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Expression of miR-223 to predict outcomes after transcatheter aortic valve implantation

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Abstract

Background: *Transcatheter aortic valve implantation (TAVI) is an established treatment for aortic stenosis (AS) in patients at increased surgical risk. Up to 29% of patients annually experience major adverse cardiac and cerebrovascular events (MACCE) after TAVI. MicroRNAs (miRNA) are currently widely investigated as novel cardiovascular biomarkers. The aim of this study was to determine the influence of TAVI on the expressions of selected miRNAs associated with platelet function (miR-125a-5p, miR-125b and miR-223), and evaluate the predictive value of these miRNAs for MACCE in 65 patients undergoing TAVI.*

Methods: *Venous blood samples for miRNA expression analysis were collected 1 day before TAVI and at hospital discharge. The expression of miR-223, miR-125a-5p, miR-125b was evaluated in platelet-depleted plasma.*

Results: *The expression of miR-223 and miR-125b increased after TAVI, compared to the measurement before ($p = 0.020$, $p = 0.003$, respectively). Among 63 patients discharged from the hospital, 18 patients experienced MACCE (29%) during the median 15 months of observation. Baseline low miR-223 expression was a predictor of MACCE in univariate Cox regression analysis (hazard ratio [HR]: 2.71, 95% confidence interval [CI]: 1.04–7.01; $p = 0.041$). After inclusion of covariates, age, gender (male), New York Heart Association class and diabetes into the multivariate Cox regression model, miR-223 did not reach statistical significance (HR: 2.56, 95% CI: 0.79–8.33; $p = 0.118$).*

Conclusions: *To conclude, miR-223 might improve risk stratification after TAVI. Further studies are required to confirm the clinical applicability of this promising biomarker.* (Cardiol J 2024; 31, 1: 111–123)

Key words: aortic stenosis, transcatheter aortic valve implantation (TAVI), microRNA, prognosis

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Introduction

Aortic stenosis (AS) is the most prevalent primary valvular heart disease in Europe and North America, with increasing occurrence due to the ageing of the population. Transcatheter aortic valve implantation (TAVI) is an established treatment in patients at increased risk of surgery [1].

Although the outcomes after TAVI are improving, with the 5-year survival rate at 48% [2], 29% of patients annually experience major adverse cardiac and cerebrovascular events (MACCE) after TAVI [3]. Factors associated with MACCE include increased body mass index, reduced left ventricular ejection fraction (LVEF), carotid or peripheral artery disease, high aortic valve calcium score and SYNTAX score [4–6]. Nevertheless, none of these factors predict post-TAVI MACCE with clinically relevant sensitivity and specificity in an individual patient.

Turbulent blood flow in AS activates platelets and triggers a chronic pro-aggregatory state. It was demonstrated that activated platelets contribute to the progression of aortic valve calcification [7]. AS is known to activate platelets, TAVI might restore their function. On the other hand, the prosthetic valve and the intervention itself might aggravate the pro-aggregatory state, thus contributing to the development of MACCE after TAVI [8]. The effect of TAVI on platelet function has not been established.

MicroRNAs (miRNAs) have gained attention as potential novel biomarkers of platelet function. MicroRNAs are small, non-coding RNAs regulating posttranscriptional gene expression [9]. Platelets are a major source of circulating miRNAs [10, 11]. Since miR-125a-5p, miR-125b and miR-223 are associated with platelet function [11–14], acute myocardial infarction (AMI) [15] and stroke [16], these miRNAs may provide new biomarkers to predict MACCE after TAVI [10]. For example, miR-125a-5p regulates early stages of megakaryocyte development [17]. MiR-125b is involved in megakaryocytes maturation, proliferation and survival [18]. MiR-223, in turn, regulates gene expression in platelets and endothelium [19].

The effect of TAVI on miRNA expression was assessed in small groups of patients (n = 5–28), providing contradictory results [20–24]. The prognostic miRNA value, after TAVI has not been evaluated to date. Studies showed that several miRNAs including miR-223 and miR-125 are participating in the vascular system as they are highly expressed by endothelial cells [12, 25]. Moreover, studies also showed that their expressions can be altered by platelet activation due to antiplatelet therapies [10, 13, 26]. The present hypothesis was that TAVI modulates the expression of platelet-associated miRNAs, and that platelet-associated miRNAs may predict MACCE after TAVI. In a previous preliminary study, differences were found in the expression of miR-223 and miR-125 in patients with and without high on-treatment platelet reactivity [27]. The goal of this study was (i) to determine the effect of TAVI on the expression of miRNA associated with platelet function (miR-125a-5p, miR-125b and miR-223), and (ii) to evaluate the predictive value of these miRNAs for MACCE after TAVI.

Methods

Study design

This was a prospective study conducted at the 1st Chair and Department of Cardiology, Medical University of Warsaw, Poland in collaboration with the Vesicle Observation Center, Amsterdam University Medical Centers, the Netherlands. The study protocol, designed in compliance with the Declaration of Helsinki, was approved by the Ethics Committee of Medical University of Warsaw (approval number: KB/128/2018, KB/4/A2021).

