Effect of Transcatheter Aortic Valve Implantation on the Immune Response Associated With Surgical Aortic Valve Replacement

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The immune response after transcatheter aortic valve implantation (TAVI) in comparison to that after surgical aortic valve replacement (SAVR) remains to be fully elucidated. In a 2-part study, we assessed laboratory data obtained before, immediately after, and 24 and 48 hours after SAVR (128 patients; age ≥80 [mean 82] years) or transfemoral TAVI (102 patients; age ≥80 [mean 86] years) performed for aortic stenosis. In-hospital mortalities were similar (3% vs 0%), but leukocyte counts and aspartate aminotransferase and creatine kinas concentrations were decreased immediately and 24 hours after surgery (all, p <0.001). We performed cytokine profiling in a SAVR group (11 patients; mean age, 77 years) and transfemoral TAVI group (12 patients; mean age, 84 years). By measuring normalized concentrations of 71 cytokines at 3 time points, we found a significant difference (defined as fold change >1.7 and p <0.05 [by Mann-Whitney U-test]) in 23 cytokines. The differentially expressed cytokines fell into 3 hierarchical clusters: cluster A (high increase after SAVR and suppressed increase after TAVI only immediately after surgery [CCL2, CCL4, and 2 others]), cluster B (high increase after SAVR and suppressed increase after TAVI at 2 time points [IL-1Ra, IL-6, IL-8, IL-10, and 5 others]), and cluster C (various patterns [TRAIL, CCL11, and 8 others]). Gene enrichment analysis identified multiple pathways associated with the inflammatory responses in SAVR and altered responses in TAVI, including cellular responses to tumor necrosis factor (p = 0.0035) and interleukin-1 (p = 0.0062). In conclusion, a robust inflammatory response follows SAVR, and a comparatively attenuated response follows TAVI. **(Am J Cardiol 2020;128:35−44)**

Transcatheter aortic valve implantation (TAVI) is an established minimally invasive treatment for symptomatic aortic stenosis (AS) in patients deemed to be at high operative risk. Although TAVI may induce a systemic inflammatory response,[1,2] various biological responses typical of surgical aortic valve replacement (SAVR) are attenuated under TAVI.[2−6] SAVR provokes multiple adverse biological reactions related to the use of cardiopulmonary bypass (CPB),[7,8] surgical trauma,[9] and other factors. CPB-related inflammation is characterized by activation of leukocytes and platelets, acute-phase protein production, thrombin- and plasmin-mediated procoagulant activity.[7,8,10] Various pro- and anti-inflammatory cytokines, including IL-1,[11] IL-6,[12] IL-8,[12] TNF-α,[12] and IL-10,[12] are reported to contribute to the postoperative inflammatory response after cardiac surgery performed under CPB. In contrast, TAVI is reported to result in IL-6, IL-8, and IL-10 concentrations that are lower than those resulting from SAVR.[3−5] For want of gene profiling and cytokine profiling studies, the detailed molecular response after TAVI has not been elucidated. We conducted a 2-part study (1) to confirm that TAVI attenuates the inflammatory response typical of SAVR, and (2) to investigate, by means of cytokine profiling, the CPB-induced inflammatory response that follows SAVR.

Methods

The Institutional Review Board of Saitama Medical Center, Jichi Medical University approved the study (approval no. S18-165). For comparison of patients' perioperative

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Data are shown as mean ± standard deviation or median (twenty-fifth, seventy-fifth percentile) values or number (%) of patients. ICU = intensive care unit; NA = not applicable; SAVR = surgical aortic valve replacement; STS = Society of Thoracic Surgery; TF-TAVI = transfemoral transcatheter aortic valve implantation.

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laboratory values, the need for informed consent was waived. For cytokine profiling, patients provided written informed consent for blood sample collection and use of their anonymized clinical information, which was obtained from hospital records.

A flow diagram of the 2-part study is given in Figure 1. TAVI was instituted at our hospital in 2014. Since then, decisions regarding which type of procedure is most appropriate have been made according to established guidelines.[13] The Part 1 study included 230 patients aged ≥80 years who had undergone elective surgery for AS between January 2009 and December 2018. The first 73 patients were treated between 2009 and 2013, when only SAVR was performed. None of the 230 patients had a hematologic disorder, autoimmune disease, or chronic kidney disease requiring hemodialysis, and none was provided perioperative extracorporeal membrane oxygenation. We divided the total 230 patients into 2 groups: those who had undergone SAVR (n = 128) and those who had undergone transfemoral TAVI (TF-TAVI; n = 102). Patients' clinical characteristics, operative variables, and in-hospital outcomes were compared between the 2 groups. Laboratory values obtained before surgery, immediately after surgery, 24 hours after surgery, and 48 hours after surgery were also compared between the 2 groups. Further, we matched patients on the basis of propensity scores to create a SAVR group and a TF-TARI group with comparable clinical characteristics. The propensity score matching yielded 52 patient pairs and we compared laboratory data and outcomes between the newly created groups. The Part 2 study included 23 patients identified among 24 patients aged ≥70 years who had undergone elective surgery for AS between April 2018 and January 2019. The operative procedure performed in each case was decided upon as described above. None of the 24 patients had a known hematologic disorder or autoimmune disease, and none was on dialysis for chronic kidney disease or required extracorporeal membrane oxygenation. Twelve of the 24 patients had undergone SAVR (n = 12), and 12 had undergone TF-TAVI (n = 12). One of the patients treated by SAVR had undergone perioperative transfusion and was thus excluded from the analysis. We divided the remaining 23 patients between those who had undergone SAVR (n = 11) and those who had undergone TAVI (n = 12). Patients' clinical characteristics, operative variables, and in-hospital outcomes were compared between the 2 groups. Laboratory values obtained before surgery, immediately after surgery, and 24 hours after surgery were also compared between the 2 groups, as were the concentrations of 71 cytokines/chemokines measured before surgery, immediately after surgery, and 24 hours after surgery. Cytokine profiling and global gene expression analysis were performed after normalization of concentrations relative to preoperative measured values.

