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ORIGINAL ARTICLE

Effects of prosthetic heart valve-associated subclinical intravascular hemolysis on endothelium and cardiovascular system: a retrospective cohort study

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Abstract

Mechanical prosthetic heart valve implantation is a widely used surgical treatment for severe symptomatic valvular heart disease, and regular follow-up of prosthetic valve function together with associated clinical and laboratory parameters is essential after implantation. Chronic subclinical intravascular hemolysis is frequently observed in this population; however, its long-term clinical implications remain incompletely defined. In this single-center, retrospective, non-randomized cohort study, 346 patients who underwent aortic and/or mitral mechanical valve replacement and met predefined inclusion and exclusion criteria were evaluated to investigate the association between subclinical hemolysis severity—assessed by follow-up serum lactate dehydrogenase (LDH) levels—and endothelial dysfunction–related clinical outcomes, includin pulmonary hypertension (PHT) and major adverse cardiovascular events (MACE). Mechanical intravascular hemolysis severity was stratified according to the median follow-up LDH (fLDH) value of 264.5 U/L, categorizing patients into non-/mild hemolysis (Group 1) and moderate hemolysis (Group 2). The mean age of the cohort was 57.5 ± 12.5 years, 65% were male, and traditional coronary artery disease risk factors were similarly distributed between groups. Over a mean follow-up duration of 9.4 ± 3.3 years 18 MACE events (5.2%) were observed, while new-onset PHT developed in 122 patients (35.3%). MACE occurred in 1 patient (0.6%) in Group 1 and in 17 patients (9.8%) in Group 2 ($p < 0.001$). In multivariable logistic regression analysis, an fLDH level ≥ 265 U/L and the presence of diabetes mellitus were associated with MACE, although effect size estimates were accompanied by wide confidence intervals due to the limited number of events. Overall, higher levels of subclinical intravascular hemolysis, reflected by elevated LDH, were associated with an increased occurrence of MACE and PHT in patients with mechanical prosthetic heart valves. Given the low eventcount and resulting statistical uncertainty, these findings should be interpreted as exploratory and hypothesis-generating rather than causal, and larger prospective studies are warranted to clarify the prognostic significance of subclinical hemolysis and its potential role in long-term risk stratification.

Keywords Intravascular hemolysis · Cardiovascular event · Lactate dehydrogenase · Prosthetic heart valve · Pulmonary hypertension

Introduction

Erythrocytes are the most abundant mammalian cells in the blood and, during their maturation process, lose their nucleus along with several organelles, including the endoplasmic reticulum, mitochondria, and ribosomes. Their primary function is the transport of oxygen and carbon dioxide between the lungs and other organs. Due to their biconcave shape, erythrocytes can partially fold onto themselves to traverse narrow lumens while also possessing a

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high surface-area-to-volume ratio, facilitating efficient gas exchange despite their relatively small cellular volume.

Erythrocytes are densely packed with hemoglobin (Hb), a protein composed of multiple tertiary-structured polypeptide chains that play a crucial role in gas exchange. Additionally, their cytoplasm contains key structural proteins such as spectrin, ankyrin, and actin, which are essential for membrane stability and deformability, as well as enzymes like carbonic anhydrase and arginase. The erythrocyte count in 1 mm^3 of blood is approximately 5.4 million in males and 4.8 million in females.

Erythropoiesis, the process of erythrocyte production, occurs in erythroblast islands within the bone marrow of adults and is tightly regulated by macrophages. This highly complex process results in the release of approximately 2 million mature erythrocytes into circulation per second. These erythrocytes, measuring 7–8 microns in diameter, must navigate through capillaries as narrow as 3 microns and endothelial slits in the red pulp of the spleen that range from 1–2 microns in width. Only erythrocytes with adequate deformability can successfully traverse these structures.

Since mature erythrocytes lack organelles and the ability to synthesize new proteins, their lifespan is limited to approximately 120 days. Throughout the circulatory cycle, senescent erythrocytes undergo degradation and are continuously replaced by newly generated erythrocytes with preserved deformability, at an estimated rate of 500,000 cells per second [1–4].

Hemolysis, characterized by the breakdown of erythrocytes, is a pathological condition encountered in various disease processes [5–12]. When hemolysis occurs within the circulatory system, it is termed intravascular hemolysis. It is well established that the release of hemoglobin and other intracellular components such as heme during intravascular hemolysis exerts toxic effects on tissues. These adverse effects have been demonstrated to occur through mechanisms involving nitric oxide (NO) imbalance, endothelial dysfunction, platelet activation, and immune system activation [13–28]. Endothelial damage or dysfunction resulting from hemolysis and other contributing factors is thought to play a pivotal role in the initiation and progression of vascular diseases, including atherosclerosis (Fig. 1,2 and Table 1). While the detrimental effects of acute and severe intravascular hemolysis are well recognized and supported by clinical studies, our knowledge regarding the impact of subclinical intravascular hemolysis remains limited [29–32].

Mechanical prosthetic heart valves, which play a crucial role in the treatment of valvular heart disease, are a significant cause of traumatic intravascular hemolysis (Fig. 3). Hemolytic anemia associated with mechanical valves was observed at a high rate (approximately 15%) in early-generation prosthetic valves, whereas modern mechanical valves exhibit this complication much less frequently. However, subclinical hemolysis, defined as mild intravascular hemolysis without accompanying anemia, remains prevalent, with reported rates ranging from 18 to 51% [33, 34].

Aims

This study aims to investigate the clinical effects of chronic subclinical intravascular hemolysis on endothelial and cardiovascular system function in patients with mechanical heart valves, as well as to assess its impact on the incidence of cardiovascular events.

