



# Correlations between the Circulating Level of Cell-Derived Microparticles and Surgical Variables in Heart Valve Surgery with Cardiopulmonary Bypass

Mehrnaz Abdolalian, MS<sup>1</sup>, Elham Khalaf-Adeli, PhD<sup>1\*</sup>, Fatemeh Yari, PhD<sup>1</sup>, Saeid Hosseini, MD<sup>2</sup>, Hooman Bakhshandeh, MD<sup>3</sup>

<sup>1</sup>Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

<sup>2</sup>Heart Valve Disease Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.

<sup>3</sup>Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.

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## Abstract

**Background:** Cell-derived microparticles (MPs) as membrane vesicles are procoagulant. They play a role in surgical hemostasis. In this study, the correlations between the circulating level of cell-derived MPs and surgical variables in heart valve surgery were investigated.

**Methods:** The present prospective case-series study was conducted in Rajaie Cardiovascular Medical and Research Center from January through March 2021. Forty patients undergoing heart valve surgery with cardiopulmonary bypass (CPB) were enrolled. Before the induction of anesthesia and 30 minutes after the administration of protamine sulfate, venous blood samples were collected. After MP isolation, the concentration of MPs was determined via the Bradford method. Flow cytometry analysis was performed to determine the MP count and phenotype. Intraoperative variables and postoperative routine coagulation tests were defined as surgical variables. Postoperative coagulopathy was defined as an activated partial thromboplastin time (aPTT)  $\geq 48$  seconds or an international normalized ratio (INR)  $> 1.5$ .

**Results:** The total concentration of MPs and the MP count increased significantly after surgery compared with before surgery. The postoperative concentration of MPs was positively correlated with the CPB time ( $P=0.030$ ,  $\rho=0.40$ ). The preoperative concentration of MPs was significantly lower in patients with higher postoperative aPTT and INR ( $P=0.003$ ,  $P=-0.50$  and  $P=0.020$ ,  $P=-0.40$ , respectively). In multivariate logistic regression analysis, the preoperative MP concentration (OR, 1.00; 95% CI, 1.00 to 1.01;  $P=0.017$ ) was considered a risk factor for postoperative coagulopathy.

**Conclusion:** The levels of MPs, especially platelet-derived MPs, rose after surgery, in correlation with the CPB time. Given the role of MPs in the induction of coagulation and inflammation, they can be considered therapeutic goals for preventing postoperative complications. In addition, the preoperative levels of MPs are a risk factor for predicting the occurrence of postoperative coagulopathy in heart valve surgery.

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**Keywords:** Cell-derived microparticles; Heart valve disease; Cardiac surgical procedure

\*Corresponding Author: Elham Khalaf-Adeli, Assistant Professor of Laboratory Hematology and Blood Banking, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, IBTO HQ, Adjacent to Milad Tower, Hemmat Expressway, Tehran, Iran. 1449613111. Tel: +98 21 88601546. Fax: +98 21 88601545. E-mail: adeli.elham@gmail.com.





## Introduction

Microparticles (MPs) are small cellular vesicles originating from the plasma membrane of an extensive range of cells, such as platelets, endothelial cells, white blood cells, and red blood cells, through cell activation or apoptosis.<sup>1</sup> MPs (diameter = 0.1–1  $\mu\text{m}^2$ ) are procoagulant due to the expression of phosphatidylserine and tissue factor and promote the coagulation cascade.<sup>2-4</sup> The presence of MPs in blood circulation is related to thrombotic events, oxidative stress, hemodynamic instability, and inflammatory response induction.<sup>5</sup> An increased level of MPs in the blood circulation has been observed in various pathological conditions, such as cardiovascular diseases, sickle-cell anemia, malaria infection, and graft-versus-host disease.<sup>5-7</sup>

Considering the physiological function of MPs, they can serve as a biomarker in diagnosing, progressing, and predicting diseases and their clinical complications like cardiovascular diseases.<sup>8,9</sup> Some studies have suggested the MP concentration in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) as a new biomarker for anticipating complications, such as acute heart failure and acute kidney injury.<sup>10,11</sup>