SAVR was performed via standard median sternotomy under general anesthesia. CPB was established after ascending aorta and bicaval venous cannulation. A left ventricular vent tube was inserted via the right upper pulmonary vein. Moderate hypothermia was induced, and combined antegrade and retrograde cold blood cardioplegia was performed intermittently to maintain cardiac arrest. TF-TAVI was performed under general anesthesia. Details of the operative procedure are given in Supplementary Methods.

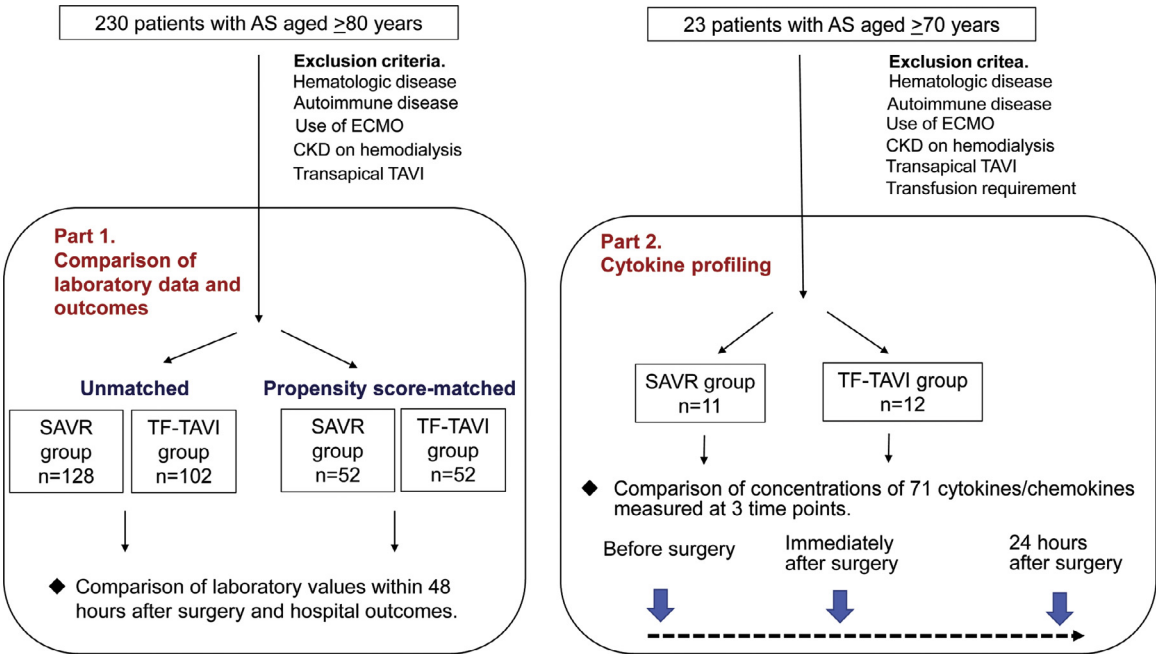

Figure 1. Flow diagram of the 2-part retrospective study. Part 1: Comparison of laboratory values and in-hospital outcomes between patients who underwent SAVR and those who underwent TF-TAVI. Patients included were aged ≥80 years and had undergone SAVR (n = 128) or TF-TAVI (n = 102) for AS. Laboratory values within 48 hours after surgery and in-hospital outcomes were compared between unmatched and propensity score-matched groups. Part 2: Patients included were aged ≥70 years and had undergone SAVR (n = 11) or TF-TAVI (n = 12) for AS. Concentrations of 71 cytokines/chemokines measured at 3 different time points were compared between these 2 patient groups. After normalization relative to the preoperative value, cytokine/chemokine expression was compared between the 2 groups, followed by bioinformatics analysis. AS = aortic stenosis; CKD = chronic kidney disease; ECMO = extracorporeal membrane oxygenation; SAVR = surgical aortic valve replacement; TF-TAVI = transfemoral transcatheter aortic valve implantation.

Peripheral arterial blood samples had been collected into ethylenediaminetetraacetic acid-containing tubes. The ethylenediaminetetraacetic acid-treated blood was centrifuged for 15 minutes at 1,500× grams. Serum was aspirated and stored in aliquots at −80°C until analysis to prevent thawing of the frozen parent samples. Serum concentrations of 71 different inflammatory proteins were measured with the use of Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panels I, II, and III (catalogue numbers HCYTMAG-60K-PX38, HCP2MAG-62K-PX23, and HCYP3MAG-63K-11, Merck Millipore, Billerica, MA). Samples were run on a Luminex 200 (Merck Millipore) according to the manufacturer's instructions and analyzed by STar Station software (Version 3.0, Applied Cytometry Systems, Sheffield, UK). Detailed information regarding measurement of the cytokines and chemokines are given in the Supplementary Methods.