Selection of participants

Patients diagnosed with severe AS and qualified for TAVI based on the Heart Team's decision were recruited. Severe AS was defined as aortic valve area (AVA) < 1.0 cm^2 or indexed AVA < 0.6 cm^2/m^2 as calculated by the continuity equation on transthoracic echocardiography (TTE). In patients with low-flow, low-gradient AS and reduced LVEF, dobutamine stress echocardiography was performed to differentiate between true severe AS and pseudo-severe AS, and in patients with low-flow, low-gradient AS and preserved LVEF, computed tomography was performed to assess aortic valve calcium score [1]. Exclusion criteria were transcatheter valve-in-valve implantation, chronic kidney disease (glomerular filtration rate < 30 mL/min), autoimmune diseases, active neoplastic disease, pregnancy, breast-feeding. All patients provided informed written consent.

Clinical data collection

The demographic and clinical data were collected during the index hospitalization.

A follow-up visit in the outpatient clinic was scheduled at 12 ± 3 months after TAVI, when control TTE was performed and data regarding

MACCE (all-cause death, cardiovascular death, myocardial infarction, stroke, transient ischemic attack [TIA], decompensation of heart failure, need for re-intervention) were recorded.

Treatment

Transcatheter aortic valve implantation was performed via femoral, subclavian, or carotid access by an interventional cardiologist (J.K., Z.H., B.R.) and a thoracic surgeon (R.W.) in a hybrid operating room. Pharmacotherapy after TAVI included dual antiplatelet therapy (acetylsalicylic acid [ASA] and clopidogrel) for 3–6 months, followed by lifelong ASA treatment in patients with no indication for oral anticoagulation (OAC), or OAC if required [1]. Other drugs were continued at the discretion of the treating physician, according to individual comorbidities.

Samples collection and handling

Blood samples were collected at two time points: 1 day before TAVI and 5–7 days following the procedure (at hospital discharge). Blood was collected in 7.5 mL ethylenediaminetetraacetic acid (EDTA) plastic tubes (S-Monovette, Sarstedt) via antecubital vein puncture using a 19-gauge needle, without tourniquet. The first 2 mL were discarded to avoid pre-activation of platelets. Within 15 minutes from blood collection, platelet-depleted plasma was prepared by double centrifugation (2500 g, 15 min, 20°C, acceleration speed 1, no brake). Supernatant was collected 10 mm above the buffy coat, re-centrifuged, mixed by pipetting, transferred to 1.5 mL low-protein binding Eppendorfs (Thermo Fisher Scientific, MA, USA), and stored in −80°C until analyzed.

RNA preparation and detection using quantitative PCR

The expression of miR-223, miR-125a-5p, miR-125b was evaluated in platelet-depleted plasma. Plasma RNA was extracted by miRVANA PARIS Kit. Total RNA was obtained as outlined above and diluted 1:10. Diluted RNA (5 μL) was reverse transcribed using the TaqMan miRNA Reverse Transcription kit (Applied Biosystems) according to the instructions of the manufacturer (Advanced miRNA assay, catalog number: A25576, Applied Biosystems). Subsequently, 3 μL of the product was used for detecting miRNA expression by quantitative polymerase chain reaction (PCR) using TaqMan miRNA Assay kits (Applied Biosystems) for the corresponding miRs on a The CFX384 Touch Real-Time PCR Detection System (BioRad Inc. Hercules, California, USA). Cel-miR-39 was spiked-in as an exogenous normalizer. Reactions were run in triplicate, and the mean value was used for all analyzes, to control variability associated with methodological reasons. MiRNA levels are expressed as 2-ΔCT [miRNA – cel-miR-39].

Endpoints

The primary end-point was the change in plasma expression of miR-223, miR-125a-5p and miR-125b before and after TAVI. The secondary endpoint was the predictive value of miR-223, miR-125a-5p and miR-125b for MACCE during the follow-up period.

Statistical analysis

Since there are no data regarding the differences in miR-223, miR-125a-5p and miR-125b before and after TAVI, power calculation for the primary end-point was based on the differences in miR-125a-5p and miR-125b expression in patients with calcified AS and healthy controls [28]. Patients with calcified AS had, on average, a 2-fold higher expression of miR-125a-5p and miR-125b, compared to controls. The required sample size was calculated by a 2-sided t-test at a significance level of 0.05 with the following assumptions: (i) mean difference between the groups = 1.0, (ii) standard deviation (SD) ± 2.0, and (iii) nominal test power = 0.8. It was estimated that a total of 64 patients should be enrolled in the study to observe a difference in miRNA expression before and after TAVI.

Statistical analysis was conducted using IBM SPSS Statistics, version 27.0 (IBM, New York, USA). MiRNA expressions data were log10-transformed for statistical analysis. Categorical variables were presented as number and percent and compared using χ^2 test. The Shapiro–Wilk test was used to assess normal distribution of continuous variables. Continuous variables were presented as mean and SD or median with interquartile range (IQR) and compared using an unpaired t-test or the Mann-Whitney U test. The predictive value of miRNAs for MACCE and the cut-offs were calculated using a receiver operating characteristic (ROC) curve. Logistic regression model incorporating miRNA expression and clinical characteristics were used to determine the best model for MACCE. A 2-sided p-value below 0.05 was considered significant.

