ORIGINAL ARTICLE

Significance of the age of transfused blood for prognosis after transcatheter aortic valve implantation

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KEY WORDS

blood transfusion, elderly, storage age of transfused blood, transcatheter aortic valve implantation

ABSTRACT

INTRODUCTION Blood transfusion after transcatheter aortic valve implantation (TAVI) is frequently required owing to the high vulnerability of this patient group and procedure-related bleeding.

OBJECTIVES We assessed the impact of postprocedural blood transfusion and the age of transfused red blood cell (RBC) units on prognosis after TAVI.

PATIENTS AND METHODS This was a single-center, observational analysis conducted between the years 2009 and 2014. The adopted endpoints were early and long-term mortality after TAVI. The risk factors for mortality included in-hospital bleeding and vascular complications, the number of transfused RBC units, transfusion of at least 2 RBC units, the age of transfused RBCs, and standard deviation of the age of RBCs.

RESULTS The study included 178 patients (mean [SD] age, 80.07 [7.47] years; range, 55–91 years). The follow-up ranged between 1 month and 5.8 years (mean [SD], 20.1 [15.2] months) after discharge; 14 early deaths (7.8%) and 27 late deaths (16.5%) were noted. In-hospital bleeding and vascular complications increased the risk of early deaths (hazard ratio [HR], 2.113; 95% CI, 1.011–4.418; $P = 0.046$ and HR, 2.265; 95% CI, 1.270–4.039; $P = 0.005$). Transfusion of younger RBCs (HR, 1.044; 95% CI, 1.004–1.085; $P = 0.028$) and a greater discrepancy in the age of transfused RBCs (HR, 1.153; 95% CI, 1.042–1.275; $P = 0.006$) were positively correlated with the risk of late deaths only in a univariate analysis. A higher number of transfused RBC units was the only independent predictor of long-term mortality (HR, 1.149; 95% CI, 1.024–1.291; $P = 0.018$).

CONCLUSIONS The higher number of RBC units transfused early after TAVI worsens long-term prognosis. Shorter-storage RBCs and a greater discrepancy in RBC age in multitransfused elderly patients after TAVI might have a deleterious effect on life expectancy.

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INTRODUCTION Blood transfusion is one of the most common therapies.[1,2] According to the Global Database on Blood Safety developed by the World Health Organization, each year nearly 85 million red blood cell (RBC) units are transfused worldwide.[1] In developed countries, most of transfused RBCs are administered to surgical and critically ill patients. Approximately 40% of intensive care patients receive at least 1 unit of RBCs during hospitalization, with a mean of 5 RBC units per patient.[2] Although blood transfusion is often a life-saving therapy, numerous studies have proved its deleterious clinical effects.[1-9] These reports also indicated the negative impact of the age of transfused RBCs.[5-9]

Concerns about the harmful impact of blood transfusion and the age of transfused RBCs seem to be justified in view of the variety of novel antithrombotic therapies, the growing number of invasive strategies, and the bleeding complications involved. One of the most rapidly developing cardiac invasive procedures is transcatheter

TABLE 1 Baseline characteristics of the study group

Characteristic		Value
Age, y, mean (SD)		80.07 (7.47)
Age >85 years, n (%)		47 (26.4)
Female sex, n (%)		119 (66.85)
Logistic EuroSCORE, %, range (mean [SD])		2.86–60.37 (22.35 [12.42])
STS risk score, %, range (mean [SD])		1.07–37.17 (8.43 [8.26])
Implantation, n (%)	Tf-AVI / Tsc-AVI	131/15 (73.59/8.42)
	Ta-AVI	25 (14.04)
	Tao-AVI	7 (3.93)
NYHA class, n (%)[a]	II	39 (21.91)
	III	103 (57.86)
	IV	27 (15.16)
Coronary artery disease, n (%)		139 (78.08)
Previous myocardial infarction, n (%)		38 (21.34)
Previous coronary intervention (total), n (%)		84 (47.19)
Previous coronary intervention, n (%)	PCI	54 (30.33)
	PCI ≤6 months pre-TAVI	36 (20.22)
	CABG	30 (16.85)
COPD, n (%)		43 (24.15)
Atrial fibrillation, n (%)		69 (38.76)
Anemia, n (%)		108 (60.67)
Permanent pacemaker: before/after TAVI, n (%)		23/54 (12.92/30.33)
Pulmonary hypertension, n (%)		84 (77.52)
Extensively calcified aorta, n (%)		34 (19.1)
Osteoporosis, n (%)		51 (57.3)
Renal impairment, n (%)		105 (58.98)
BMI, kg/m^2, mean (SD)		26.72 (5.18)

a Data for 9 patients were not reported.