Valvular heart diseases are known as major heart problems worldwide. Valve repair and replacement constitute 2 therapeutic methods used with CPB for treating valvular heart diseases as single or multiple valve surgeries.<sup>5</sup> The platelet count, the activated partial thromboplastin time (aPTT), the prothrombin time (PT), and the international normalized ratio (INR) are routinely utilized to monitor postoperative homeostasis among individuals undergoing valve surgery.<sup>12</sup> Some researchers have reported the association of the low plasma levels of MPs in trauma patients with lower thrombin generation.<sup>13</sup> Based on the results of *in vitro* studies, the existence of procoagulant MPs affects thrombin generation significantly, and an enhancement in MP formation during blood bag storage reduces the clotting time.<sup>14,15</sup>

In the present study, we assessed the relationship between the plasma MP concentration and surgical variables, including perioperative variables and coagulation tests, among patients undergoing heart valve surgery with CPB.

## Methods

The present prospective case-series study was conducted among 40 adult candidates for heart valve surgery in Rajaie Cardiovascular Medical and Research Center between January and March 2021. The study protocol was approved by the Research Ethics Committees of the High Institute for Education and Research in Transfusion Medicine (IR.TMI.REC.1398.026), and written informed consent was obtained from each patient. The inclusion criteria consisted of Plavix and warfarin discontinuation 5 and 3 days before surgery,

respectively. Individuals with emergency surgery, heart valve surgery combined with other operations, and bleeding disorders were excluded.

Venous blood samples (6 mL) were taken and transferred into the sodium citrate anticoagulant prior to (before inducing anesthesia) and after surgery (30 minutes after neutralizing the effect of heparin by administering protamine sulfate). Clinical characteristics and laboratory results were obtained from the clinical record of the subjects.

The surgical variables considered in this study included the CPB time, the aortic cross-clamp time, the operation time, and the valve surgery type as perioperative variables and aPTT and INR as routine coagulation tests. Furthermore, aPTT  $\geq 48$  s or INR  $\geq 1.5$  was defined as the laboratory criterion for coagulopathy.

MP-rich plasma was obtained by centrifuging the blood samples at 1200g twice and then collecting the supernatant. Afterward, the resultant supernatant was centrifuged at 16000g for 10 minutes, and its MP-containing deposition was extracted, washed using phosphate buffer saline (PBS), and centrifuged at 16000g for 10 minutes. The washing step was repeated 3 times, followed by the dissolution of the final deposition in 50  $\mu\text{L}$  of PBS. The MP concentration was measured in the derived deposition via the Bradford method in comparison with the pre-prepared standard curve.<sup>16</sup>

The number of the extracted MPs was counted using the yellow-green microbead (1.0  $\mu\text{m}$ , Polysciences, Germany, concentration:  $4.55 \times 10^{10}$  particle/ $\mu\text{L}$ ). Then, their final number in each microliter was determined using flow cytometry analysis in proportion to the microbead count. Briefly, 5  $\mu\text{L}$  of the microbead suspension (diluted 1/500 with PBS) was added into the MP-containing samples. After the flow cytometry, the absolute number of MPs in each microliter (count/ $\mu\text{L}$ ) was calculated.

The phenotypes of platelet-derived MPs, monocyte-derived MPs, and red cell-derived MPs were respectively identified using the monoclonal antibodies of CD41-FITC (Dako, Denmark, IgG1  $\kappa$ , 2  $\mu\text{L}$ ), CD14-PE (BD PharMingen, USA, IgG2a  $\kappa$ , 5  $\mu\text{L}$ ), and CD235a-PE (BD PharMingen, USA, IgG2b  $\kappa$ , 3  $\mu\text{L}$ ). In this regard, 30  $\mu\text{L}$  of plasma was incubated with the abovementioned antibodies for 35 minutes at 4 °C in the dark. The stained suspension was immediately analyzed using a flow cytometer (Partec CyFlow).

