aforementioned variables, strongly suggesting that patient characteristics do not explain the effects on platelets observed with the valve prostheses. Using Perimount as a reference, maximal decreases in postoperative platelet count were significantly greater for all three Sorin bioprostheses (all: $P < 0.001$), but were not different from the 3f Enable sutureless valve ($P = 0.10$) (Fig. 2). Furthermore, there were no differences among the three Sorin valves for platelet decreasing effects ($P = 0.07$–0.94) after adjustment, thus allowing grouping for combined analyses. Platelet counts were significant lower in patients receiving any Sorin valve compared with a non-Sorin bioprostheses ($P < 0.001$). In fact, use of any Sorin product caused on average 13% (11–14%) greater decrease of platelet counts, compared with non-Sorin valves. All independent variables in this multivariable linear regression model explain 35% of the variability in platelet drop relative to baseline.

Sensitivity analyses

Restricting the analysis to patients without any platelet transfusion ($n = 1207$), the same patterns were observed. Using Perimount as reference, there is a significantly greater decrease in platelet count for all three Sorin valves, with estimates of 11% (CI 7–14), 12% (CI 11–13) and 7% (CI 3–11) for Perceval, SOLO and Pericarbon Freedom, respectively. There is no difference between Perimount and Enable, and a significantly greater increase of platelets for the ATS group (Fig. 2). The overall effect of Sorin valves for significantly greater reductions in platelets was found to be 12% (CI 10–13) compared with non-Sorin valves.

Excluding ATS and thus restricting the analysis to include only biological valves, we found estimates of 12% (CI 9–16), 13% (CI 12–14) and 7% (CI 3–11) for Perceval, SOLO and Pericarbon Freedom, respectively. There is no difference between Perimount and Enable. The overall effect of Sorin valves for significantly greater reductions in platelets was found to be 12% (CI 10–14) compared with non-Sorin valves.

Complications

The overall in-hospital mortality in the total cohort (database, before applying the exclusion criteria) was not different among valves ($P = 0.453$). Postoperative need for transfusion ranged from 31.7% to 61.8% of patients for RBC, and from 4.0% to 14.6% of patients for platelets (Table 1). Logistic regression analysis identified age [OR 1.02 (1.01–1.04) per year, $P < 0.001$], female gender [OR 1.59 (1.21–2.08), $P = 0.001$], BSA [OR 0.17 (0.09–0.32) per m^2, $P < 0.001$] and ECC time [OR 1.74 (1.41–2.15) per hour, $P < 0.001$] as significant predictors for RBC transfusion. The corresponding predictors for platelet transfusion were BSA [OR 0.35 (0.15–0.80) per m^2, $P = 0.014$], ECC time [OR 2.74 (2.17–3.47), $P < 0.001$] per hour and preoperative platelet count [OR 0.995 (0.993–0.997), $P < 0.001$], but not age ($P = 0.48$), gender ($P = 0.89$) nor isolated procedure ($P = 0.17$). Importantly, after adjustment all three Sorin valves were associated with lower requirements for RBC [OR 0.56 (0.41–0.75), $P < 0.001$] and platelet [OR 0.49 (0.33–0.74), $P = 0.001$] transfusions, strongly suggesting that the use of Sorin valves (Pericarbon Freedom, SOLO or Perceval S) is not associated with excess bleeding as measured by need for blood product transfusions. Furthermore, and in support of these findings, hospital stay was not different among valve groups for total LOS ($P = 0.939$), nor for LOS in the intensive care unit or intermediate care ($P = 0.106$).