Data analysis after cytokine measurement, including normalization to the preoperative measured value, was performed with the use of Subio Platform (https://www.subioplatform.com). Briefly, the raw data table contained many empty cells, meaning not detected. To avoid errors in calculation, we replaced values <0.5 with 0.5 and used 0.25 for values not detected. The data were converted to log2 ratios, that is, against the preoperative values, for each patient. We compared, between the SAVR group and the TF-TAVI group, the measured cytokine concentrations at 2 times points: immediately after surgery and 24 hours after surgery. The criteria for extracting differentially expressed cytokines were fold changes greater than 1.7 and Mann-Whitney U-test-derived p values <0.05. Hierarchical clustering was performed on the basis of the gene expression data. Differentially expressed genes were subjected to

Table 1
Clinical characteristics, operative variables, and in-hospital outcomes of the total Part 1 study patients, per group (SAVR vs TF-TAVI)

Variable	SAVR (n = 128)	TF-TAVI (n = 102)	p Value
Age (years)	82 (80, 83)	86 (83, 88)	<0.001
Women	76 (59%)	73 (72%)	0.054
Cause of the aortic stenosis			
Atherosclerosis	106 (83%)	100 (98%)	0.001
Bicuspid valve	20 (16%)	2 (2%)	0.001
Hypertension	100 (78%)	81 (79%)	0.81
Diabetes mellitus	33 (26%)	25 (25%)	0.83
Cerebrovascular disease	12 (9%)	15 (15%)	0.21
Coronary artery disease	30 (23%)	33 (32%)	0.13
Chronic obstructive pulmonary disease	4 (3%)	3 (3%)	1.0
Preoperative therapy			
Ca blocker	63 (49%)	53 (52%)	0.68
Angiotensin II receptor blocker	57 (45%)	44 (43%)	0.83
Angiotensin-converting enzyme	12 (9%)	13 (13%)	0.42
β blocker	26 (20%)	34 (33%)	0.025
Statin	57 (45%)	53 (52%)	0.26
Aspirin	30 (23%)	41 (40%)	0.006
Echocardiographic variables			
Left ventricular diastolic diameter (mm)	46 (42, 50)	47 (43, 51)	0.28
Left ventricular systolic diameter (mm)	28 (26, 32)	29 (26, 35)	0.11
Left ventricular ejection fraction (%)	65 (58, 72)	68 (61, 72)	0.096
Maximum pressure gradient (mm Hg)	87 (72, 112)	88 (71, 109)	0.97
Mean pressure gradient (mm Hg)	54 (42, 70)	53 (41, 68)	0.84
Aortic valve area (cm^2)	0.66 (0.53, 0.79)	0.64 (0.51, 0.76)	0.38
STS predicted risk of surgical mortality (%)	6.8 (5.0, 8.5)	6.8 (4.2, 8.3)	0.49
Operative variables			
Operation time (minutes)	289 (255, 348)	127 (111, 149)	<0.001
Cardiopulmonary bypass time (minutes)	139 (114, 171)	NA	NA
Aorta clamp time (minutes)	118 (94, 143)	NA	NA
Intraoperative blood loss (ml)	350 (236, 550)	15 (0. 50)	<0.001
Amount of blood transfused (ml)	560 (0. 1280)	0 (0, 0)	<0.001
Coronary artery bypass grafting	19 (15%)	NA	NA
Mitral surgery	14 (11%)	NA	NA
Tricuspid valve surgery	15 (12%)	NA	NA
Ascending aorta replacement	4 (3%)	NA	NA
In-hospital outcomes			
Mortality	4 (3%)	0 (0%)	0.19
Length of ICU stay (days)	4 (3, 5)	2 (2, 2)	<0.001
Length of hospital stay (days)	16 (14, 19)	8 (7, 10)	<0.001
Prolonged ventilation (>48 hours)	17 (13%)	0 (0%)	<0.001
Complete atrioventricular block	1 (0.8%)	8 (8%)	0.016
Re-exploration for bleeding	4 (3%)	0 (0%)	0.20
Stroke	1 (0.8%)	1 (1%)	1.0

enrichment analysis performed with the DAVID functional annotation tool (https://david.ncifcrf.gov/) to identify pathways related to altered inflammatory responses in patients who underwent TAVI.

Study data are shown as mean ± standard deviation values, median (twenty-fifth and seventy-fifth percentiles) values, or the number (percentage) of patients. Between-group differences were analyzed by chi-square test or Mann −Whitney U-test, as appropriate. A logistic regression model was fit with the operative procedure as the response variable and age, gender, hypertension, diabetes, cerebrovascular disease, coronary artery disease, chronic obstructive pulmonary disease, preoperative medical therapy, and echocardiographic measures (left ventricular ejection fraction, mean pressure gradient, and aortic valve area) as predictor variables to generate the propensity scores. All statistical analyses were performed with SPSS 26.0 for Windows software (IBM Corp., Armonk, NY), and $p < 0.05$ was considered significant.

Results

Clinical characteristics, operative variables, and in-hospital outcomes of the total Part 1 study patients are shown per group in Table 1. Patients in the TF-TAVI group were significantly older than those in the SAVR group. Co-morbidities did not differ significantly between the groups. Use of β blockers and use of aspirin were significantly more common in the TF-TAVI group than in the SAVR group. In-hospital mortality was 3% (4/128) in the SAVR group and 0% (0/102) in the TF-TAVI group ($p = 0.19$). ICU and hospital stays were significantly shorter for patients in the TF-TAVI group than for those in the SAVR group. Laboratory values of the total Part I study patients are shown per group in Supplementary Table 1 and Figure 2. There were slight but significant between-group differences in preoperative white blood cell (WBC) and platelet counts, with counts being lower in the TF-TAVI group than in the SAVR group. Similarly, preoperative aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK) concentrations were significantly lower in the TF-TAVI group than in the SAVR group. Relative to the preoperative WBC count and AST, LDH, and CK concentrations, postoperative values were increased in both groups, but much more so in the SAVR group than in the TF-TAVI group (Figure 2).

Characteristics of the Part 1 propensity score-matched study patients are shown per group in Supplementary Table 2. In accordance with results of the unmatched analysis, operative variables and ICU and hospital stays differed significantly between the 2 propensity score-matched groups. Laboratory values are shown per propensity score-matched group in Supplementary Table 3. With the exception of the CK concentration, preoperative laboratory values did not differ between the groups. Comparison of variables between the 2 propensity score-matched groups showed the postoperative WBC count and AST, ALT, LDH, and CK concentrations to be significantly decreased in the TF-TAVI group.