Abbreviations: BMI, body mass index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; STS, Society of Thoracic Surgeons; Ta-AVI, transapical aortic valve implantation; Tao-AVI, transaortic aortic valve implantation; Tf-AVI, transfemoral aortic valve implantation; Tsc-AVI, transsubclavian aortic valve implantation

aortic valve implantation (TAVI). This rescue procedure is dedicated to high-risk elderly patients with severe aortic stenosis.[10-15] Given the vulnerability of this population, TAVI is burdened with a risk of periprocedural vascular complications and demand for blood transfusion.[10,12-15] The impact of the age of transfused RBCs on prognosis after TAVI has not yet been investigated. Therefore, the aim of our study was to assess the impact of postprocedural blood transfusion and the age of transfused RBCs on early and long-term prognosis after TAVI.

PATIENTS AND METHODS

This single-center, retrospective, and observational analysis included consecutive patients who underwent TAVI in our center between the years 2009 and 2014. The mean (SD) follow-up was 20 (15.2) months (range, 1 month to 5.8 years) after discharge. The adopted endpoints were 30-day and long-term mortality after TAVI. The potential risk factors for mortality included the number of blood transfusion procedures, the number of transfused RBC units, transfusion of at least 2 RBC units, the age of transfused RBCs, and the SD of the age of RBCs. The data were obtained from databases and blood transfusion records. Additionally, the impact of in-hospital bleeding and vascular complications on prognosis was estimated. The age of RBCs was defined as the days elapsed between donation and transfusion, both dates included. The SD of the age of RBCs was defined as the difference in the age of transfused RBCs in patients who received at least 2 units of RBCs. Early deaths were those that occurred during the first 30 days after TAVI; late deaths were incidents that occurred at a later date. The RBCs were buffy-coat depleted, with less than 1.2×10^9 white blood cells (WBCs) per unit. After preparation, all RBCs were stored in saline-adenine-glucose-mannitol (SAGM; 376 mOsm/l) for a maximum of 42 days at a temperature of 4°C. Bleeding and vascular complications were classified according to the Valve Academic Research Consortium-2 criteria.[10]

Transfusions were performed at the discretion of operators and the attending physician in the case of significant blood loss during the procedure, significant overt bleeding after TAVI, and significant periprocedural hemoglobin drop.

Each patient provided written informed consent for blood product transfusion before TAVI. The study protocol complied with the Declaration of Helsinki and was approved by the local ethics committee.

Statistical analysis

All analyses were performed using the SAS system (SAS 9.4, SAS Institute Inc., Campus Drive, Cary, North Carolina, United States). Categorical data were expressed as frequencies and percentages; continuous variables were presented as means with SD. Comparisons were done with the χ^2 test or the Fisher exact test for categorical variables and with the nonparametric Wilcoxon test for continuous variables. The impact of the assessed variables on mortality was determined with the Cox regression analysis with estimated hazard ratio (HR) and 95% CI and by constructing the receiver operating characteristic (ROC) curve of the sensitivity versus 1-specificity and calculating the area under the ROC curve (AUC) with 95% CIs. Optimal cut-off values were chosen based on the intersection of sensitivity and specificity, as well as positive and negative predictive values.

The independent risk factors for mortality, isolated in a multivariable logistic regression analysis, were adjusted for clinical characteristics as potential confounding variables. For all statistical tests, a significance level of a P value of 0.05 or lower was used, which was also the P level for entry in the multivariable analysis.