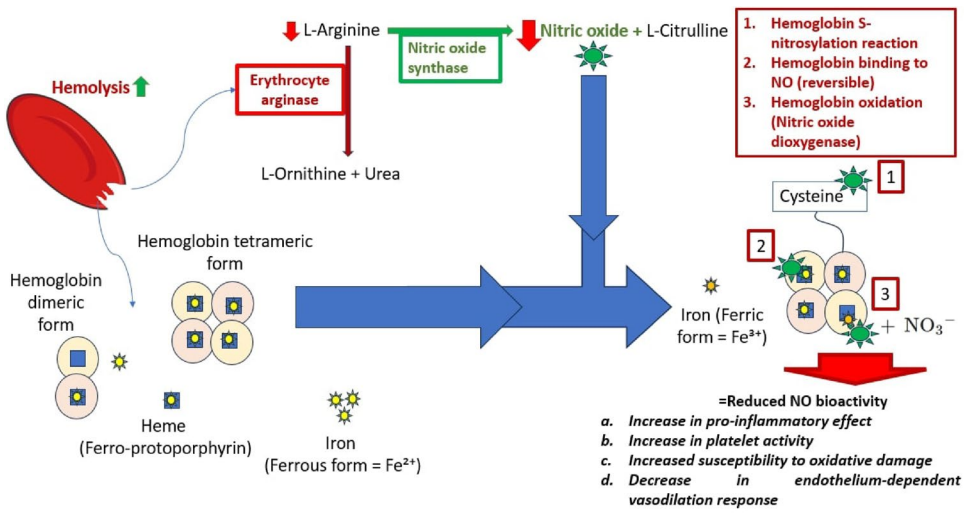


Fig. 1 Pathophysiology of Intravascular Hemolysis

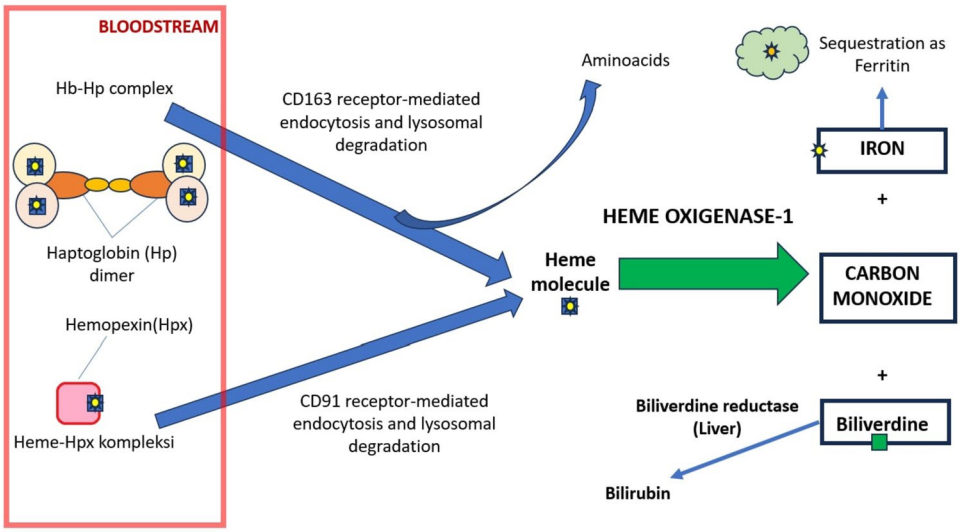


Fig. 2 Defense mechanisms against the harmful effects of free hem and hemoglobin in the circulatory system

Table 1 Harmful effects of free hemoglobin and hem molecule and defense mechanisms

Harmful effects	Defense mechanisms
• Development of atherosclerosis and pulmonary hypertension • Lipid peroxidation • Increased susceptibility to oxidative damage • Pro-inflammatory effects and platelet activation • Cellular dysfunction due to hemosiderin accumulation	• Compartmentalization within erythrocytes • Cellular uptake of hemoglobin-haptoglobin complex • Cellular uptake of hem-hemopexin complex • Antioxidant enzymes (e.g., HO-1) • Degradation of the hem molecule via α1-microglobulin

α, alpha; HO-1, heme oxygenase-1 enzyme

Materials and methodsx

Study design

The medical records of 1,783 patients who underwent valve surgery at Ankara University Heart Center between January 2004 and December 2021 were reviewed. Patients who had undergone mechanical heart valve implantation and had regular follow-up data without complications in the postoperative period were included in the study.

Since one of the primary endpoints of our study was to compare the incidence of atherosclerosis and coronary events between groups, 311 patients with preoperatively diagnosed severe coronary artery disease were excluded. Additionally, patients with bioprosthetic valves (512 patients) were excluded from the study due to the lower incidence of early postoperative hemolysis and the increased risk of cardiovascular events and hemodynamic complications associated with structural valve deterioration over long-term follow-up (≥5–10 years). After a detailed retrospective evaluation of the remaining patients, 346 patients who met the inclusion and exclusion criteria were selected, and their clinical, laboratory, and echocardiographic data were collected for statistical analysis.

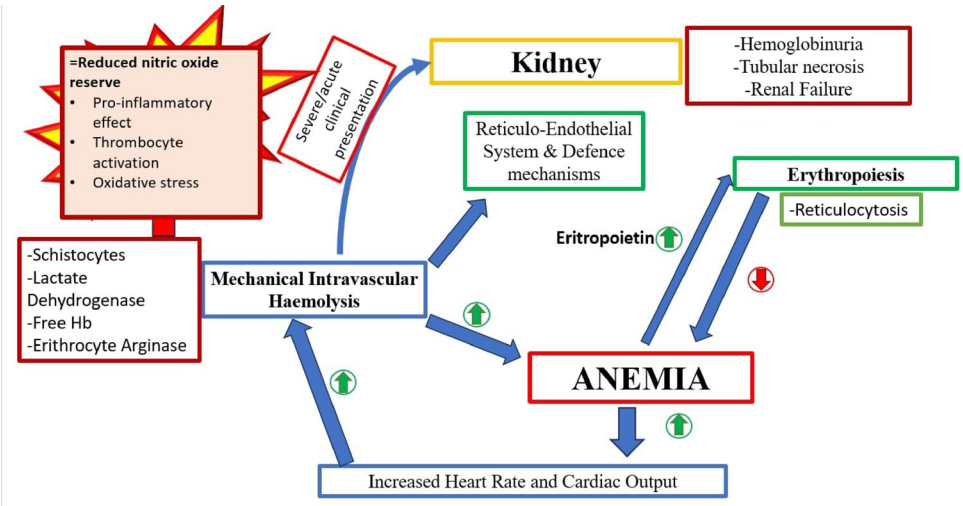


Fig. 3 Pathophysiology of mechanical intravascular hemolysis

Baseline echocardiographic parameters were recorded by reviewing transthoracic echocardiography (TTE) reports obtained immediately before surgery and during the first postoperative follow-up. Follow-up echocardiographic parameters were retrospectively obtained from the most recent recorded TTE report for each patient. Clinical and laboratory data were extracted from electronic hospital records.