Clinical characteristics of the Part 2 study patients are shown per group in Table 2. Age differed significantly between the 2 groups, but other clinical characteristics, including preoperative medical therapy and echocardiographic variables, did

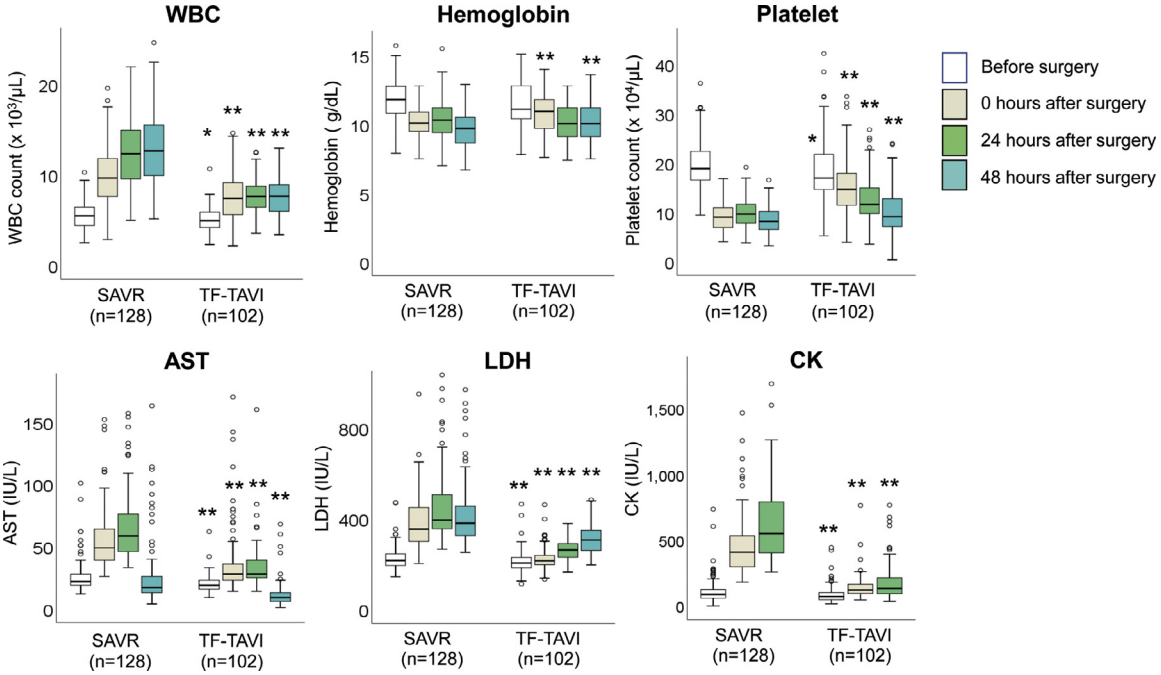

Figure 2. Box and whisker plots of hematologic values, liver enzyme concentrations, and creatinine kinase concentrations measured at 4 different time points in the Part 1 study patients, per group (SAVR [n = 128] vs TF-TAVI [n = 102]). Median (horizontal line dissecting the box), interquartile range (length of the box), and minimum and maximum (whisker ends) values are shown. Outliers are shown as circles. p values were obtained by Mann-Whitney U-test. AST = aspartate aminotransferase; CK = creatinine kinase; LDH = lactate dehydrogenase; SAVR = surgical aortic valve replacement; TF-TAVI = transfemoral transcatheter aortic valve implantation; WBC = white blood cells. *p <0.05, **p <0.01.

Table 2
Clinical characteristics, echocardiographic variables, operative variables, and in-hospital outcomes of the Part 2 study patients, per group (SAVR vs TF-TAVI)

Variable	SAVR (n = 11)	TF-TAVR (n = 12)	p Value
Age (years)	77 (74, 81)	84 (81, 85)	0.002
Women	5 (36%)	7 (67%)	0.30
Bicuspid aortic valve	1 (9%)	0 (0%)	0.96
Height (cm)	156 (154, 160)	156 (146, 160)	0.69
Weight (kg)	55 (50, 69)	51 (43, 66)	0.45
Hypertension	8 (73%)	7 (58%)	0.78
Diabetes mellitus	5 (46%)	1 (8%)	0.12
Coronary artery disease	2 (18%)	1 (8%)	0.93
Chronic obstructive pulmonary disease	1 (9%)	0 (0%)	0.96
Preoperative therapy			
Ca blocking agent	4 (36%)	4 (33%)	0.88
Angiotensin II receptor blocker	5 (46%)	5 (42%)	0.86
Angiotensin-converting enzyme	1 (9%)	2 (17%)	1.0
β blocking agent	0 (0%)	4 (33%)	0.12
Statin	7 (64%)	5 (42%)	0.53
Aspirin	1 (9%)	2 (17%)	1.0
STS predicted risk of surgical mortality (%)	4.0 (3.4, 5.5)	6.1 (4.2, 8.6)	0.079
Echocardiographic variables			
Left ventricular diastolic diameter (mm)	44 (42, 49)	50 (40, 54)	0.57
Left ventricular systolic diameter (mm)	27 (24, 33)	29 (23, 38)	0.69
Left ventricular ejection fraction (%)	70 (59, 74)	70 (55, 73)	0.79
Maximum pressure gradient (mm Hg)	97 (71, 124)	87 (74, 109)	0.54
Mean pressure gradient (mm Hg)	60 (38, 74)	51 (44, 67)	1.0
Aortic valve area (cm^2)	0.82 (0.50, 0.93)	0.72 (0.53, 0.82)	0.49
Operative variables			
Operation time (minutes)	270 (244, 289)	126 (108, 144)	<0.001
Cardiopulmonary bypass time (minutes)	113 (100, 152)	NA	NA
Aorta clamp time (minutes)	87 (80, 109)	NA	NA
Intraoperative blood loss (ml)	409 (370, 475)	20 (5, 50)	<0.001
Amount of transfusion (ml)	0	0	1.0
In-hospital outcomes			
Death	0 (0%)	0 (0%)	1.0
Length of ICU stay (days)	5 (4, 6)	2 (2, 2)	<0.001
Length of hospital stay (days)	15 (13, 20)	8 (7, 9)	<0.001
Prolonged ventilation >48 hours	0 (0%)	0 (0%)	1.0
Complete atrioventricular block	0 (0%)	0 (0%)	1.0
Re-exploration for bleeding	2 (18%)	0 (0%)	0.22
Stroke	0 (0%)	0 (0%)	1.0