COMMENT

This study identified each of the Sorin valves, the stentless Pericarbon Freedom, SOLO and the sutureless Perceval S, as independent predictors for significantly greater decreases in postoperative platelet counts, being associated with a reduction of 13% below that of non-Sorin bioprostheses. Consequently, our findings support the concept that the anticalcification treatment with HCA is the underlying cause of the more severe postoperative thrombocytopaenia associated with Sorin valves. The severe SOLO-associated decrease in postoperative platelets was incidentally discovered in 2008 [9, 11], and has since been confirmed in comparisons with stented Perimount [8, 10, 11, 15], Mitroflow [9] and Mosaic [7] valves. Unsupported speculations regarding the causative mechanisms of SOLO-induced platelet reductions persist, including acute or chronic toxic effects of the SOLO valve [8, 9] or its storage solution [8, 9, 16], as well as direct haemodynamic effects of the prosthesis on platelets [9, 11, 12]. Our study identified female gender, higher age, lower BSA, combined procedures and longer ECC time as independent predictors for more severe postoperative decrease in platelet count. However, in contrast to a previous report of a significant association between larger valve size and decreased odds of postoperative thrombocytopaenia [12], valve size was not predictive of greater decreases in platelet count in our study. Conflicting results from other studies [8, 12, 15] can be explained with varying inclusion and exclusion criteria, case numbers, and definitions. We calculated relative changes from baseline in order to account for individual variations in preoperative status. In contrast, other authors deliberately defined absolute cut-off values (i.e. 30 000 or 50 000) [8, 12, 16] for postoperative thrombocytopaenia, which, however, strongly depends on baseline values and thus only partially reflects causal effects. Most importantly, after adjustment for confounders and using the Perimount as a reference, maximal platelet decreases were significantly greater for Pericarbon Freedom, SOLO and Perceval S bioprostheses compared with all other valves. CPB and postoperative treatment protocols were identical for all investigated bioprostheses, and bias was partly eliminated through adjustment for all aforementioned patient- and procedure-related variables. Furthermore, sensitivity analyses excluding patients with platelet transfusions and mechanical valves resulted in nearly identical results, strongly supporting the evidence that the observed effects are valve-dependent. Our findings add to current knowledge in demonstrating an identical, platelet-lowering effect for three

bioprostheses of varying design from the same manufacturer with identical tissue and anticalcification treatment. Apparently, use of any Sorin valve caused 13% (10–14%) greater platelet reduction (relative to baseline) compared with non-Sorin valves as the single, strongest, independent predictor for thrombocytopaenia.

Blood damage [Reynold (turbulent) shear stress (σ_R)] is lowest with pericardial valves (due to their large EOA and low gradients) [17], and completely absent in the stentless Toronto valve (St Jude Medical), suggesting generally favourable, low-stress profiles [18]. Contrary to our observations, platelet-damaging effects are expected to persist if haemodynamic stress is causal. Thus, causal haemodynamic stress appears highly unlikely in this setting given the large EOA in a fully, unobstructed opening of a correctly sized stentless valve, low gradients [6], and performance that is close to a normal, native aortic valve under resting and stress conditions [19].

Platelet activation is the initial step of the aggregation process that results in diffuse platelet consumption and decreased platelet count [1]. The quantitative change in platelets in patients receiving SOLO was complemented with qualitative analyses of platelet function and their capacity for haemostasis before and after AVR with SOLO. Despite the finding of thrombocytopaenia in 70% of patients, no change in postoperative platelet function, nor impairment in thrombi generation with fibrinogen, was detectable [20]. In agreement with our data, it is important to emphasize that despite the common finding of postoperative thrombocytopaenia with SOLO, increases in bleeding, thromboembolic events and transfusion requirement, have not been reported after many thousands of implants [6–12, 15, 16].

In summary, there is no evidence of either a patient-specific or a design-dependent cause for the unique effect of more severe postoperative platelet lowering observed with the Pericarbon Freedom, SOLO and Perceval S bioprostheses alike. Rather, it can be logically argued that platelet-lowering effects result from a specific feature shared exclusively by the three Sorin bioprostheses, such as the unique anticalcification treatment with HCA. Furthermore, given that the nadir of thrombocytopaenia is observed on the first postoperative day after AVR followed by full recovery, platelet lowering is transient and persistent toxicity is unlikely.