The study population was categorized into two groups according to follow-up serum LDH (fLDH) levels, which serve as a surrogate marker for the presence and severity of mechanical intravascular hemolysis (MIH). Although ROC-curve analysis indicated that an LDH threshold slightly above 300 U/L provided better discrimination for adverse outcomes, to avoid potential bias we selected the median fLDH value of 265 U/L as an objective and clinically meaningful cut-off—also aligning with the upper limit of normal LDH values reported in many laboratories [35]. Patients with fLDH < 265 U/L were classified as Group I (no/mild hemolysis), whereas those with fLDH ≥ 265 U/L were categorized as Group II (moderate hemolysis). Individuals with established hemolytic anemia or fLDH > 800 U/L, indicative of severe hemolysis, were excluded from the analysis (n = 63). Due to the retrospective design and incomplete laboratory records, additional hemolysis markers (e.g., haptoglobin, indirect bilirubin, reticulocytes, or free hemoglobin) could not be systematically evaluated.

This study aimed to investigate the cardiovascular effects of chronic subclinical hemolysis by comparing the incidence of clinical events such as pulmonary hypertension and atherosclerotic vascular events between groups. The primary study endpoints were major adverse cardiovascular events (MACE) and pulmonary pressure measurements. MACE was defined as a composite of nonfatal acute coronary syndrome (ACS), ischemic or hemorrhagic cerebrovascular events (CVE), cardiovascular mortality, significant clinical arrhythmias, and elective revascularization. Significant clinical arrhythmia was defined as bradyarrhythmia or tachyarrhythmia requiring permanent pacemaker (PPM) or implantable cardioverter-defibrillator (ICD) implantation.

New-onset pulmonary hypertension (PHT) was defined as a mean pulmonary artery pressure (mPAP) of ≥ 25 mmHg during follow-up in patients without a prior diagnosis of PHT. mPAP values were calculated using the commonly applied Chemla formula (mPAP = 0.61 × sPAP + 2 mmHg), where systolic pulmonary artery pressure (sPAP) was determined non-invasively using the echocardiographic Bernoulli equation, based on tricuspid regurgitant jet velocity and estimated right atrial pressure (RAP).

The study flowchart and inclusion/exclusion criteria are presented in Fig. 4 and Table 2.

Sample size calculation and study power analysis

Since the study is retrospective in nature, no sample size calculation was performed. However, a post-hoc analysis was conducted based on the number of MACE events, as well as the mean and standard deviation of fLDH in Groups I and II. According to this calculation, the statistical power of the study (1- β error) was determined to be 88.73%. 5.3. Statistical Methods Used Data analysis was performed using the SPSS (Statistical Package for Social Sciences) version 25 software. The normality of the distribution of variables was assessed using both visual (histograms and probability plots) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk tests). Descriptive statistics were presented as mean and standard deviation for normally distributed numerical variables, median and extreme values

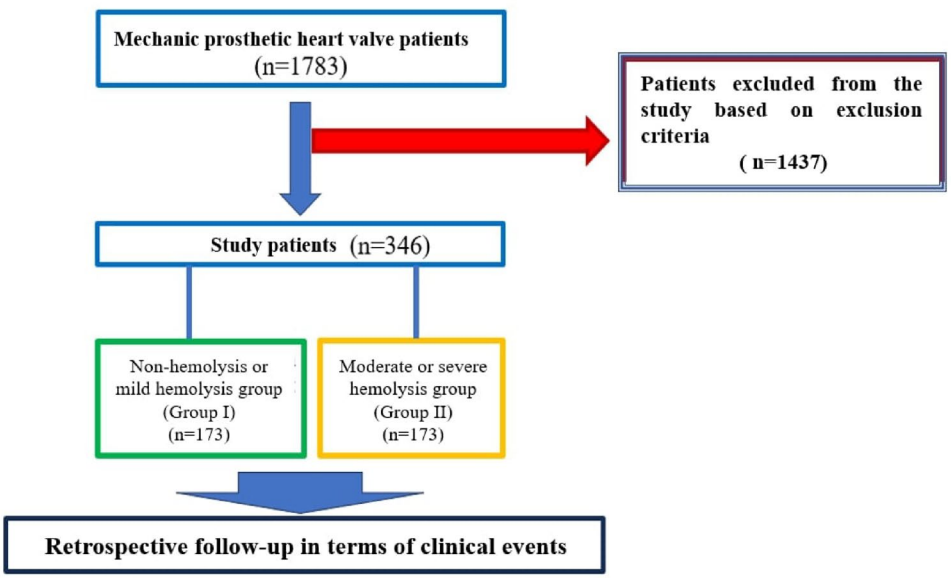


Fig. 4 Study flowchart

Table 2 Inclusion and exclusion criteria of the study

Inclusion Criteria
Patients aged between 18–75 years
Patients who underwent mechanical aortic and/or mitral valve replacement
• Patients aged between 18–75 years
• Patients who underwent mechanical aortic and/or mitral valve replacement
Exclusion Criteria
• Hemolytic anemia
• Severe paravalvular leak
• Patients who underwent revascularization before or during valve surgery
• Patients diagnosed with coronary artery disease in preoperative coronary angiography before valve surgery
• Patients who developed a major adverse cardiovascular event (MACE) during the same hospitalization after valve surgery
• Patients diagnosed with or not ruled out for prosthetic heart valve complications (e.g., pannus, prosthetic valve endocarditis) requiring intervention during follow-up
• Patients with postoperative echocardiographic and/or laboratory follow-up of less than two years or with incomplete follow-up
• Presence of severe liver disease
• Presence of severe kidney disease
• Patients diagnosed with active malignancy or those in whom malignancy could not be clinically ruled out
Patients without follow-up serum lactate dehydrogenase (LDH) levels or those whose LDH values were only obtained during active infection/autoimmune disease (patients with LDH values deemed unsuitable for the study)