Results

Figure 1 shows the study design and flow chart. Out of 135 patients who underwent TAVI be-

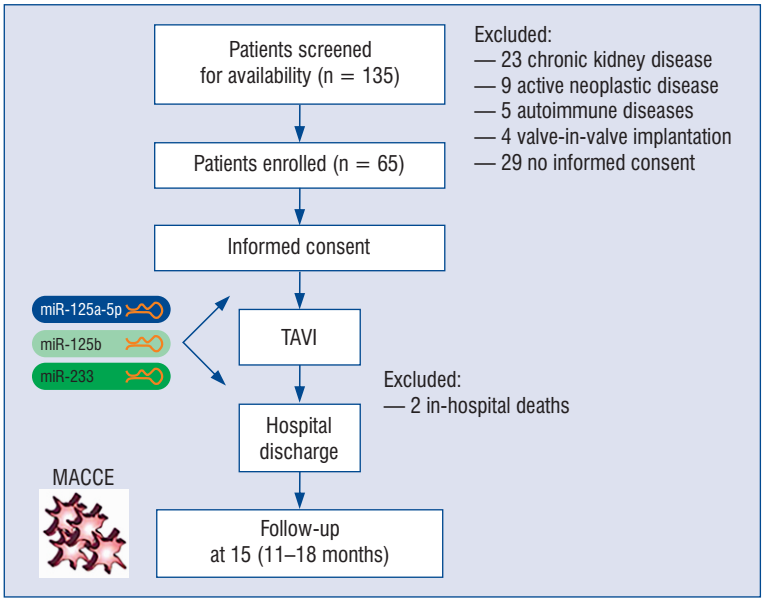

Figure 1. Study design and flow chart; MACCE — major adverse cardiac and cerebrovascular events; TAVI — transcatheter aortic valve implantation.

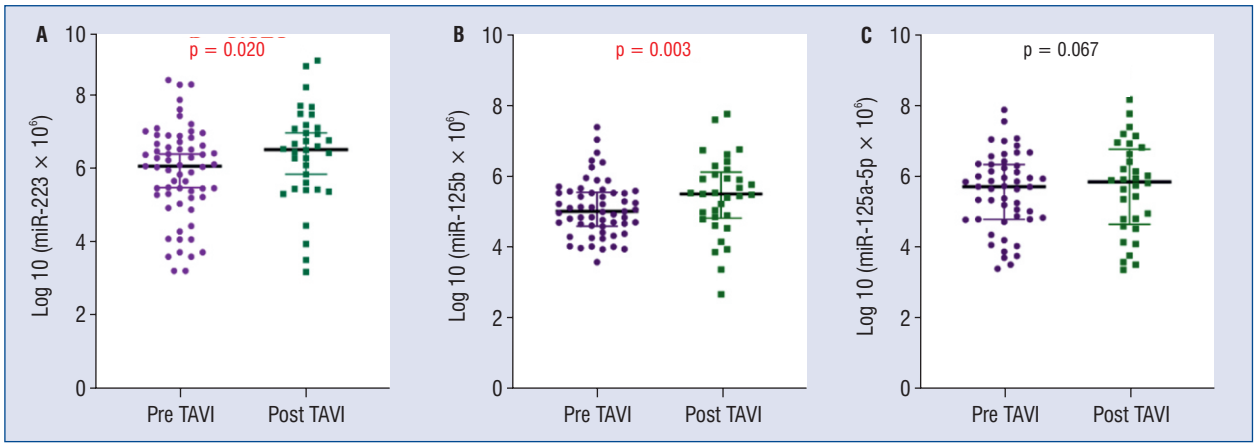

Figure 2. Comparison of plasma miRNAs expression before and after transcatheter aortic valve implantation (TAVI); **A.** miR-223; **B.** miR-125b; **C.** miR-125a-5p.

tween November 2018 and June 2020, 65 patients were enrolled in the study and 63 patients attended the follow-up. The median time to follow-up was 15 months (IQR 11–18 months).

Expression of miRNAs before and after TAVI

The expression of miR-223 and miR-125b increased after TAVI, compared to the measurement before ($p = 0.020$, $p = 0.003$, respectively; Fig. 2). There was a trend towards the increased expression of miR-125a-5p after TAVI ($p = 0.067$)

Expression of miRNAs after TAVI according to the antiplatelet and OAC treatment

Compared to the pre-TAVI measurement, expressions of miR-223 and miR-125a-5p increased after TAVI in patients taking P2Y12 inhibitors ($p = 0.045$, $p = 0.006$, respectively) (Fig. 3A, C). Concentration of all miRNAs decreased after TAVI in patients taking OACs ($p = 0.014$, $p = 0.047$,