Values are median (twenty-fifth, seventy-fifth percentiles) or number (%) of patients. ICU = intensive care unit; NA = not applicable; SAVR = surgical aortic valve replacement; STS = Society of Thoracic Surgery; TF-TAVI = transfemoral transcatheter aortic valve implantation.

not. Operative variables and in-hospital outcomes are shown per group in Table 3. There was no in-hospital death in either group. Laboratory values of the Part 2 study patients are also shown per group in Table 3. Preoperative laboratory values did not differ significantly between the groups. The preoperative differential WBC count also did not differ significantly between the 2 groups (data not shown). The WBC count increased postoperatively in both the SAVR group and TF-TAVI group, and the platelet count decreased postoperatively in both groups. The between-group difference in the WBC count at 24 hours was significant.

Raw cytokine/chemokine levels are shown per group in Supplementary Table 4. Of the 71 cytokines/chemokines assayed, 8 (11%), CCL2, CCL8, CCL22, fibroblast growth factor-2, IL-5, leukemia inhibitory factor (LIF), TGF-α, and thrombopoiesis stimulating factor, differed significantly between the 2 groups preoperatively. Non-normalized concentrations of 42% (30/71) of cytokines/chemokines differed significantly between groups at least once after surgery: 17 (24%) immediately after surgery and 21 (30%) 24 hours after surgery. Concentrations of 8 cytokines/chemokines, CCL2, G-CSF, IL-1Ra, IL-6, IL-8, IL-10, LIF, and TRAIL, differed significantly both immediately after and 24 hours after surgery. Representative non-normalized cytokines/chemokine concentrations are shown in Figure 3. Circulating G-CSF, IL-1Ra, IL-6, IL-8, and IL-10 concentrations were elevated postoperatively in both groups, however, these elevations were not as great in the TF-TAVI group as in the SAVR group. TRAIL and CCL11/eotaxin concentrations increased immediately after surgery in both groups but then decreased to below preoperative levels in both groups by 24 hours after surgery. The reductions seen at 24 hours were not as sizable in the TF-TAVI group as they were in the SAVR group. The tumor necrosis factor alpha (TNF-α) concentration immediately after surgery was higher in the SAVR group

Table 3
Laboratory values of the Part 2 study patients, per group (SAVR vs TF-TAVI)

Variable	SAVR (n = 11)	TF-TAVI (n = 12)	p Value
Before surgery			
White blood cell count (x10^3/μl)	5.6 (4.8, 6.4)	5.0 (3.9, 6.4)	0.38
Hemoglobin (g/dL)	13.0 (11.6, 13.7)	12.1 (11.2, 12.9)	0.38
Hematocrit (%)	39.1 (35.5, 40.7)	36.7 (35.3, 40.3)	0.41
Platelet count (x10^4/μl)	20.0 (15.4, 23.9)	18.7 (15.7, 24.7)	0.93
Aspartate aminotransferase (IU/L)	19 (17, 24)	19 (16, 25)	0.79
Lactate dehydrogenase (IU/L)	196 (176, 253)	215 (200, 232)	0.49
Creatinine (mg/dl)	0.83 (0.57, 0.89)	0.86 (0.64, 0.98)	0.70
Creatinine kinase (IU/L)	83 (62, 100)	70 (53, 124)	0.57
Immediately after surgery			
White blood cell count (x10^3/μl)	8.1 (7.5, 12.7)	6.9 (5.3, 10.0)	0.056
Hemoglobin (g/dl)	10.3 (9.5, 11.2)	11.6 (10.8, 12.5)	0.018
Hematocrit (%)	32.8 (28.5, 34.2)	36.0 (32.6, 37.7)	0.031
Platelet count (x 10^4/μl)	10.7 (10.0, 14.4)	15.4 (13.6, 17.1)	0.019
Aspartate aminotransferase (IU/L)	44 (42, 53)	34 (25, 45)	0.021
Lactate dehydrogenase (IU/L)	405 (331, 453)	222 (199, 252)	<0.001
Creatinine (mg/dl)	0.69 (0.56, 0.84)	0.68 (0.60, 0.92)	0.78
Creatinine kinase (IU/L)	524 (484, 727)	124 (76, 210)	<0.001
Creatine kinase-MB isoenzyme (IU/L)	47 (40, 63)	11 (8, 21)	<0.001
24 hours after surgery			
White blood cell count (x10^3/μl)	11.4 (10.9, 16.0)	8.1 (5.7, 9.2)	<0.001
Hemoglobin (g/dl)	10.5 (8.7, 11.1)	10.9 (9.9, 11.7)	0.09
Hematocrit (%)	30.4 (26.4, 33.4)	33.5 (31.2, 35.4)	0.049
Platelet count (x10^4/μl)	10.7 (8.9, 12.6)	12.3 (11.7, 15.0)	0.14
Aspartate aminotransferase (IU/L)	50 (43, 68)	31 (27, 41)	0.002
Lactate dehydrogenase (IU/L)	463 (389, 501)	264 (247, 291)	<0.001
Creatinine (mg/dl)	0.92 (0.64, 1.08)	0.77 (0.65, 0.86)	0.48
Creatinine kinase (IU/L)	576 (534, 950)	151 (77, 201)	<0.001
Creatine kinase-MB isoenzyme (IU/L)	35 (21, 42)	6 (4, 13)	0.001

Values are median (twenty-fifth, seventy-fifth percentiles). SAVR = surgical aortic valve replacement; TF-TAVI = transfemoral transcatheter aortic valve implantation.