for non-normally distributed numerical variables, and frequency tables for ordinal and categorical variables. For comparisons between groups, Student's t-test was used for normally distributed numerical variables, while the Mann–Whitney U test was applied for non-normally distributed numerical variables. Categorical variables were analyzed using either the Chi-square test or Fisher's exact test, as appropriate. Correlation analysis between fLDH and pulmonary artery pressure (PAP) was performed using Spearman's correlation test. When significant threshold values were identified, the corresponding positive likelihood ratios and Youden indices were calculated, and the most appropriate one was selected. MACE-free and PHT-free survival rates were estimated using Kaplan–Meier survival analysis. The impact of the two groups on survival was assessed using the log-rank test. To identify independent risk factors associated with MACE occurrence and the presence and severity of hemolysis based on fLDH groups, multivariate analysis was conducted. Variables found to be statistically significant in univariate analyses were included in the model, and logistic regression analysis was applied. Among highly correlated variables, those with a higher p-value or lower clinical significance were excluded from the models. Model fit was evaluated using the Hosmer–Lemeshow test. A p-value of < 0.05 was considered statistically significant.

Results

A total of 346 patients who underwent mechanical prosthetic heart valve surgery at Ankara University Heart Center between January 2004 and December 2021 were included in the study. These patients had no detected prosthetic valve dysfunction and had regular follow-up data available.

The mean age of the patients was 57.5 ± 12.5 years, and 65% of the study population consisted of male patients. There was no significant difference between Group I and Group II in terms of hypertension, diabetes mellitus, smoking, family history of coronary artery disease (CAD), or hyperlipidemia. However, preoperative electrocardiographic (ECG) assessment revealed that patients in Group II had a higher prevalence of atrial fibrillation (AF).

The demographic and clinical characteristics of the study population are summarized in Table 3.

An analysis of the patients' laboratory parameters revealed no significant differences between the groups, except for the red cell distribution width (RDW), which was found to be higher in Group II patients ($p = 0.016$). The preoperative lactate dehydrogenase (LDH) levels were similar between both groups and remained within the normal reference range (Table 4).

An evaluation of the patients' regularly used medications during follow-up revealed that the use of aspirin ($p < 0.001$), statins ($p = 0.013$), angiotensin-converting enzyme inhibitors (ACEIs) / angiotensin II receptor blockers (ARBs) ($p < 0.001$), and digoxin ($p = 0.007$) was significantly higher in Group II (Table 5).

Among the patients included in the study, the most common valve pathology was severe mitral stenosis (MS), observed in 37% of cases. In terms of valve disease etiology, rheumatic valve disease ranked first (58.1%), followed by congenital valve diseases (25.4%), the majority of which consisted of bicuspid aortic valve stenosis cases.

The most frequently performed valve replacement procedures, from most to least common, were:

- Isolated mitral valve replacement (MVR) (55.5%)
- Isolated aortic valve replacement (AVR) (23.1%)
- AVR combined with aortic root replacement (ARR) (11.6%)

When assessing the relationship between valvular heart disease (VHD) etiology and hemolysis severity, rheumatic valve disease was found to be significantly more common in Group II patients ($p < 0.001$).

Regarding the relationship between valve implantation procedures and hemolysis severity, patients who underwent isolated AVR had significantly lower hemolysis severity

Table 3 Demographic and clinical characteristics of the study population

Parameter	General Population (*n* = 346)	Group I (*n* = 173)	Group II (*n* = 173)	P
Gender, Male (n, %)	225 (65)	76 (43,9)	149 (86,1)	< 0,001
Age (years) [1]	57,5 ± 12,5	58,3 ± 11,8	56,8 ± 13,2	0,38
Body Mass Index (kg/m^2) [2]	27,4 (22–36,9)	26,7 (22–31,3)	28,2 (22,1–36,9)	0,003
Hypertension (n, %)	62 (17,9)	37 (21,4)	25 (14,5)	0,093
Diabetes Mellitus (n, %)	40 (11,6)	24 (13,9)	16 (9,2)	0,18
Hyperlipidemia (n, %)	16 (4,6)	8 (4,6)	8 (4,6)	> 0,99
Family History of Coronary Artery Disease (n, %)	31(9)	12(6,9)	19(11)	0,258
Smoking (n, %)	59(17,1)	35(20,2)	24 (13,9)	0,099
Baseline LVEF(%)[1]	61,3 ± 5,7	62,3 ± 5,2	60,3 ± 6,1	< 0,001
Last LVEF Measurement (%)[1]	60,3 ± 7,6	62 ± 7	58,6 ± 7,8	< 0,001
Systolic Blood Pressure (mmHg) [1]	122,7 ± 10,7	122,5 ± 10,3	122,9 ± 11,1	0,38
Diastolic Blood Pressure (mmHg) [1]	78,3 ± 7,2	78,1 ± 7,7	78,4 ± 6,7	0,55
Preoperative ECG Rhythm (n, %) AF	82 (23,7)	14 (8,1)	68 (39,3)	< 0,001

[1] = M

[2] = Median value (IQR = Interquartile Range, Q1-Q3)

AF, atrial fibrillation, ECG, electrocardiogram, LVEF, left ventricular ejection fraction, SR, sinus rhythm

compared to those who underwent isolated MVR or AVR combined with MVR ($p < 0.001$) (Table 6).