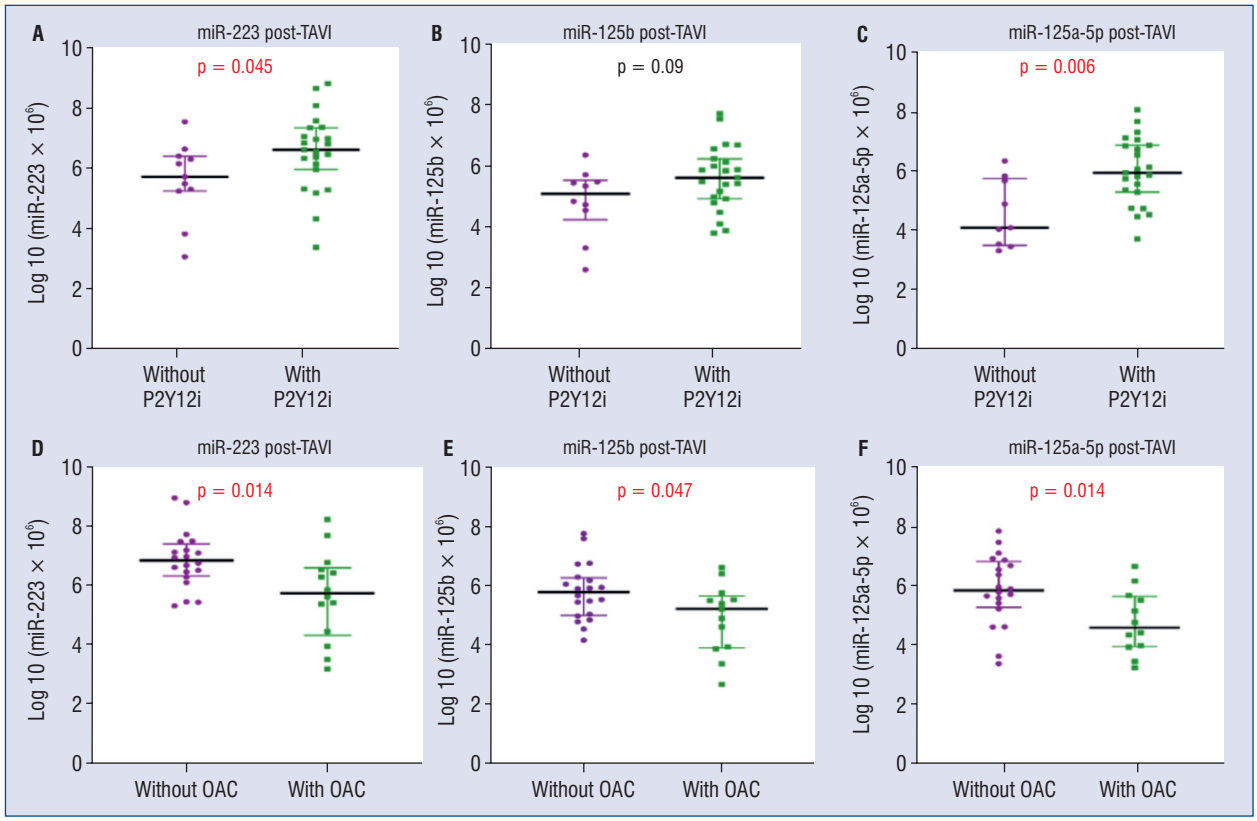

Figure 3. Post-transcatheter aortic valve implantation (TAVI) miRNAs expressions in regard to antiplatelet treatment and oral anticoagulant (OAC); **A.** miR-223 regarding P2Y12 inhibitors (P2Y12i); **B.** miR-125b regarding P2Y12i; **C.** miR--125a-5p regarding P2Y12i; **D.** miR-223 regarding OAC; **E.** miR-125b regarding OAC; **F.** miR-125a-5p regarding OAC.

p = 0.014, respectively; Fig. 3D–F). There was no significant difference between miRNA expression with or without ASA. Patients initially treated with antiplatelet drugs and OAC had comparable miRNAs expressions (data not shown).

Decreased baseline expression of miR-223 is associated with adverse outcomes

There were 2 in-hospital deaths. Among 63 discharged patients, 18 (29%) patients experienced MACCE: 2 (3.2%) all-cause deaths, 7 (11.1%) cardiovascular deaths, 2 (3.2%) TIA, 6 (9.5%) readmissions due to decompensated heart failure and 1 (1.6%) need for valve re-intervention. There were no AMI or strokes.

Patient characteristics are presented in Table 1. Patients who experienced MACCE were older (median age 84.0 vs. 81.0 years, p = 0.060) and were more frequently male (72.2% vs. 33.3%, p = 0.005). There were no other differences between the groups.

The procedural characteristics and device success rate were comparable in both groups (94.4% vs. 100.0%, p = 0.111). The incidence of procedural complications (life-threatening or disabling bleeding, major vascular complication, new permanent pacemaker implantation) were similar in both groups.

At follow-up, the mean LVEF and mean aortic valve gradient were comparable in both groups (60% vs. 50.5%, p = 0.075 and 8.5 mmHg vs. 8.7 mmHg, p = 0.804, respectively). No significant correlations between the studied miRNAs expressions and pressure gradient via the aortic valve were observed (data not shown).

The baseline miR-223 expression was lower in patients who experienced MACCE, compared to those who did not (p = 0.006; Fig. 4A) and discriminated between these two groups of patients (area under ROC curve [AUC] = 0.72, p = 0.006; Fig. 4B). MiR-125b and miR-125a-5p expression was comparable between patients with and without MACCE (p = 0.109, p = 0.118, respectively; Fig. 4C, 3F).

Table 2 shows the statistical estimates for the prediction of MACCE by baseline miR-223

Table 1. Comparison of baseline characteristics between patients who experienced MACCE and those who did not during a median follow-up of 15 months.