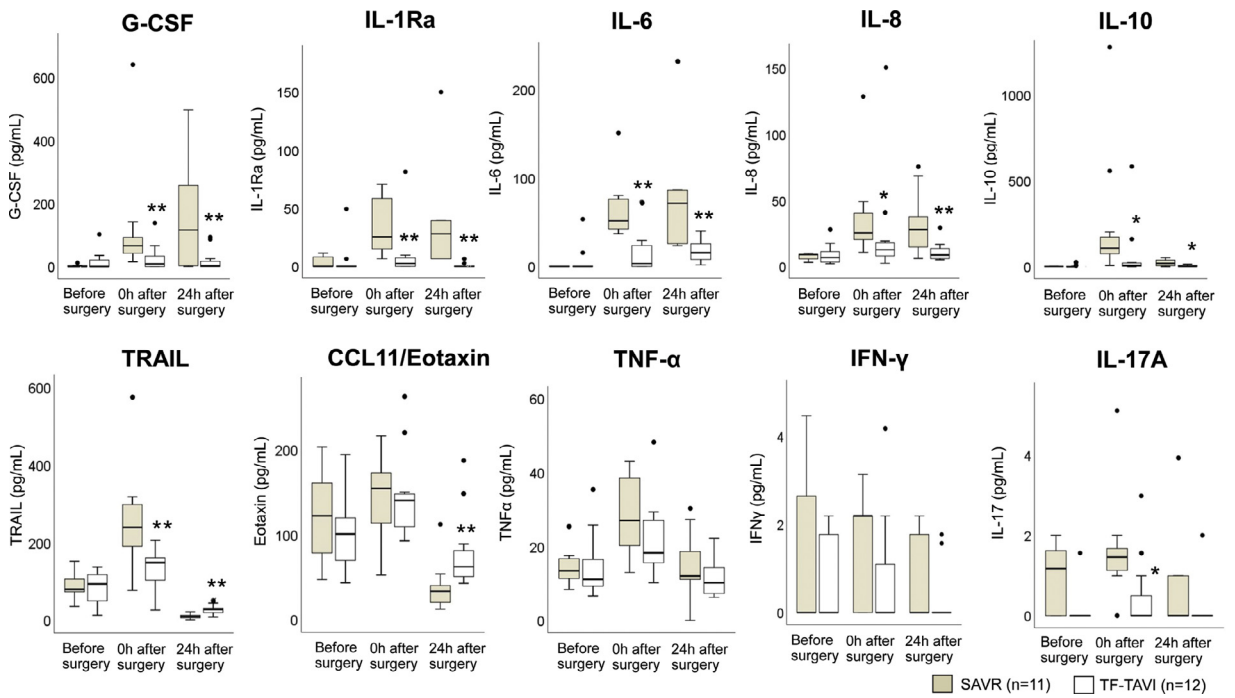

Figure 3. Box and whisker plots of perioperative serum cytokine and chemokine concentrations of the Part 2 study patients, per group (SAVR [n = 11] vs TF-TAVI [n = 12]). Median (horizontal line dissecting the box), interquartile range (length of the box), and minimum and maximum (whisker ends) values are shown. Outliers are shown as circles. p values were obtained by Mann-Whitney U-test. SAVR = surgical aortic valve replacement; TF-TAVI = transfemoral transcatheter aortic valve implantation; WBC = white blood cells. *p <0.05, **p <0.01.

Table 4
Differentially expressed cytokines and chemokines identified after normalization to preoperative values

Cytokine	Immediately after surgery		24 hours after surgery	
	p Value	Fold change	p Value	Fold change
Interleukin 6	0.000	47.37	0.001	10.55
Granulocyte-colony stimulating factor	0.001	26.07	0.012	36.12
Interleukin 1 receptor antagonist	0.003	10.05	0.001	39.14
Interleukin 10	0.014	8.31		
C-C motif chemokine ligand 4/Macrophage inflammatory protein-1β	0.023	7.92		
Interleukin 11	0.014	5.47	0.047	3.55
C-C motif chemokine ligand 2/Monocyte chemoattractant Protein 1	0.023	2.41		
Interleukin 8/C-X-C motif chemokine ligand 8	0.019	1.99	0.006	2.37
Interleukin 12p40	0.008	1.98		
C-C motif chemokine ligand 8/Monocyte chemoattractant protein 2	0.042	1.83	0.048	2.39
TNF-related apoptosis-inducing ligand	0.002	1.74	0.000	0.24
Interleukin 28a			0.000	110.97
C-C motif chemokine ligand 26/Eotaxin-3			0.046	6.92
X-C motif chemokine ligand 1/ Lymphotactin			0.021	2.78
C-C motif chemokine ligand 20/ Macrophage Inflammatory Protein-3			0.008	2.13
Leukemia inhibitory factor	0.014	0.20		
C-C motif chemokine ligand 17/Thymus and activation regulated chemokine	0.031	0.54		
Thymic stromal lymphopoietin	0.040	0.58	0.047	0.58
Fractalkine			0.047	0.39
C-C motif chemokine ligand 11/Eotaxin-1			0.001	0.39
C-C motif chemokine ligand 19			0.010	0.45
Interleukin 5			0.021	0.48
Interleukin 12p70			0.035	0.54

than in the TF-TAVI group, but the difference was not significant ($p = 0.056$).