Due to variations in prosthetic valve sizes, which could influence differences in baseline and follow-up echocardiographic parameters (such as postoperative transvalvular gradient levels), the study population was stratified into subgroups based on whether they underwent isolated aortic valve replacement (AVR) or isolated mitral valve replacement (MVR) for a more precise analysis.

Although baseline systolic pulmonary artery pressure (sPAP) values were similar between Group I and Group II, follow-up transthoracic echocardiography (TTE) assessments revealed that sPAP was significantly higher in Group II compared to Group I ($p < 0.001$).

Table 4 Laboratory characteristics recorded during the follow-up period of study patients

Parameter	General population (*n* = 346)	Group I (*n* = 173)	Group II (*n* = 173)	P
Hemoglobin (g/dl)[1]	13,8 ± 1,6	13,9 ± 1,5	13,8 ± 1,6	0,39
RDW (%)[1]	14,15 ± 1,5	13,98 ± 1,45	14,32 ± 1,54	0,016
Platelet (× 10^9/L)[2]	240 (32–562)	240 (32–562)	240,5 (32–487)	0,63
Leukocyte (× 10^9/L)[2]	6700 (3500–15100)	6840 (3500–15100)	6470 (4000–11700)	0,22
Serum Creatinine (mg/dL^2)	0,82 (0,5–1,8)	0,82 (0,5–1,5)	0,84 (0,5–1,8)	0,55
Glomerular Filtration Rate (mL/min/1.73 m^2)[2]	92 (28–190)	93 (45–190)	90 (28–190)	0,91
Alanine Aminotransferase (U/L)[2]	22 (10–171)	22 (10–160)	22 (13–171)	0,59
Aspartate Aminotransferase (U/L)[2]	21 (15–98)	21 (15–98)	21 (17–90)	0,53
Total bilirubin (mg/dL)[2]	0,57 (0,3–2,1)	0,57 (0,3–1,7)	0,570 (0,3–2,1)	0,70
Direct bilirubin (mg/dL)[2]	0,17 (0,1–0,6)	0,17 (0,1–0,5)	0,16 (0,1–0,6)	0,41
LDL-c (mg/dl)[2]	111 (12–323)	113 (12–226)	107 (47–323)	0,29
HDL-c (mg/dl)[2]	42 (15–127)	40,4 (15–73,8)	44,5 (20–127)	0,075
Prothrombin time (INR)[2]	2,62 (1,8–4,1)	2,64 (1,9–4,1)	2,6 (1,8–3,4)	0,13
Preoperative Serum Lactate Dehydrogenase Level (U/L)[2]	181 (117–463)	180 (117–463)	197 (130–320)	0,33

[1] = Mean ± Standard Deviation

[2] = Median value (IQR = Interquartile Range, Q1-Q3)

INR, international normalized ratio, HDL-c, high-density lipoprotein cholesterol, LDL-c, low-density lipoprotein cholesterol, RDW, red cell distribution width

Table 5 Medications regularly used by the study population during follow-up

Medications	General population (*n* = 346)	Group I (*n* = 173)	Group II (*n* = 173)	P
Aspirin (n, %)	16 (4,6)	0 (0)	16 (9,2)	< 0,001
Calcium Channel Blocker (n, %)	16 (4,6)	8 (4,6)	8 (4,6)	> 0,99
Beta-blocker (n, %)	329 (95,1)	164 (94,8)	165 (95,4)	> 0,99
ACEi/ ARB (n, %)	72(20,8)	20(11,6)	52(30,1)	< 0,001
Statin (n, %)	48 (13,9)	16 (9,2)	32 (18,5)	0,013
Loop Diuretics (n, %)	201 (58,1)	99 (57,2)	102 (59)	0,74
MRA (n, %)	184 (53,2)	95 (54,9)	89 (51,4)	0,52
Digoxin (n, %)	8 (2,3)	0 (0)	8 (4,6)	0,007
Amiodarone (n, %)	32 (9,2)	16 (9,2)	16 (9,2)	> 0,99

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; Ca, calcium; MRA, Mineralocorticoid receptor antagonist

Table 6 Characteristics of valve diseases and surgical procedures applied to the study patients

Characteristics	General population ($n=346$)	Group I ($n=173$)	Group II ($n=173$)	P
VHD (n, %)				<0,001
MS	128 (37)	67 (38,7)	61 (35,3)	
MR	64 (18,5)	23 (13,3)	41 (23,7)	
AS	72 (20,8)	49 (28,3)	23 (13,3)	
AR	40 (11,6)	24 (13,9)	16 (9,2)	
MS+AS	8 (2,3)	0 (0)	8 (4,6)	
AS+AR	8 (2,3)	7(4)	1 (0,6)	
AR+MS	9 (2,6)	0 (0)	9 (5,2)	
MR+AoRA	16 (4,6)	2 (1,2)	14 (8,1)	
AR+MR	1 (0,3)	1 (0,6)	0 (0)	
VHD etiology (n, %)				<0,001
Rheumatic	201 (58,1)	82 (47,4)	119 (68,8)	
Congenital	88 (25,4)	48 (27,7)	40 (23,1)	
Degenerative	48 (13,9)	34 (19,7)	14 (8,1)	
Infective Endocarditis	9 (2,6)	9 (5,2)	0 (0)	
Surgical Procedures (n, %)				<0,001
MVR	192 (55,5)	90 (52)	102 (59)	
AVR (±AoRR)	120 (34,7)	80 (46,2)	40 (23,1)	
AVR+MVR	34 (9,8)	3 (1,7)	31 (17,9)	

AS, Aortic stenosis; AR, aortic regurgitation; MS, mitral stenosis; MR, mitral regurgitation; AoRA, aortic root aneurysm; VHD, valve heart disease; AVR, Aortic valve replacement; MVR, mitral valve replacement; AoRR, aortic root replacement

Table 7 Procedural and echocardiographic follow-up characteristics of aortic valve replacement patients only