RESULTS

Impact of blood transfusion and storage time of red blood cells on 30-day mortality

The study included 178 consecutive patients after

TABLE 2 Univariable and multivariable analyses of the impact of bleeding, vascular complications, blood transfusion, and the age of transfused red blood cells on 30-day mortality (Cox regression analysis)

Variable	30-day mortality		*P* value	Univariable analysis	Multivariable analysis[c]
	Yes (n = 14)[a]	No (n = 164)		HR (95% CI); *P* value	HR (95% CI); *P* value
Bleeding complications, n (%)	9 (69.23)	99 (60.36)	0.7	2.11 (1.011–4.418); 0.046	1.14 (0.412–3.128); 0.80
Vascular complications, n (%)	9 (69.23)	79 (48.17)	0.2	2.27 (1.270–4.039); 0.005	2.09 (0.905–4.857); 0.08
Blood transfusion, n (%)	10 (76.92)	91 (55.48)	0.45	2.59 (0.715–9.442); 0.14	–
RBC units, range (mean [SD])	1–13 (3.23 [3.81])	1–17 (1.76 [2.49])	0.08	1.13 (0.994–1.279); 0.06[b]	–
Transfusion ≥2 RBC units, n (%)	7 (53.84)	75 (45.73)	0.73	1.36 (0.457–4.048); 0.58	–
RBC age, d, range (mean [SD])	9–26 (17.35 [4.97])	7–38 (19.56 [7.62])	0.56	1.04 (0.984–1.088); 0.18	–
SD for RBCs age, d, range (mean [SD])	0.707–5.77 (2.88 [1.89])	0.707–16.46 (3.37 [3.92])	0.22	1.00 (0.817–1.243); 0.94	–

a One patient died during transcatheter aortic valve implantation and was excluded from the analysis.
b Per RBC unit
c Adjusted for female sex as the only relevant confounding variable in the model

Abbreviations: HR, hazard ratio; RBC, red blood cells; SD, standard deviation

TABLE 3 Univariable and multivariable analyses of the impact of bleeding, vascular complications, blood transfusion, and the age of transfused red blood cells on long-term mortality (Cox regression analysis)

Variable	Long-term mortality		*P* value	Univariable analysis	Multivariable analysis[b]
	Yes (n = 27)	No (n = 135)		HR (95% CI); *P* value	HR (95% CI); *P* value
Bleeding complications, n (%)[c]	17 (62.96)	82 (60.74)	0.91	1.26 (0.798–2.001); 0.31	–
Vascular complications, n (%)[c]	16 (59.25)	61 (45.18)	0.18	1.44 (0.950–2.188); 0.08	–
Blood transfusion, n (%)	18 (66.66)	73 (45.06)	0.53	1.71 (0.768–3.809); 0.18	–
RBC units, range (mean [SD])	1–17 (3.07 [4.33])	1–8 (1.514 [1.88])	0.03	1.16 (1.051–1.273); 0.003[a]	1.15 (1.024–1.291); 0.018
Transfusion ≥2 RBC, n (%)	59 (43.7)	16 (59.25)	0.38	1.79 (0.832–3.888); 0.13	–
RBC age, d, range (mean [SD])	18.94 (7.3) (7–38)	25.56 (11.65) (14–38)	0.04	1.04 (1.004–1.085); 0.028	1.03 (0.984–1.073); 0.21
SD of RBC age, d, range (mean [SD])	0.57–13.67 (3.01 [3.67])	0–16.46 (5.52 [4.6])	0.003	1.15 (1.042–1.275); 0.006	1.09 (0.949–1.242); 0.23

a Per RBC unit
b Adjusted for female sex as the only relevant confounding variable in the model
c Excluding events in patients who died within 30 postprocedural days

Abbreviations: see TABLE 2

TAVI (mean [SD] age, 80.07 [7.47] years; range, 55–91 years; 119 women [66.9%]). The baseline characteristics of the study population are presented in TABLE 1.

Procedure-related and postprocedural blood transfusion was performed in 101 patients (56.7%); 1 to 19 units of RBCs were required (mean [SD], 1.87 [2.62]). Blood transfusion was performed mainly within the first 2 days after TAVI (mean [SD], 1.81 [1.78] days). The mean (SD) hemoglobin concentration before transfusion was 9.67 (1.38) mg/dl. We noted 14 early deaths (7.8%), which occurred 1 to 29 days after TAVI (mean [SD], 11.78 [11.43] days).