The fitness of interval variables to normal distribution was assessed via the 1-sample Kolmogorov–Smirnov test. The data were described as the median (the interquartile range [IQR<sub>25-75%</sub>]) for interval variables and frequencies (percentages) for categorical variables.

The Wilcoxon signed-rank test was applied to compare pre and postoperative MP levels. In addition, the correlation between the interval variables was assessed using the Spearman correlation coefficient ( $\rho$ ). Crude and adjusted associations between the existence of coagulopathy and MP levels were investigated using logistic regression models.

A P value <0.050 was considered statistically significant. The data were analyzed with IBM SPSS Statistics 22 for Windows (IBM Inc, Armonk, NY).

## Results

Table 1 summarizes the patients' characteristics at the beginning of the study and preoperative variables. Valve repair and valve replacement were performed on 12 (30.0%) and 28 (70.0%) patients, respectively.

The median values concerning pre and postoperative MP concentrations and cell-derived MP enumeration (count/ $\mu\text{L}$ ) are presented in Table 2, which shows that the postoperative concentration was 4 times the preoperative concentration.

Further, a significant increase was observed in the count of CD41<sup>+</sup> platelet-derived MPs, CD14<sup>+</sup> monocyte-derived MPs, and CD235a<sup>+</sup> red cell-derived MPs after surgery (P<0.001, P=0.002, and P=0.003, respectively). Among the studied phenotypes, the highest enhancement was 3.5-fold for platelet-derived MPs (P<0.001).

Despite the lack of a significant relationship between the postoperative MP concentration and the operation time, the concentration was significantly and positively correlated with the CPB time (P=0.030, P=0.40). Furthermore, no significant correlation was found with the aortic cross-clamp time.

Additionally, the postoperative red cell-derived MP count in subjects undergoing valve repair and multiple valve surgery was higher than that in subjects with valve

Table 1. Preoperative demographic, clinical, and lab characteristics of the study population

Characteristics	Total (n=40) Median (IQR <sub>25-75%</sub> )	n (%)
Age (y)	54.00 (40.20-62.00)	
Sex (%)		
Male		14 (35.0)
Female		26 (65.0)
Type of Valve		
Single-valve		27 (67.5)
Multiple-valve		13 (32.5)
BMI (kg/m <sup>2</sup> )	26.90 (22.60-29.60)	
BSA (m <sup>2</sup> )	1.75 (1.68-1.86)	
Hb (g/dL)	13.20 (12.10-13.90)	
HCT (%)	38.60 (37.20-41.20)	
RBC ( $\times 10^6/\text{mm}^3$ )	4.60 (4.30-5.00)	
WBC (cells/ $\text{mm}^3$ )	7500.00(6400.00-8600.00)	
PLT( $\times 10^3/\text{mm}^3$ )	215.00 (169.00-269.00)	
PT(s)	13.70 (12.50-14.50)	
aPTT(s)	32.00 (29.60-35.70)	
INR	1.12 (1.04-1.21)	
Cr (mg/dL)	0.90 (0.80-1.10)	22.5 (9)
EF (%)	55.00(46.00-55.00)	35.0 (14)
Diabetes (%)		
Hypertension (%)		

BMI, Body mass index; BSA, Body surface area; Hb, Hemoglobin; HCT, Hematocrit; RBC, Red blood cell; WBC, White blood cell; PLT, Platelet; PT, Prothrombin time; aPTT, Activated partial thromboplastin time; INR, International normalized ratio; Cr, Creatinine; EF, Ejection fraction

Interquartile range, IQR<sub>25-75%</sub>

Table 2. Wilcoxon analysis for the comparison of pre and postoperative microparticle levels

Characteristics	Before Median (IQR <sub>25-75%</sub> )	After Median (IQR <sub>25-75%</sub> )	P
Concentration ( $\mu\text{g}/\text{mL}$ )	54.30 (37.00-204.00)	218.00 (60.20-305.00)	<0.001
CD41 <sup>+</sup> PMP (ct/ $\mu\text{L}$ )	4529.00(2197.00-9047.00)	15893.00 (8208.00-51042.00)	<0.001
CD14 <sup>+</sup> MMP (ct/ $\mu\text{L}$ )	3701.00 (561.00-6020.00)	5446.00 (2356.00-8451.00)	0.002
CD235 <sup>+</sup> RMP (ct/ $\mu\text{L}$ )	33488.00 (19929.00-46017.00)	52096.00 (36524.00-82452.00)	0.003