Parameters	Group I ($n=49$)	Group II ($n=31$)	P
Baseline Echocardiographic Parameters			
LVEF (%)[1]	61,9±4,7	60,8±4,3	0,16
LA diameter (cm)[1]	4,4±0,4	4±0,7	0,40
LVEDD (cm)[1]	5,1±0,4	4,9±0,4	0,004
IVSd (cm)[1]	0,9±0,1	1,1±0,2	0,001
sPAP (mmHg)[1]	27,1±3,5	29,4±5,7	0,11
Valve Brand (n, %)			<0,001
Sorin	16 (32,7)	24 (77,4)	
St Jude	33 (67,3)	7 (22,6)	
Valve size (n, %)			<0,001
19 mm	7 (14,3)	9 (29)	
21 mm	16 (32,7)	0 (0)	
23 mm	8 (16,3)	16 (51,6)	
25 mm	18 (36,7)	6 (19,4)	
≤21 mm	23 (46,9)	9 (29)	
Follow-up Duration (years)[1]	10±1,7	6,9±3,1	<0,001
Echocardiographic Parameters at the End of Follow-up			
LVEF (%)[1]	63,5±2,3	62,3±2,5	0,032
LA diameter (cm)[1]	4,5±0,5	4,2±0,8	0,24
LVEDD (cm)[1]	5,2±0,4	4,8±0,4	<0,001
IVSd (cm)[1]	1±0,2	1,1±0,2	0,002
sPAP (mmHg)[1]	28,8±4,4	33,2±6,5	0,002
Mean gradient (mmHg)[1]	11±4,2	9,8±1,8	0,84
Transvalvular AR (n, %)			>0,99
0° degree	24 (49)	16 (51,6)	
1° degree	25 (51)	15 (48,4)	
Paravalvular AR (n, %)			>0,99
0° degree	49(100)	31(100)	
1° degree	0 (0)	0 (0)	

[1] = Mean ± standart deviation

AR, aortic regurgitation; LVEF, left ventricular ejection fraction; LA, left atrium; LVEDD, left ventricular end-diastolic diameter; IVSd, interventricular septum diastolic thickness; sPAP, systolic pulmonary artery pressure

Similarly, in both AVR and MVR subgroups, follow-up sPAP values were significantly higher in Group II compared to Group I ($p<0.001$).

A detailed analysis of the echocardiographic parameters for patients undergoing isolated AVR and isolated MVR is presented in Table 7 and Table 8.

To assess the effect of mechanical valve implantation site on the development of pulmonary hypertension (PHT), postoperative and follow-up transthoracic echocardiography (TTE) measurements of systolic pulmonary artery pressure (sPAP) were analyzed across surgical subgroups.

While baseline sPAP values differed between isolated MVR and isolated AVR groups, the trend of sPAP elevation over follow-up was similar in both groups. However, in patients who underwent both AVR and MVR, a significantly steeper sPAP increase was observed compared to those with single-valve replacement.

In all three groups, follow-up sPAP values showed a statistically significant increase compared to baseline sPAP ($p<0.001$), indicating progressive PHT development over time (Fig. 5).

As illustrated in Fig. 6, a significant association was identified between the location of valve replacement, the number of implanted valves, and follow-up lactate dehydrogenase (LDH) levels. Patients who underwent isolated MVR had higher fLDH levels compared to those who underwent isolated AVR ($p<0.001$). The highest follow-up LDH levels were observed in the subgroup that underwent both AVR and MVR, indicating the greatest degree of hemolysis in this group.

To assess factors that might modify the effect of single or double valve replacement on hemolysis severity, further analysis was conducted. Diabetes mellitus (DM) diagnosis, baseline and follow-up left ventricular ejection fraction (LVEF), and baseline sPAP were identified as parameters that could influence this relationship ($p<0.05$) (Table 9).

In the analysis of new-onset PHT, the mean pulmonary artery pressure (mPAP) calculated using the Chemla formula was used as a reference, based on clinical studies

Table 8 Procedural and echocardiographic follow-up characteristics of mitral valve replacement patients only

Parameters	Grup I ($n=90$)	Grup II (n=102)	*P*
Baseline Echocardiographic Parameters			
LVEF (%)[1]	61,5±6,1	61,8±3,7	0,22
LA diameter (cm)[1]	4,7±0,8	4,6±0,6	0,81
LVEDD (cm)[1]	5±0,5	5±0,4	0,56
IVSd (cm)[1]	0,9±0,1	0,9±0,1	0,44
sPAP (mmHg)[1]	30,3±3,5	30,5±3,6	0,5
Mean gradient (mmHg)[1]	10,1±2,8	11,2±3,2	0,006
Transvalvular MR (n, %)			0,042
0° degree	34 (37,8)	54 (52,9)	
1° degree	56 (62,2)	48 (47,1)	
Paravalvular MR (n, %)			0,008
0° degree	90 (100)	94 (92,2)	
1° degree	0 (0)	8 (7,8)	
Valve brand (n, %)			<0,001
ST-Jude	0 (0)	16 (15,7)	
Sorin	90 (100)	86 (84,3)	
Valve size (n, %)			<0,001
27 mm	46 (51,1)	66 (64,7)	
28 mm	4 (4,4)	12 (11,8)	
29 mm	24 (26,7)	16 (15,7)	
30 mm	0 (0)	8 (0,8)	
31 mm	16 (17,8)	0 (0)	
≤28 mm	50 (55,6)	78 (76,5)	
Follow-up Duration (years)[1]	9,6±4,4	9,7±3,1	0,52
Echocardiographic Parameters at the End of Follow-up			
LVEF (%)[1]	60,1±9,2	58,7±6,7	<0,001
LA diameter (cm)[1]	4,8±0,9	5±0,6	0,005
LVEDD (cm)[1]	5,1±0,6	5,1±0,5	0,50
IVSd (cm)[1]	0,9±0,1	0,9±0,1	0,080
sPAP (mmHg)[1]	33,3±7	40,4±5,9	<0,001
Mean gradient (mmHg)[1]	4,4±1,2	5,4±1,2	<0,001
Transvalvular AR (n, %)			0,008
0° degree	19 (21,1)	29 (28,4)	
1° degree	71 (78,9)	65 (63,7)	
2° degree	0 (0)	8 (7,8)	
Paravalvular MR (n, %)			0,008
0° degree	90 (100)	94 (92,2)	
1° degree	0 (0)	8 (7,8)	