Only serious bleeding (odds ratio [OR], 2.113; 95% CI, 1.011–4.418; $P = 0.046$) and vascular complications (OR, 2.265; 95% CI, 1.270–4.039; $P = 0.005$) predicted 30-day mortality. The results are presented in TABLE 2.

Impact of blood transfusion and storage time of red blood cells on long-term mortality A total of 162 patients (91.0%) were followed (14 early deaths and 2 patients were lost to follow-up); 118 patients (71.95%) were followed longer than 12 months. We noted 27 late deaths (16.6%) that occurred between 63 days and 3.4 years (mean [SD], 497.66 [389.11] days) after TAVI. Postprocedural blood

TABLE 4 Multivariable analysis of the impact of transfused red blood cell units on long-term mortality, adjusted for clinical characteristics (Cox regression analysis)

Variable	Multivariable analysis HR (95% CI)	*P* value
RBC units, mean (SD)	1.14 (1.0345–1.2618)	0.01
Logistic EuroSCORE, %	1.02 (0.9920–1.0493)	0.16
STS score, %	0.98 (0.796–1.0375)	0.32
Age, y, mean (SD)	1.02 (0.9530–1.0960)	0.54
Female sex	0.41 (0.1821–0.9254)	0.03
Preprocedural anemia[a]	2.30 (0.8766–6.0463)	0.09

a Defined as a hemoglobin level of less than 12.0 g/dl for women and less than 13.0 g/dl for men

transfusion was performed in 91 participants (56.2%) discharged from the hospital. These patients received from 1 to 17 units of RBCs (mean [SD], 1.76 [2.49] units) (TABLE 3).

Patients who died during follow-up were those who had been transfused with a greater number of RBC units soon after TAVI (HR, 1.157; 95% CI, 1.051–1.273; $P = 0.003$). Participants who died after discharge had received significantly younger RBCs than those who survived. Shorter storage time was associated with worse distant prognosis by more than 4% per each day of storage less (HR, 1.044; 95% CI, 1.004–1.085; $P = 0.028$). A greater age discrepancy in RBC units in multitransfused patients was related to a higher risk of late death. Each additional day of RBC age variation increased the risk of late death by over 15% (HR, 1.153; 95% CI, 1.042–1.275); $P = 0.006$). However, in the multivariable analysis, only the number of RBC units transfused soon after TAVI remained an independent predictor of long-term mortality (HR, 1.149; 95% CI, 1.024–1.291; $P = 0.018$) (TABLE 3). The adverse effect of the number of transfused RBC units remained significant after adjustment for clinical risk factors (TABLE 4).

The ROC analysis confirmed that the number of transfused RBC units was a significant predictor of late mortality (AUC, 0.599; 95% CI, 0.4819–0.7174; $P = 0.017$), with 3 units of RBCs as a cut-off value (FIGURE 1). The younger RBCs increased the risk of late deaths (AUC, 0.635; 95% CI, 0.518–0.741; $P = 0.05$), with a cut-off value of 18.5 days or less for storage time (FIGURE 2). The greater discrepancy in the age of transfused RBCs increased the risk of late deaths (AUC, 0.690; 95% CI, 0.5550–0.8224; $P = 0.006$). The cut-off value was 0.7 day for recipients with multiple RBC transfusions (FIGURE 3).

DISCUSSION

A population of patients undergoing TAVI is a specific group of elderly individuals with severe aortic stenosis and a number of comorbidities, which makes these patients high-risk or inoperable with classic surgical aortic valve replacement.[10-15] Given the vulnerability of this population, TAVI often results in bleeding and vascular complications that translate into a substantial rate of blood transfusion.[10-15] According to current registries and multicenter studies, from 40% to 80% of patients undergoing TAVI required blood transfusion during or soon after the procedure.[12-15] According to those reports, blood transfusion was related with increased mortality in the TAVI population. However, there have been no studies investigating the negative impact of the age of transfused RBCs in this population.