CD, Cluster of differentiation; PMP, Platelet-derived microparticle; MMP, Monocyte-derived microparticle; RMP, Red cell-derived microparticle

Interquartile range, IQR<sub>25-75%</sub>



Table 3. Univariate and multivariate analyses of risk factors for postoperative coagulopathy

	Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P
Pre-con of MPs	1.00 (1.00-1.01)	0.014	1.00 (1.00-1.01)	0.017
CPB time	0.99 (0.97-1.00)	0.320		
Aortic cross-clamp time	0.97 (0.95-1.00)	0.130		
Operation time	0.99 (0.98-1.01)	0.720		

Pre, Preoperative; Con, Concentration; MPs, Microparticles; CPB, Cardiopulmonary bypass

replacement ( $P=0.020$ ) and single valve surgery ( $P=0.010$ ).

The Spearman rank correlation analysis was utilized to examine the relationship between the preoperative MP concentration and the results of postoperative routine coagulation tests. Patients with lower MP concentrations had longer aPTT and higher INR values after surgery ( $P=0.003$ ,  $P=-0.50$  and  $P=0.020$ ,  $P=-0.40$ , respectively). The results of the univariate logistic regression analysis suggested the preoperative MP concentration as a risk factor for postoperative coagulopathy (as defined in the method). In the multivariate logistic regression analysis, the preoperative MP concentration (OR, 1.00; 95% CI, 1.00 to 1.01;  $P=0.017$ ) was considered a risk factor for postoperative coagulopathy after the assumption of intraoperative variables (the CPB time, the aortic cross-clamp time, and the operation time) as confounding variables (Table 3).

## Discussion

Several studies have evaluated cell-derived MPs under *in vitro* and various clinical conditions. In the present study, we focused on the relationship between cell-derived MPs and surgical variables (perioperative variables and routine coagulation tests) in valve surgery with CPB.

Given that cell activation in the production of MPs from circulating cells, surgery-caused trauma seemingly plays an effective role in MP generation during surgery. Ikeda et al reported that more platelet-derived MPs were formed in patients undergoing the open abdominal procedure after surgery because of promoting cell activation. They also pointed out that minimally invasive procedures could prevent excessive cell activation by considering the results obtained for patients with colorectal cancer undergoing laparoscopy as cited by Nishiguchi et al<sup>17</sup> and Leung et al.<sup>18</sup> Nonetheless, they mentioned the low sample size as the limitation of their investigation and reiterated the need for further research.<sup>19</sup> Park et al<sup>20</sup> introduced tissue damage in patients with blunt trauma as a factor enhancing MP production. Regarding the present study, MP generation significantly increased after surgery. Based on the results of our correlation analysis, the increase was related to the CPB time, and it was not associated with the operation time. It seems that the role of CPB in forming cell-derived MPs was greater than that of trauma in patients undergoing cardiac surgery.<sup>21</sup> Jian et

al<sup>11</sup> found no significant changes in the MP concentration after CPB. It is worth noting that they measured MP levels at 12 hours after CPB, while it was immediately determined after CPB in the present study. The high concentrations of cell-derived MPs in patients undergoing heart valve surgery lead to oxidative stress induction, inflammatory responses, endothelial dysfunction, and hemodynamic instability.<sup>5</sup>

The results of red cell-derived MP, platelet-derived MP, and monocyte-derived MP generation indicated that the maximum enhancement in the production was related to platelet-derived MPs despite elevations in all 3 phenotypes after surgery. As expected, the platelet count fell as the platelet-derived MP level rose ( $P<0.001$ , 156000.00 [145000.00–169000.00] and 215000.00 [169000.00–269000.00]). This decrease represents platelet activation due to the contact of blood with the CPB circuit and the consumption of platelets, associated with platelet-derived MP formation.