	Total populations (n = 63)	No MACCE (n = 45)	MACCE (n = 18)	P
Baseline characteristics				
Age [years]	81.0 (77.5–84.0)	81.0 (77.0–83.0)	84.0 (80.0–85.0)	0.060
Gender, male	28 (44.4%)	15 (33.3%)	13 (72.2%)	**0.005**
BMI [kg/m^2]	27.3 ± 3.8	27.5 ± 4.2	26.9 ± 3.1	0.661
Co-morbidities				
Hypertension	49 (81.7%)	34 (79.1%)	15 (88.2%)	0.408
Diabetes mellitus	22 (36.7%)	16 (37.2%)	6 (35.3%)	0.890
Atrial fibrillation	16 (26.7%)	10 (23.3%)	6 (35,3%)	0.342
Prior stroke/TIA	9 (15. 0%)	7 (16.3%)	2 (11.8%)	0.659
Prior myocardial infarction	13 (21.7%)	9 (20.9%)	4 (23.5%)	0.826
Prior PCI	27 (45.0%)	18 (41.9%)	9 (52.9%)	0.437
Prior CABG	3 (5.0%)	2 (4.7%)	1 (5.9%)	0.844
COPD	8 (13.3%)	5 (11.6%)	3 (17.7%)	0.537
Heart failure (NYHA III/IV)	15 (27.3%)	10 (25.6%)	5 (51.3%)	0.908
EuroSCORE II [%]	4.2 (3.3–5.4)	4.2 (3.2–5.3)	4.5 (3.7–5.8)	0.447
CKD > 3a	11 (18.3%)	6 (14.0%)	5 (29.4%)	0.163
Laboratory data				
Hemoglobin [g/d]	11.8 ± 2.0	11.9 ± 2.1	11.6 ± 1.4	0.512
Creatinine [mg/dL]	1.3 (1.0–1.6)	1.3 (1.0–1.6)	1.3 (1.0–1.5)	0.948
Estimated GFR [mL/min/1.73 m^2]	45.5 (36–57.7)	45 (35–57)	47 (43–61)	0.583
NT-proBNP	1811 (508–3901)	1777 (394.5–3804)	2670.5 (1584–9080)	0.199
Echocardiography before TAVI				
Ejection fraction [%]	57 (46–63)	57 (46–63)	55 (42–63)	0.868
V max [m/s]	4.1 (3.5–4.5)	4.1 (3.6–4.4)	4.1 (2.1–4.6)	0.589
Gradient max	68 (42.5–80.5)	70 (47–80)	44 (31–83)	0.486
Gradient mean	43 (31–51)	43 (31.5–51.5)	43 (15–51)	0.604
AVA (VTI)	0.7 (0.6–0.9)	0.7 (0.6–0.9)	0.9 (0.6–0.9)	0.309
AVAi	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.7 (0.4–1.0)	0.053
Low-flow, low-gradient AS	15 (23.8%)	12 (26.7%)	3 (16.7%)	0.400
Procedural characteristics				
Access site:				0.676
Transfemoral	55 (91.7%)	41 (95.4%)	14 (82.4%)	0.787
Subclavian	3 (5%)	2 (4.4%)	1 (6.7%)	0.732
Carotid	2 (3,3%)	2 (4.4%)	0 (0%)	0.378
Prosthesis size [mm]:				0.907
23	1 (1.7%)	1 (2.4%)	0 (0%)	0.521
25	13 (22%)	9 (21.4%)	4 (23.5%)	0.860
26	2 (3.4%)	2 (4.8%)	0 (0%)	0.360
27	14 (23.7%)	10 (23.8%)	4 (23.5%)	0.982
29	18 (30.5%)	13 (31%)	5 (29.4%)	0.806
31	0 (0%)	0 (0%)	0 (0%)	1.000
34	11 (18.6%)	7 (16.7%)	4 (23.5%)	0.610
Valve type:				0.672
EvolutR	22 (37.3%)	17 (40.5%)	5 (29.4%)	0.350
EvolutPRO	5 (8.5%)	3 (7.1%)	2 (11.8%)	0.610
Portico	32 (54.2%)	22 (52.4%)	10 (58.8%)	0.821

→

Table 1 (cont.). Comparison of baseline characteristics between patients who experienced MACCE and those who did not during a median follow-up of 15 months.

	Total populations (n = 63)	No MACCE (n = 45)	MACCE (n = 18)	P
Device success	62 (98.4%)	45 (100%)	17 (94.4%)	0.111
Procedure complications				
Life-threatening or disabling bleeding*	1 (1.6%)	1 (2.2%)	0 (0%)	0.524
Major vascular complication*	5 (7.9%)	4 (8.8%)	1 (5.5%)	0.658
Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
New pacemaker	7 (11.1%)	5 (11.1%)	2 (11.1%)	1.000
Echocardiography at follow-up				
Ejection fraction [%]	55 (46–65)	60 (46–65)	50.5 (40–55)	0.075
Peak AV gradient [mmHg]	17.2 ± 5.4	17 ± 5.2	17.5 ± 5.9	0.848
Mean AV gradient [mmHg]	8.5 ± 2.7	8.5 ± 2.8	8.7 ± 2.5	0.804
Paravalvular leak type 3 or 4	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Post-TAVI procedure concomitant medications				
Beta-blockers	51 (81%)	37 (82%)	14 (78%)	0.950
ACE inhibitors	40 (64%)	27 (60%)	13 (72%)	0.177
MRA	17 (27%)	12 (27%)	5 (28%)	0.883
Diuretics	53 (84%)	27 (82%)	16 (89%)	0.115
Statins	49 (78%)	36 (80%)	13 (72%)	0.675
Proton pump inhibitors	68 (74%)	30 (67%)	13 (72%)	0.445
Antidiabetic drugs	22 (35%)	16 (37%)	6 (35%)	0.890
Acetylsalicylic acid	47 (75%)	37 (82%)	10 (56%)	0.046
P2Y12 inhibitor	44 (70%)	35 (78%)	9 (50%)	0.031
Anticoagulant	22 (35%)	14 (31%)	8 (44%)	0.242
miRNAs relative expressions:				
miR-223 pre-TAVI	1.14 (0.16–5.22)	1.64 (0.29–7.96)	0.20 (0.005–2.06)	0.006
miR-223 post-TAVI	3.92 (0.82–30.15)	4.26 (0.61–14.37)	1.27 (0.007–15.79)	0.196
miR-125b pre-TAVI	0.08 (0.29–0.15)	0.12 (0.05–0.46)	0.05 (0.02–0.19)	0.109
miR-125b post-TAVI	0.34 (0.07–2.40)	0.30 (0.07–0.99)	0.32 (0.01–4.71)	1.000
miR-125a-5p pre-TAVI	0.20 (0.06–0.73)	0.44 (0.06–2.98)	0.30 (0.01–0.68)	0.118
miR-125a-5p post-TAVI	0.59 (0.05–7.09)	0.30 (0.07–0.99)	0.32 (0.01–4.71)	0.654