Differentially expressed cytokines, identified after normalization to preoperative values, are shown in Table 4. Fourteen cytokines differed significantly in concentration immediately after surgery, and 17 differed significantly 24 hours after surgery. In looking over these results comprehensively and applying hierarchical clustering (Figure 4), we identified 23 differentially expressed cytokines. The 23 inflammatory proteins fell into 3 hierarchical clusters:

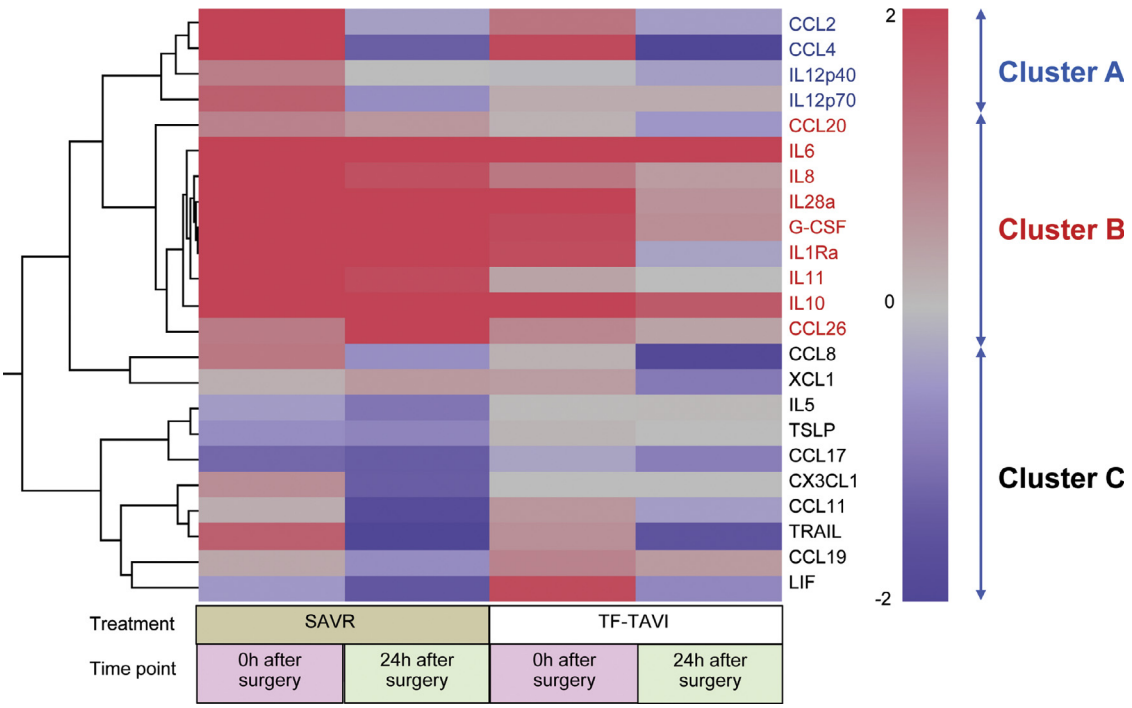

Figure 4. Hierarchical clustering of differentially expressed cytokines and chemokines. SAVR = surgical aortic valve replacement; TF-TAVI = transfemoral transcatheter aortic valve implantation.

Table 5
Top 10 pathways identified in relation to altered inflammatory responses in patients who underwent TF-TAVI

Pathway	p Value
Cellular response to tumor necrosis factor	0.0035
Cellular response to interleukin-1	0.0062
Monocyte chemotaxis	0.0101
Cellular response to interferon-gamma	0.0101
Positive regulation of extracellular signal-regulated kinase 1 and extracellular signal-regulated kinase 2 cascade	0.0393
CC chemokine, conserved site	0.0492
Cellular response to lipopolysaccharide	0.0509
Cytokine-cytokine receptor interaction	0.0510
Neutrophil chemotaxis	0.0589
CCR chemokine receptor binding	0.0697

TF-TAVI = transfemoral transcatheter aortic valve replacement.

cluster A, characterized by highly increased expression immediately after surgery in the SAVR group and slightly increased expression immediately after surgery in the TF-TAVI group and decreased expression 24 hours after surgery in both groups (CCL2, CCL4, IL-12p40, and IL-12p70); cluster B, characterized by highly increased expression immediately after surgery and at 24 hours in the SAVR group and slightly increased expression immediately after surgery and at 24 hours in the TAVI group (CCL20, IL-6, IL-8, IL-28A, G-CSF, IL-1Ra, IL-11, IL-10, and CCL26); and cluster C, characterized by various other patterns, for example, by highly decreased expression after SAVR and slightly decreased expression after TAVI or by highly increased expression after TAVI and slightly increased expression after SAVR (CCL8, XCL1, IL-5, TSLP, CCL17, CXCL1, CCL11, TRAIL, CCL19, and LIF).

We performed gene enrichment analysis for functional annotation of the 23 differentially expressed cytokines/chemokines. The top 10 related pathways identified by gene ontology analysis are shown in Table 5. Multiple pathways were shown to be related to the postoperative inflammatory response after SAVR and altered response after TAVI, including cellular response to TNF ($p = 0.0035$), cellular response to interleukin-1 ($p = 0.0062$), and monocyte chemotaxis ($p = 0.0101$).

Discussion

Our study confirmed minimal invasiveness of TAVI from the standpoint of postoperative inflammatory response. Our main findings were as follows: (1) TF-TAVI attenuated postoperative alterations in laboratory values caused by conventional SAVR, (2) TF-TAVI suppressed the postoperative elevation in concentrations of multiple cytokines/chemokines (G-CSF, IL-1Ra, IL-6, IL-8, and IL-10) that follow conventional SAVR, and (3) cytokines involved in the inflammatory response after conventional SAVR were identified. These are TNF-α and IL-1 and may be a potential therapeutic target. To the best of our knowledge, our study is the first reported cytokine profiling and bioinformatics analysis-based investigation of the postoperative inflammatory response triggered by TAVI.