[1]=Mean±standart deviation

MR, mitral regurgitation; LVEF, left ventricular ejection fraction, LA, left atrium; LVEDD=Left Ventricular End-Diastolic Diameter, IVSd=Interventricular Septum Diastolic Thickness, sPAP=Systolic Pulmonary Artery Pressure

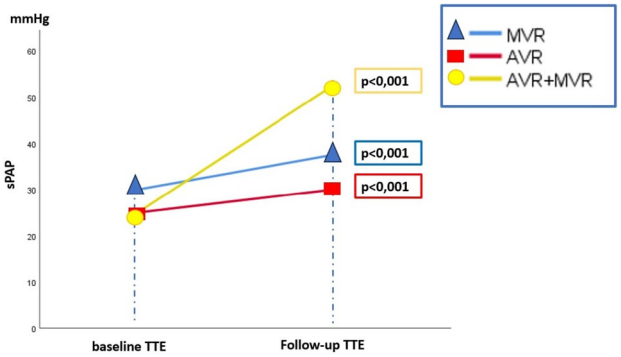


Fig. 5 Follow-up course of sPAP in valve surgery subgroups (sPAP=systolic pulmonary artery pressure, TTE=transthoracic echocardiography, AVR=aortic valve replacement group, MVR=mitral valve replacement group, AVR+MVR=Patients undergoing simultaneous aortic and mitral valve replacement)

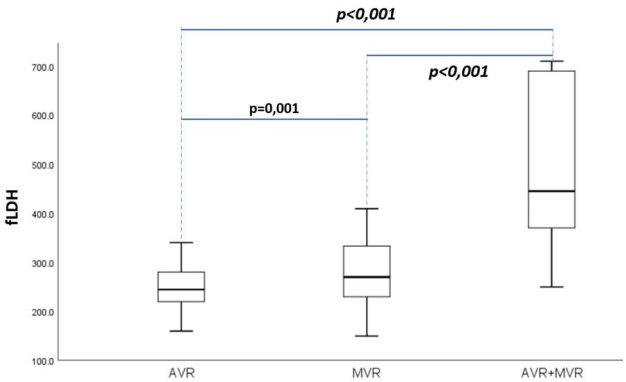


Fig. 6 Comparison of follow-up serum lactate dehydrogenase (fLDH) Levels in patients who underwent isolated aortic valve replacement, isolated mitral valve replacement, and simultaneous aortic and mitral valve replacement, using boxplot

Table 9 Correlation analysis between pulmonary hypertension parameters and follow-up lactate dehydrogenase levels in study patients

Parametre	fLDH (U/L)			
	All Patients (n=346)		LVEF≥50% (n=322)	
	r_s	*p*	r_s	*p*
Baseline sPAP (mmHg)	0,166	0,002	0,061	0,280
Follow-up sPAP (mmHg)	0,368	<0,001	0,313	<0,001
ΔsPAP (mmHg)	0,390	<0,001	0,363	<0,001

sPAP, systolic pulmonary artery pressure; Δ, delta/change; LVEF, left ventricular ejection fraction; tLDH, follow-up lactate dehydrogenase level; rs, spearman correlation coefficient

demonstrating its correlation with invasive pulmonary hypertension (PH) diagnosis. A cutoff value of≥25 mmHg was accepted for defining PHT. PHT was detected in 82 patients (47.4%) in Group II, whereas 40 patients (23.1%) in Group I developed PHT. This difference between the two groups was found to be statistically significant ($p<0.001$).

Correlation analysis demonstrated that follow-up LDH levels correlated with both baseline and follow-up sPAP values, as well as with the difference between these values (ΔsPAP, i.e., the change in sPAP over time). To determine whether left heart pathologies, the most common cause of PHT, influenced this correlation, a separate analysis was performed including only patients with preserved ejection fraction (LVEF≥50%, n=322). In this subgroup, follow-up sPAP and ΔsPAP values remained significantly correlated.

Additionally, a significant relationship was observed between increasing LDH levels and rising pulmonary pressures (Fig. 7). (Table 9 provides a detailed breakdown of these findings.)

In the univariate analysis, parameters that were statistically significant in predicting hemolysis severity were included in a logistic regression model for multivariate analysis. Multivariate analysis identified the following as independent predictors of hemolysis severity:

- Male sex (*OR* = 8.48, 95% CI: 3.79–18.97, p < 0.001)
- Use of Sorin mechanical valves (*OR* = 5.45, 95% CI: 2.54–11.70, p < 0.001)
- Preoperative atrial fibrillation (AF) rhythm (*OR* = 2.74, 95% CI: 1.47–5.10, p = 0.002)

When evaluating clinical events, patients in Group II had significantly higher rates of major adverse cardiovascular events (MACE) and newly developed PHT compared to Group I:

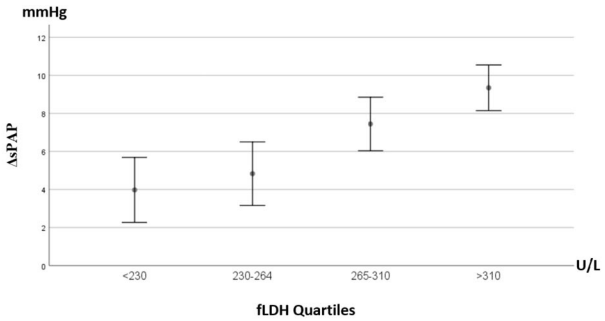
- MACE occurred in 17 patients (9.8%) in Group II, whereas it was observed in only 1 patient (0.6%) in Group I (p < 0.001). When individual MACE components were evaluated based on the number of affected patients, no patient experienced acute coronary syndrome in either group. Stroke occurred in 4 patients (2.3%) in Group II, while no patient in Group I developed stroke. Likewise, significant arrhythmia was observed in 4 patients (2.3%) in Group II and in none of the patients in Group I. Elective coronary revascularization was required in 8 patients (4.6%) in Group II, whereas no patient in Group I underwent revascularization. Cardiovascular mortality occurred in 1 patient (0.6%) in each group.
- Newly developed PHT was also significantly more frequent in Group II.