*According to the Valve Academic Research Consortium (VARC). Bold p value indicates significantly different (< 0.05). Data are shown as number (percentage), median (interquartile range), mean ± standard deviation; ACE — angiotensin-converting enzyme; AS — aortic stenosis; AV — atrioventricular; AVA — aortic valve area; AVAi — aortic valve area index; BMI — body mass index; CABG — coronary artery bypass graft surgery; COPD — chronic obstructive pulmonary disease; CKD — chronic kidney disease; GFR — glomerular filtration rate; MACCE — major adverse cardiac and cerebrovascular events; MRA — mineralocorticoid receptor antagonists; NT-proBNP — N-terminal pro-B-type natriuretic peptide; NYHA — New York Heart Association; PCI — percutaneous coronary intervention; TAVI — transcatheter aortic valve implantation; TIA — transient ischemic attack; VTI — velocity time integral

expression below the cut-off value, determined based on the ROC curve. MiR-223 at admission predicted MACCE with 78% sensitivity and 61% specificity. MiR-223 expression levels after TAVI procedure were not predictive of MACCE (data not shown).

To check whether the baseline expression of miR-223 is an independent predictor of MACCE, the baseline expression of miR-223 was incorporated in the univariate Cox regression analysis. Low baseline expression of miR-223 was associated with MACCE in univariate analysis (HR: 2.71, 95% CI: 1.04–7.01; p = 0.041). However, after including the covariates (age, gender [male], New York Heart Association [NYHA] class and diabetes ([4–6]) into the multivariate Cox regression model, miR-223 did not reach statistical significance [HR: 2.56, 95% CI: 0.79–8.33; p = 0.118; Table 3).