In absence of any evidence for excess activation, platelet-consuming aggregation, haemolysis or flow-dependent mechanical damage, we hypothesize that temporary chemistry-induced platelet lysis leads to lower counts with Sorin valves. These bioprostheses undergo antimineralization treatment with HCA, a reactive biochemical compound and intracellular oxidation product of the sulphur-containing amino acid homocysteine, which is usually not measurable in peripheral blood. Using high-performance liquid chromatography and Ultratrax tissue homogenizer (Omni TH tissue homogenizer, OC/TH 220), our group determined that HCA concentrations in the SOLO valve and its storage solution (unpublished data) are comparable to those present in blood from untreated patients with homocystinuria, an inborn metabolic error associated with severe hyperhomocysteinaemia and premature cardiovascular complications including thromboembolic events [21]. Recently, short-term exposure to HCA has been shown, via activation of *N*-methyl-D-aspartic acid (NMDA)-type glutamate receptors, to increase intracellular levels of ionized calcium and reactive oxygen species, which lead to expression of pro-apoptotic genes, progressive cellular degeneration and cell lysis within hours [22, 23]. NMDA membrane receptors are expressed in megakaryocytes and platelets; cell membrane receptor density depends on cellular functional status and increases massively under conditions of activation [23, 24]. In fact, only 30-min incubation with HCA was necessary to activate lymphocytes [23]. The HCA-dependent hyperactivation of NMDA receptors with subsequent lysis may be limited to a sub-group of platelets that are particularly susceptible to damage and stimulation after CPB. Furthermore, the non-persistent damaging effect would be limited to the short time of the fully exposed prosthesis surface following implantation until the onset of protein denaturation and fibrinogen adsorption. Recovery of platelet counts following the nadir further argues for a short, non-persistent, valve-dependent effect. Further investigation is required to fully characterize the precise molecular mechanism(s), underlying Sorin-associated platelet reductions.

Limitations

This study shares the limitations associated with a retrospective design and single-centre experience, which limits the generalizability of the results; particularly, some patients had to be excluded because of missing baseline platelet counts thus results concern primarily subjects with adherence to protocol. Because the defined end-point excluded early re-exploration, and the database, as well as protocols, did not always provide exact information on reasons for reintervention, we abstained from presenting data on the frequency of re-exploration for bleeding. However, our data do not suggest any unexpected variation of re-exploration rates for bleeding between valves in multivariate analysis, particularly between Sorin- and non-Sorin bioprostheses. Alternatively, we presented data for the administration rate (%) of platelet and RBC transfusions, representing overall procedure-associated bleeding prevalence albeit not proving a causal relationship with use of a specific valve. The available records did not provide the exact data to separate intraoperative and postoperative platelet transfusions with potential temporal influence on the nadir. The sensitivity analysis after exclusion of all patients with platelet transfusion at any time point, however, gave nearly identical results strongly supporting the evidence for valve-dependent effects. Due to the long enrolment period for this large study management of blood products, in part, being based on individual experience, may have changed over time. We observed a marginal time effect that was accounted for by including a time variable into the multivariable model albeit not affecting the overall results. Because all patients undergoing surgery with use of CPB are exposed to heparin, HIT-II is a logical suspicion in the postoperative setting when platelet counts are unexpectedly low. HIT typically manifests with an initial postoperative rise in platelet count and a subsequent fall 5–10 days after heparin exposure, and is caused by heparin platelet factor 4 antibodies, which lead to platelet activation, hypercoagulability and unexpected thrombotic events [25]. Although the changes in platelet counts are fundamentally different after SOLO implantation with early-onset and persisting thrombocytopaenia in the absence of thromboembolic events, we cannot exclude that some patients with low platelet counts due to HIT-II were missed. However, given the very low incidence of HIT-II $\approx$ 0.3% in patients undergoing cardiac surgery [25], even routine testing would not have changed the overall outcome of this study.

CONCLUSION

In conclusion, all Sorin valves (Pericarbon Freedom, SOLO and Perceval S; individually and grouped) were associated with a significantly greater decrease of 12.9% in postoperative platelet count compared with non-Sorin prostheses. Because the only common characteristic of all three Sorin valves is their unique anticalcification treatment, our findings argue, in consequence, for a mechanism caused by the chemical treatment using HCA.

Conflict of interest: none declared.

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