We found that the transfusion of younger RBCs, stored for less than 18.5 days, deteriorates distant prognosis. So far, only 2 other analyses confirmed our result that shorter-storage RBCs are an independent predictor of death.[16-18] Middelburg et al[16] and Schulman et al[17] demonstrated that transfusions of fresh RBCs, stored for less than 10 days, resulted in an almost 2-fold increase in the rate of 1-year mortality as compared with RBCs stored for more than 24 days. Additionally, Lean-Noval et al[18] proved that the transfusion of fresh RBCs significantly worsened brain oxygenation during the first 24 hours after trauma.

On the other hand, numerous observational studies reported that transfusion of longer-storage RBCs was a predictor of postoperative infection, multiorgan failure, and mortality.[6,19] These outcomes were explained by the fact that stored RBCs undergo constant, time-related structural and biochemical changes known as storage lesions, which result in greater O_2 affinity, increase in fragility, reduction of deformability, and procoagulant properties of older RBCs.[20-24]

One possible explanation of why fresh RBCs deteriorate long-term prognosis after TAVI could be the high amount of allogenic WBCs in the blood of recipients, since transfused RBCs were only buffy-coat depleted. An excessive amount of donor WBCs increases the risk of systemic inflammatory response, infectious risk associated with immunomodulation, and organ failure.[25] However, these adverse effects of allogenic WBCs are observed shortly after transfusion,[25] and we did not observe such a correlation in our study.

We could speculate that the adverse effect of fresh, leukodepleted RBCs on long-term prognosis might be the result of the transfusion-associated microchimerism (TA-MC). TA-MC, a transient presence of allogenic WBCs in the blood of recipients, is a well-known phenomenon.[25-28] Several studies reported significantly prolonged TA-MC.[26-29] Enduring TA-MC has been observed in elderly patients, in those with acute trauma and severe hemorrhage, or after transplantation.[26-29] In these patients, TA-MC occurs most frequently in the case of transient immunologic insufficiency, which promotes tolerance to donor WBCs. Bloch et al[29] demonstrated that trauma hemorrhage is related to deficits in interleukin-2 production and T-cell proliferative capacity in the murine model, and to an altered lymphocyte response in trauma patients. In these subjects, TA-MC persisted for at least 18 months, and in some cases, it was observed even decades after transfusion. The potent predictors for long-lasting TA-MC were the short RBC storage time, altered immune response,