Previously reported studies have compared the inflammatory response triggered by SAVR and that triggered by TAVI. Lindman et al reported severe systemic inflammatory response syndrome (SIRS) developed in 6% of TAVI patients ($n = 264$) and 11% of SAVR patients ($n = 747$; $p = 0.02$).[2] Our study confirmed that TAVI significantly attenuates the elevations in WBC count, liver enzymes, and CK and the reductions in hemoglobin and platelet count associated with SAVR. Several groups have compared postoperative inflammation by measuring IL-6, IL-8, and IL-10, showing the concentrations of these conventionally measured cytokines to be decreased after TAVI, in comparison to concentrations after SAVR.[3,4] Furthermore, 2 groups of investigators recently measured a wide range of cytokines using a cytokine panel assay and found the concentrations of IL-1Ra, IL-6, IL-8, IL-10, CCL2, CCL5, TNF-α, and CXCL1 to be significantly decreased after TF-TAVI, relative to concentrations after SAVR.[5,6] Likewise, we found, after normalization of values, significantly decreased expression of several of these cytokines, that is, IL-1Ra, IL-6, IL-8, IL-10, and CCL2, in our TF-TAVI group. By hierarchical clustering analysis, we found most of the reported differentially expressed cytokines fell into cluster A or cluster B. Common to these 2 clusters is the fact that the cytokine levels were significantly decreased in the TAVI group at at least 1 postoperative time point. Cluster C cytokines followed various other patterns, suggesting more complicated and diverse changes in the postoperative inflammatory response after TAVI in comparison to that after SAVR.

We found cluster B cytokines to be mildly elevated in the TF-TAVI group. Sinning et al analyzed outcomes among 152 patients who underwent TAVI (TF-TAVI; $n = 140$, trans-subclavian TAVI; $n = 12$) and reported that SIRS after TAVI was related to elevation of proinflammatory cytokines, including IL-6 and IL-8. Regarding the effect of SIRS on outcomes of TAVI, occurrence of SIRS was associated with 30-day mortality (18.0% vs 1.1%, $p < 0.001$) and was shown to be an independent predictor of 1-year mortality (hazard ratio 4.3, 95% confidence interval 1.9 to 9.9; $p < 0.001$).[1] Several cytokine studies have suggested the transapical approach, in comparison to the transfemoral approach, may increase the postoperative inflammatory responses.[3,5] Careful monitoring and hemodynamic management are important for patients who show postoperative inflammatory symptoms and/or signs after TAVI.

Our bioinformatics analysis showed involvement of multiple pathways in the postoperative inflammatory response to SAVR. Cellular responses induced by TNF and IL-1 were the top 2 inflammatory pathways identified in our study. TNF-α is a proinflammatory cytokine and is reported to play a key role in development of CPB-induced lung injury[14] and acute kidney injury.[15] We found that the TNF-α concentration tended to increase immediately after SAVR. In a previous study, the TNF-α concentration was shown to increase immediately after aortic declamping, reaching its peak 1.5 to 2 hours after reperfusion.[12] Using a rabbit model of CPB, Yu et al demonstrated TNF-α neutralizing antibody alleviates CPB-induced pulmonary inflammation.[16] Like TNF-α, IL-1 is a proinflammatory cytokine and is reported to be increased in CPB-induced lung injury.[17] Anti-inflammatory treatment neutralizing IL-1 has not been reported in either a human or animal model of CPB. A new treatment reducing cytokine activity (CytoSorb, Cytosorbents, Monmouth Junction, NJ)

has been applied during cardiac surgery performed under CPB. However, use of this system has not been associated with a decrease in pro- or anti-inflammatory cytokines or improvement in relevant clinical outcomes after cardiac surgery.[18] Although neutralization of cytokine activity can be considered as a possible therapeutic target in conventional cardiac surgery, further studies are needed before clinical application can be realized.

Our study had several limitations. First, it was a single center study, and the sample size was relatively small. A large-scale multicenter study is needed to confirm our findings. Second, in both Part 1 and Part 2 of our study, median age of patients in the TF-TAVI group was significantly greater than that of patients in the SAVR group. Elderly patients are likely to be in a chronic inflammatory state due to age-related diseases, including atherosclerosis,[19] and age-associated inflammation may lead to dysregulation of the innate immune response characterized by impaired cell migration and effector functions.[20] Although the Part 1 unmatched analysis and propensity score-matched analysis yielded similar results, the altered postoperative inflammatory response in our TAVI group might have been influenced by a basic immune status specific to elderly patients. Third, some patients in our study were regular users of aspirin and/or statin, which are known to influence immune activity. We did not investigate the effect of preoperative medical therapy on patients' postoperative inflammatory response.

In conclusion, our study showed a significant decreased in peak WBC count, liver enzymes, and CK concentrations in patients who underwent TF-TAVI. Cytokine profiling demonstrated that a robust postoperative inflammatory response follows SAVR but that a significantly attenuated response follows TAVI. Although the immune response provoked by surgical trauma and use of CPB is complicated, pathway analysis suggested that several key cytokines, including TNF-α and IL-1, are involved in the upregulation of inflammatory reactions after SAVR. These cytokines may be a potential therapeutic target.

Disclosures

The authors have no conflicts of interest to disclose.

Author Contributions

Naoyuki Kimura: Conceptualization, Methodology, Formal analysis, Data curation, Software, Writing, Original draft preparation, Writing-Review & Editing, Funding acquisition.

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Supplementary materials

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