To our knowledge, no study has focused on red cell-derived MP variations in heart valve surgery. The results of the present study suggested a rise in the postoperative red cell-derived MP count. In addition, the platelet-derived MP count was greater among patients undergoing valve repair than those with valve replacement, which may be attributed to the higher complexity of the repair operation.<sup>22</sup> A large body of research conducted on red cell-derived MPs in the context of blood transfusion has considered red cell-derived MPs to be the red blood cell storage lesion.<sup>14,23</sup> Some studies have highlighted the clinical role of red cell-derived MPs under different conditions, such as paroxysmal nocturnal hemoglobinuria, sickle-cell anemia, and malaria infection.<sup>7,24,25</sup> In these clinical conditions, red cell-derived MPs affect nitric oxide bioavailability and vascular complications because of the presence of free plasma hemoglobin. Moreover, red cell-derived MPs are procoagulant due to the expression of phosphatidylserine.<sup>26</sup> Further studies are required to elucidate the clinical relationship between enhanced red cell-derived MPs and heart valve surgery, especially repair and replacement. Furthermore, we observed increased levels of monocyte-derived MPs after surgery. Various studies have shown the clinical significance of leukocyte-derived MPs in the secretion of inflammatory cytokines and vascular damage.<sup>27</sup> Given the role of MPs in the induction of coagulation and inflammation, they can be considered therapeutic goals for the prevention of postoperative complications.

Some researchers have reported a direct relationship between a rise in the plasma MP level and thrombotic events, such that an increase in the MP concentration enhances thrombin generation and thrombosis risk.<sup>28-30</sup> Nevertheless, the results of some clinical studies have revealed that a low MP level is accompanied by the symptoms of bleeding.<sup>31-33</sup> Regarding the present study, patients with lower preoperative MP concentrations exhibited higher INR and longer aPTT values after surgery. Further, preoperative MP levels were greater in non-coagulopathic subjects when considering the criteria provided to define postoperative coagulopathy in this study. (See the Material and Methods.) Hashemi et al<sup>14</sup> found a lower clotting time in samples extracted from blood bags with high MP concentrations. The results of the present study are consistent with those reported by Matijevic et al,<sup>13</sup> who showed a lower bleeding rate among trauma patients with a higher MP level. Furthermore, the preoperative MP concentration was negatively correlated with postoperative bleeding, although the correlation was nonsignificant, which may be ascribed to the small sample size. Given that the production of more MPs due to interventions can be considered a risk factor for thrombosis, it seems that high preoperative MP levels can benefit patients and be effective in controlling surgery-induced coagulopathy.

In this study, we highlighted the importance of MP levels in surgery-induced coagulopathy. As far as we know, red cell-derived MP variations have not been studied in heart valve surgery. Still, more studies are needed in this field.

We used the flow cytometry method to phenotype MPs. It is introduced as a highly specific and sensitive laboratory method to detect, identify, and count specific cells.<sup>34</sup> Furthermore, we determined the concentration of MPs using the Bradford method. As is shown in Table 2, the results obtained from the Bradford method were consistent with those obtained from the flow cytometry technique. Although flow cytometry is more specific, it seems that the Bradford method can be used as a fast method for MP evaluation given its availability and lower costs.

The salient limitation of the current investigation is its relatively small sample size and the inclusion of just a single type of surgery. We suggest that future investigations analyze other cellular markers in the flow cytometry method and evaluate the procoagulant activity of MPs using functional assays.

## Conclusion

The levels of MPs, especially platelet-derived MPs, increased after surgery in correlation with the CPB time. Given the role of MPs in the induction of coagulation and inflammation, they can be considered therapeutic goals for the prevention of postoperative complications. In addition, the pre-surgical levels of MPs can be used for predicting

postoperative coagulopathy in heart valve surgery. It seems that high pre-surgical levels of MPs confer an advantage insofar as they reduce the occurrence of coagulopathy in candidates for valve surgery with CPB.

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