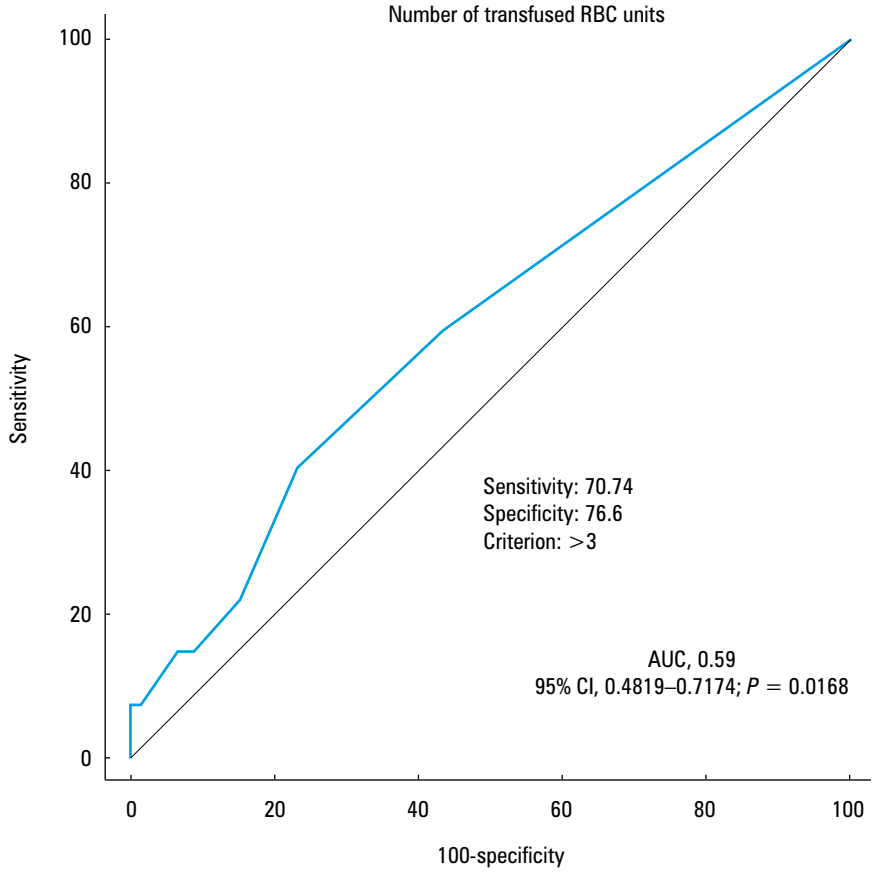

FIGURE 1 Receiver operating characteristic curve analysis of the impact of transfused red blood cell count on long-term prognosis
Abbreviations: AUC, area under the receiver operating characteristic curve

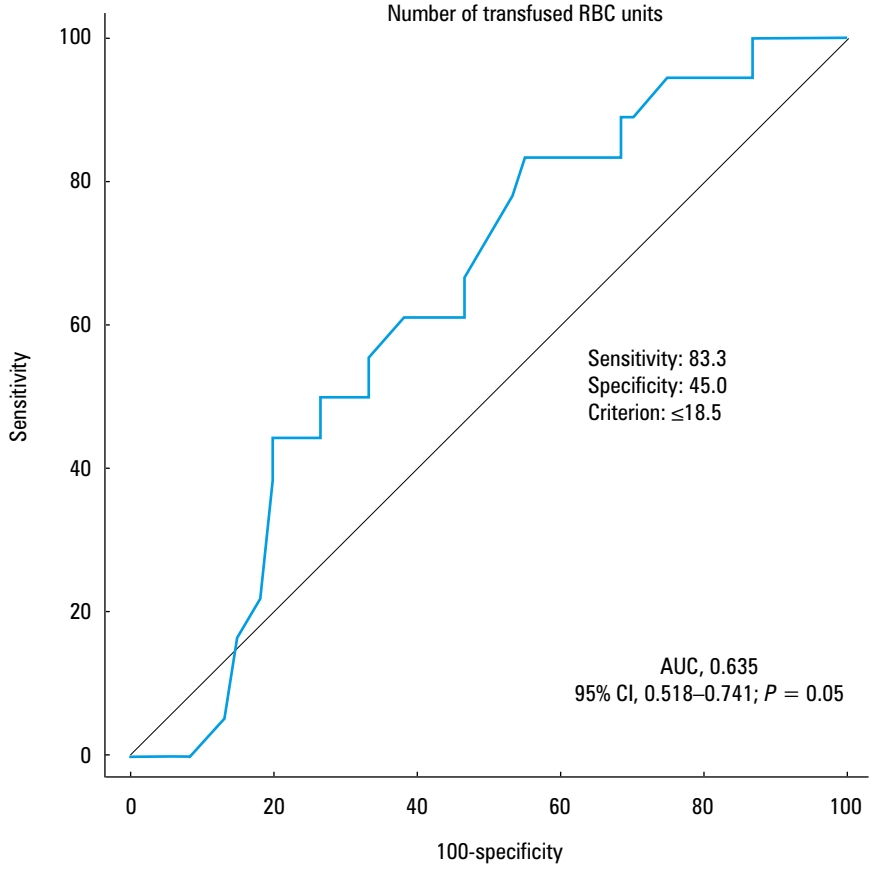

FIGURE 2 Receiver operating characteristic curve analysis of the impact of transfused red blood cell (RBC) age on long-term prognosis
Abbreviations: see **FIGURE 1**

and a large number of transfused RBC units.[26-29] Additionally, Lapierre et al[28] proved that TA-MC was associated with increased production of interleukins 10 and 12. Therefore, TA-MC may induce chronic immune disturbances and be relevant for long-term survival.