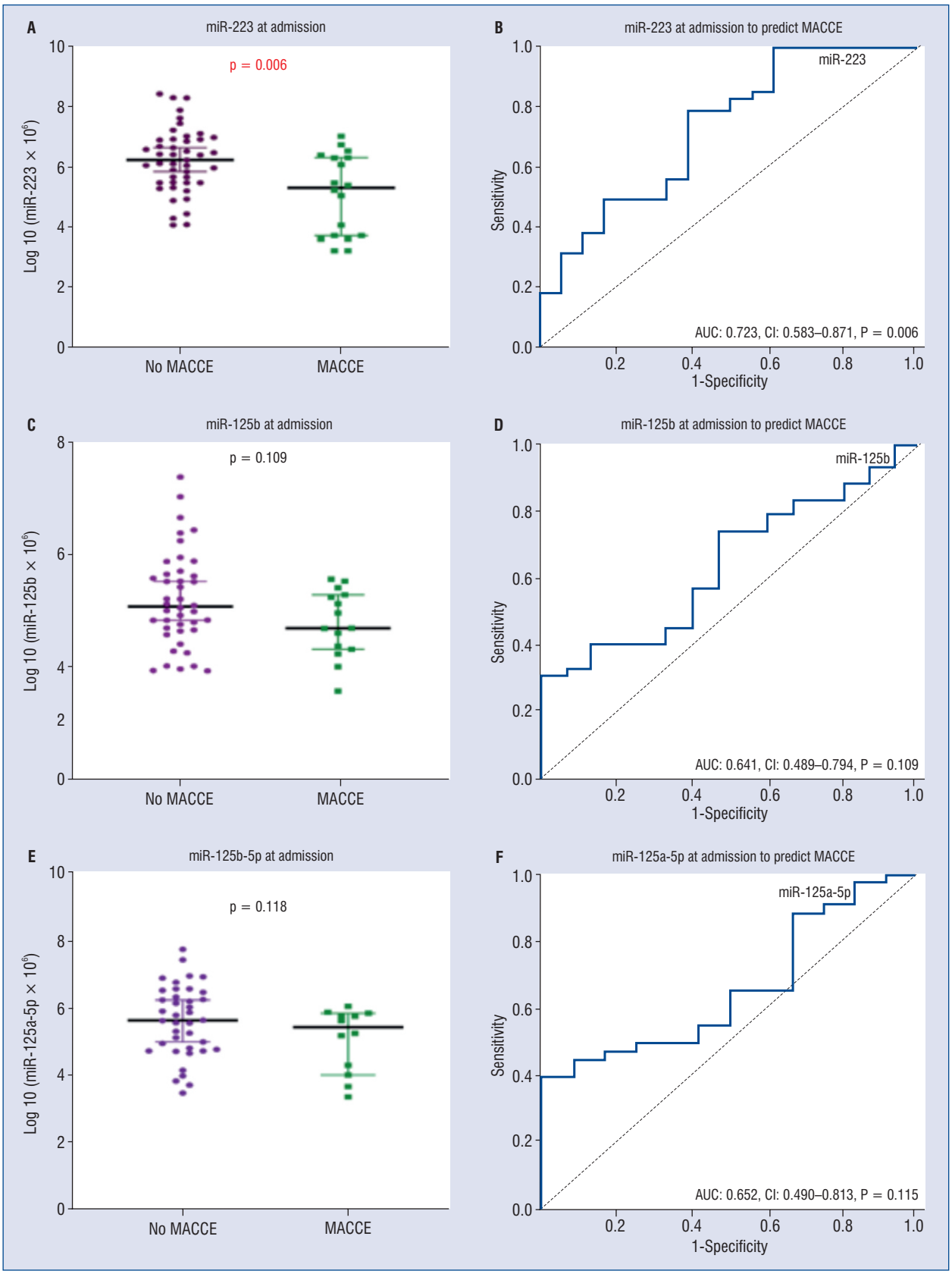

Figure 4. Expressions of baseline miRNAs (miR) in plasma of patients at admission before transcatheter aortic valve implantation in patients with and without major adverse cardiac and cerebrovascular events (MACCE) during a median follow-up of 15 months; **A, B.** (ROC-curve): MiR-223; **C, D.** (ROC-curve): MiR-125b; **E, F.** (ROC-curve): MiR-125a-5p; AUC — area under the curve; CI — confidence interval; ROC — receiver operating characteristic.

Table 2. Statistical estimates for prediction of major adverse cardiac and cerebrovascular events by baseline miR-223 expression.

miRNA	AUC (95% CI)	P	Cut-off	Sensitivity	Specificity	PPV	NPV	PLR
Baseline miR-223	0.72 (0.58–0.87)	0.006	0.285	78%	61%	83%	53%	2

AUC — area under the curve; CI — confidence interval; PPV — positive predictive value; NPV — negative predictive value; PLR — positive likelihood ratio

Table 3. Univariate and multivariate Cox regression analysis for prediction of major adverse cardiac and cerebrovascular events by low baseline miR-223 expression.

miRNA	Cox regression	Hazard ratio	95% confidence interval		P
			Lower	Upper	
Low baseline miR-223 expression	Univariate	2.713	1.043	7.054	0.041
	Multivariate*	2.560	0.787	8.329	0.118

*After adjustment for age, gender (male), NYHA class, and diabetes. Bold p value indicates significantly different (< 0.05).

Figure 5 shows the Kaplan-Meier analysis of event-free survival for MACCE in patients after TAVI stratified according to miR-223 baseline plasma expression, with low expression defined as expression below the established cut-off (7 out of 18 patients who experienced MACCE), based on the ROC curve analysis. Patients with low miR-223 expression at admission had a lower chance of event-free survival during follow-up, compared to patients with high miR-223 expression (p = 0.033 for the log-rank test).

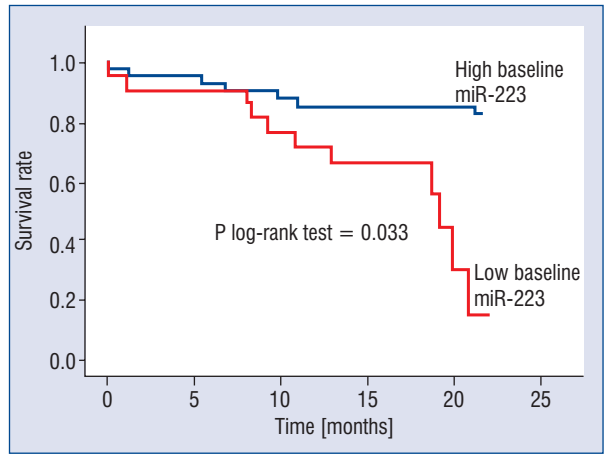

Figure 5. The Kaplan-Meier survival analysis for major adverse cardiac and cerebrovascular events (MACCE) after transcatheter aortic valve implantation in patients with high or low miR-223 at admission.

Discussion

The main findings of the present study are: (i) the expression of miR-223 and miR-125b increased after TAVI, compared to the baseline, and (ii) low baseline expression of miR-223 was a predictor of MACCE in univariate Cox regression analysis.

High shear stress in AS, is associated with endothelial dysfunction and increased platelet activation [29]. AS is associated with distinct miRNAs expression changes [30]. The resolution of high shear stress through TAVI was shown to have an anti-inflammatory effect, which was proposed to be a novel therapeutic benefit of TAVI [31].

The effect of TAVI on miRNA expression was evaluated by other authors providing contradictory results [1, 21–24]. The current study provides evidence that the expression of miR-223, miR-125b and potentially miR-125a-5p changes after TAVI, suggests their involvement in the adaptation to altered hemodynamic conditions after TAVI.

MiR-125a-5p plays a role in atherosclerosis [32] and stroke [33]. In the present study, there was only a trend towards increased expressions of miR-125a-5p after TAVI, and it did not predict MACCE. However, the lack of statistical significance might be due to the small sample size and no strokes during the observation period.