The enduring TA-MC might concern the TAVI population. In the case of TAVI-related vascular

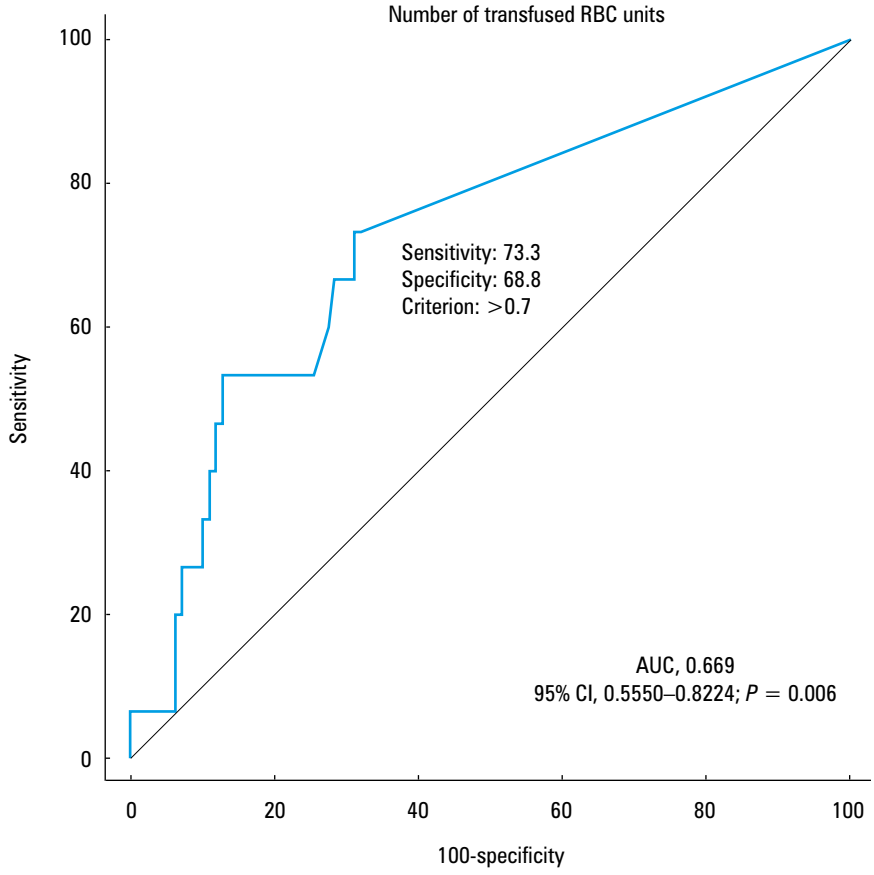

FIGURE 3 Receiver operating characteristic curve analysis of the impact of the discrepancy in red blood cell age on long-term prognosis
Abbreviations: see **FIGURE 1**

complications and excessive bleeding, potential immunosuppression after TAVI could be favorable for donor WBC survival. It is also likely that these elderly patients have an impaired immune system at baseline. Multiple RBC transfusions after TAVI supply a large load of allogenic WBCs that may exhaust the already altered clearance processes and may lead to durable TA-MC, further immune system disturbances, and worse distant prognosis.

The amount of donor WBCs is substantially reduced with leukoreduction.[25] Filtered, fresh RBCs must contain less than 1×10^6 of WBCs per unit.[25,30] Leukoreduction is clinically relevant since it eliminates inflammatory cytokines, reduces human leukocyte antigen (HLA) immunization, febrile nonhemolytic transfusion reactions, leukotropic virus transmission, organ dysfunction, and mortality.[25] Transfusion of such RBCs is recommended mainly for HLA-immunized patients, fetal and newborn transfusions, and prevention of cytomegalovirus infection.[30] Unfortunately, patients subjected to cardiac invasive procedures (excluding heart transplantation and implantation of cardiac assist devices) are administered only buffy-coat depleted RBCs with a greater WBC load. Therefore, our findings might suggest that TAVI patients, as potential multiple RBC recipients, could take advantage of leukoreduced RBCs.

Additionally, we found that the discrepancy in the age of transfused RBCs might be relevant to late mortality in patients with TAVI. This may be a logical solution for the dilemma of shorter- versus longer-storage RBCs. The crucial issue may be the discrepancy in storage time of RBCs and not the storage time itself. As described above, there is a difference in the effect of fresh and old RBCs. When acting simultaneously, these 2 different adverse mechanisms may prove to be an overload for the already impaired immune system of elderly patients undergoing TAVI.

In fact, the cut-off value for the detrimental storage time of RBCs shown in our study is rather convergent with a standard practice for RBC transfusion (the oldest compatible units available in the blood bank) reported in the most recent randomized trials: RECESS and ABLE.[31,32] Moreover, the results of these trials failed to prove any impact of the storage time of RBCs on prognosis in critically ill patients.[31,32] Therefore, both the above outcomes of our analysis should be treated with caution.

Only the number of RBC units transfused seems to have a significant effect on prognosis after TAVI, with the most prominent risk of death in the case of transfusion of more than 3 RBC units. This is in accordance with numerous clinical intensive care studies.[2-9] These analyses confirmed that blood transfusion is harmful, increasing the risk of adverse immune stimulation and mortality in a dose-dependent manner.[2-9] In fact, our cut-off value for 3 units of RBCs is in line with that reported by Donadee et al.[20] On the basis of preclinical experiments, the authors speculated that transfusion of 3 to 4 units of longer-storage RBCs could be detrimental via its vasoconstrictor

effect and has the potential to entirely inhibit systemic nitric oxide signaling.[20]

Nevertheless, unfavorable storage lesions occur in RBCs immediately after collection.[22,23,33,34] Bennett-Guerrero et al[33] showed that freshly collected RBCs were already dysfunctional in their hypoxic vasoactivity due to the *S*-nitrosohemoglobin and vasodilator *S*-nitrosothiol deficiency.[33] The RBC-dependent vasodilation was already altered after 3 hours of storage and remained impaired for the next 6 storage weeks.[33] Similarly, other researchers reported that during the first week of storage intracellular supplies of adenosine triphosphate were significantly depleted and RBC cytoskeletal and membrane proteins were oxidatively modified and degraded.[22,23,33,34]

Furthermore, according to European Union and Food and Drug Administration standards on blood transfusion, at least 75% of donor erythrocytes should remain in circulation 24 hours after transfusion.[30] Thereby, each RBC unit contains from 25% to 30% of the oldest donor erythrocytes, which are removed from circulation via the reticuloendothelial system. Taking into account that each RBC unit contains 1.5×10^{12} erythrocytes, after transfusion of 4 RBCs, the recipient received approximately 1 unit of the oldest erythrocytes that will have to be consumed by 10^{11} phagocytes.[30] Additionally, the oldest erythrocytes are sensitive to storage and may contribute to adverse effects of even fresh RBCs owing to microparticles formation, release of hemoglobin, other intracellular components, and iron overload.[20-22] Thus, in the case of multiple RBC transfusions, at least temporary alteration of the recipient immune system seems likely. Additionally, autoantibody formation occurs most frequently in multiple RBC recipients.[25,30] The greater the number of transfused RBCs, the greater the variability of donor WBC population in the recipient circulation, which increases the risk of the donor–recipient HLA mismatch, various types of TA-MC, and detrimental immune stimulation.

Despite the novel approach, our study has a number of limitations. The observational design of the study as well as the small sample size did not allow estimation of the impact of exclusively old versus exclusively young RBCs on prognosis. Moreover, owing to the small number of noted endpoints, the proper adjustment of analyzed risk factors with all clinical confounders that could be relevant for prognosis was impossible. Therefore, to diminish this limitation we used the logistic EuroSCORE and the Society of Thoracic Surgeons risk score as confounding variables, which included proven clinical predictors of mortality. The small sample size might be also responsible for the high prevalence of reported bleeding, vascular complications, and blood transfusion, and lack of the correlation between these events and long-term mortality. Therefore, the results of our study should be interpreted with caution and must be verified in a prospectively designed large sample size trial of a population undergoing TAVI.

In conclusion, the higher number of RBC units transfused soon after TAVI worsens long-term prognosis. Moreover, the transfusion of shorter-storage RBCs and a greater discrepancy in the age of RBCs might have a deleterious effect on life expectancy in patients undergoing TAVI. Finally, in the TAVI population, the transfusion of leukoreduced RBCs might be beneficial.

Contribution statement KC-J designed the study, performed the research, and wrote the paper. MZ-K was involved in data collection. WP contributed to the analysis and interpretation of data. MŁ, AW, and JS were scientific supervisors of this research; they contributed to the design of the study and reviewed the manuscript critically for important intellectual content. All authors edited and approved the final version of the manuscript.

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