The influence of miR-125b on the cardiovascular system remains conflicting. Some authors showed deleterious effects miR-125b, including intravascular calcification [34], hypoxia-induced cardiomyocyte injury [35], among others [16,

36, 37]. Other authors reported beneficial effects of miR-125b, including protection against ischemia-reperfusion injury [37, 38] or alleviation of infarction-induced cardiomyocytes apoptosis [39]. Therefore, it is difficult to determine whether the increased expression of miR-125b after TAVI, found in the present study and by other authors [40], is a response to the procedure, or a part of the anti-inflammatory effect.

MiR-223 — one of the most abundant miRNAs in platelets — has been investigated in platelet function and thrombotic events [41]. In a murine model, elevated expression of miR-223 were observed in thrombin-activated platelets, suggesting that miR-223 might reflect platelet activation [42]. In cultured endothelial cells, miR-223 decreased tissue factor expression and procoagulant activity, implying its potential protective function following endothelial injury [43]. Decreased miR-223 was an independent predictor of MACCE in coronary artery disease patients receiving antiplatelet treatment [41]. However, the association between miR-223 and cardiovascular disease (CVD) remains complex. Downregulation of miR-223 in CVD patients was previously demonstrated, suggesting miR-223 might have a protective role in CVD [44]. Anti-inflammatory ability of miR-223 against various diseases has been published in literature. Wang et al. [45] showed exosomal miR-223 plays a role in cardio-protection through down-regulation of *Sema3A* and *Stat3* genes, which are direct targets of miR-223-5p and -3p. Moreover, during cerebral ischemia, cysteinyl leukotrienes are largely secreted and their receptors are also increased in activated microglia, previous studies showed that miR-223-3p may exert anti-inflammatory effect through inhibiting cysteinyl leukotrienes receptors [46, 47]. Besides the cerebrovascular protection properties, studies also aimed to analyze the molecular mechanism of miR-223 in myocardial infarction. MiR-223-3p mimics showed decreased myocarditis and apoptosis after myocardial infarction and improved cardiac function by targeted inhibition of *FBXW7* expression in in vitro analysis [48]. It was also suggested that miR-223 may serve as a potential target in AMI treatment in an animal study [49]. Furthermore, in a large cohort of coronary artery disease patients, upregulated miR-223 expression was a predictor of cardiovascular death [15]. On the other hand, a contradictory study reported an increase in miR-223 expression in patients with AMI and stroke [50]. Hence, the increase in miR-223 expression could either be a protective mechanism against an acute cardiovascular event in these patients, or contribute to cardiovascular injury. In the current study, low baseline expression of miR-223 was a predictor of MACCE in univariate analysis, supporting the protective effect of this miRNA on the cardiovascular system. However, the direct link between the decreased expression of miR-223 and adverse outcomes requires further investigation.

Lower miR-223, miR-125a and miR-125b expressions were also found in patients treated with OAC therapy after TAVI, and higher levels of miR-125a and miR-125b in patients taking P2Y12 inhibitors. Recent meta-analysis demonstrated that miR-223 is increased in patients with atrial fibrillation treated with OAC [51]. It was also shown that miR-223-3p is an independent predictor of thrombotic events and can be used for ischemic risk stratification after AMI [52]. Similarly, elevated miR-125a-5p, miR-125b-5p were early biomarkers in ischemic stroke [53]. The present observations may indicate that in some patients after TAVI, therapy with OAC might be more beneficial than single antiplatelet therapy (SAPT) or dual antiplatelet therapy (DAPT), which is in line with our recent meta-analysis showing that the use of OAC after TAVI is associated with a lower risk for subclinical leaflet thrombosis, compared with SAPT and DAPT [54].

Limitations of the study

The main limitation of this study is the small sample size, making the study underpowered to detect the predictive value of the investigated miRNAs for the individual components of the composite end-point. Second, since one miRNA can bind to various mRNAs and can be regulated by other miRNAs [55], the differences in miRNA expression before and after TAVI, and in patients with and without MACCE do not allow drawing conclusions regarding the causal association between the investigated miRNA and the development of adverse events. Moreover, the present study did not have blood collection and the miRNAs analysis right after, and a few months after the TAVI procedure in the population, which also limited monitoring of miRNAs expression levels. Third, given the hypothesis-generating study design, the analysis was limited to miRNA associated with platelet function, based on a preliminary analysis [27]. MiRNA sequencing might enable determining novel miRNAs with higher predictive value for post-TAVI MACCE. Fourth, since platelet reactivity in the study was not evaluated, we cannot exclude that the inverse relationship between

miR-223 expression and post-TAVI MACCE is related with poor response to DAPT in the patients [56]. Fifth, miR-223 is also related to systemic endothelial damage and platelet status-related biomarkers, not measured in this study [57]. Finally, all TAVI procedures were done by the same team, which eliminated the bias due to various expertise levels, but also limited the general results applicability. Altogether, results herein, should be confirmed in a larger, multi-center study before miRNAs can be used in risk stratification after TAVI in clinical practice.

Conclusions

Expression of miR-223 and miR-125b increased after TAVI, compared to the measurement before TAVI. Baseline low expressions of miR-223 is a promising marker of increased risk of MACCE after TAVI. Because the present study is limited by a small sample size, a composite end-point and lack of statistical significance in multivariate analysis, the results should be interpreted with caution.

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