However, no significant difference in overall mortality was found between the two groups (p > 0.99).

A detailed analysis of clinical events is presented in Table 10 and Fig. 8.

In univariate analysis, parameters that could be statistically significant for MACE prediction were included in the model, and logistic regression analysis was performed. In this analysis, modeling was conducted with independent variables that were considered clinically significant. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal fLDH threshold for predicting MACE, identifying a cut-off value of ≥300 (AUC: 0.775, 95% CI: 0.716–0.834; p < 0.001) (Fig. 9). A fLDH value ≥ 265 was identified as a highly significant variable in increasing the risk of MACE development (OR = 37.18, 95% CI: 3.24–426.35, p = 0.004). Additionally, the presence of a diagnosis of diabetes mellitus (DM) was found to be statistically significant in increasing the risk of MACE development (OR = 27.14, 95% CI: 1.22–601.72, p = 0.037) (Table 11).

Table 11 shows the results of multivariable analysis models for MACE prediction.



F

Fig. 7 Analysis of systolic pulmonary hypertension change level (ΔsPAP) and follow-up lactate dehydrogenase (fLDH) quartiles using a mean value and confidence interval bar chart

Table 10 Analysis of clinical events in study patients by hemolysis groups

Clinical Event	Group I (n = 173)	Group II (n = 173)	*P*
MACE (n, %)	1 (0,6)	17 (9,8)	<0,001
MACE Type (n, %)			
Acute coronary syndrome	0(0)	0(0)	
Stroke	0 (0)	4 (2,3)	
Significant arrhythmia	0 (0)	4 (2,3)	
Elective coronary revascularization	0 (0)	8 (4,6)	
Cardiovascular mortality	1 (0,6)	1 (0,6)	
Newly developed PHT (n, %)	40 (23,1)	82 (47,4)	<0,001

MACE, major adverse cardiovascular event; PHT, pulmonary hypertension

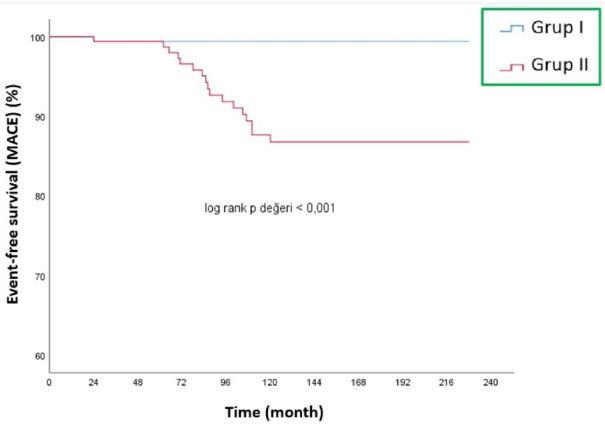


Fig. 8 Analysis of event-free survival curves for MACE (major adverse cardiovascular events) development in groups I and II

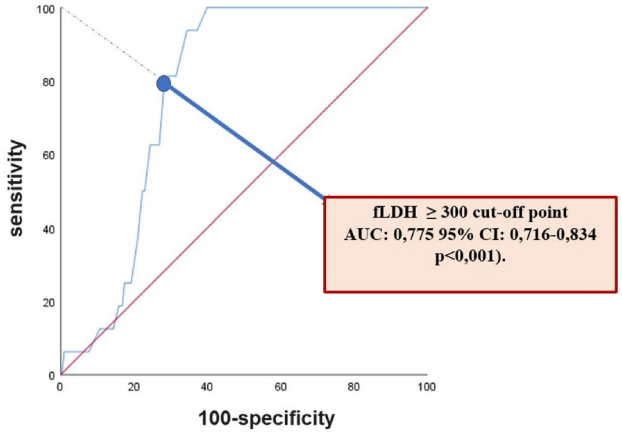


Fig. 9 ROC curve analysis performed to identify the lactate dehydrogenase levels during follow-up that predict the development of major adverse cardiovascular events (MACE)

Table 11 Multivariate analysis of parameters that can be used for predicting MACE in study patients

Parameter	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Hypertension	6,72 (2,40–18,83)	<0,001	0,63 (0,04–9,79)	0,63
Diabetes mellitus	12,40 (4,32–35,60)	<0,001	27,14 (1,22–601,72)	0,037
Preoperative AF (+)	4,06 (1,44–11,49)	0,008	1,04 (0,27–4,09)	0,96
fLDH≥265 U/L	18,74 (2,47–142,49)	0,005	37,18 (3,24–426,35)	0,004

MACE, major adverse cardiovascular event; OR, odds ratio; CI, confidence interval; fLDH, follow-up serum lactate dehydrogenase level; AF, atrial fibrillation

Discussion

In this retrospective, single-center clinical study, we aimed to investigate the cardiovascular effects/associations of subclinical hemolysis, which is commonly encountered after metallic prosthetic valve surgery, despite the absence of any issues with valve function. In our study, where follow-up LDH levels were used to determine the presence and severity of hemolysis, we observed a positive correlation between higher LDH levels and increased pulmonary artery pressures. Additionally, although the event frequency was low during the follow-up period, clinical events were observed more frequently in patients with higher LDH levels.

Although Cox proportional hazards modeling is an appropriate method for evaluating time-to-event outcomes, the small number of MACE events in our cohort raised concerns regarding model instability and overfitting. For this reason, Cox regression and spline-based sensitivity analyses for LDH were not performed in the present pilot study. Likewise, the limited sample size did not allow meaningful stratification by valve position. These advanced analyses will be incorporated in future studies with larger and adequately powered populations.

Subclinical microangiopathic hemolysis (MIH) has been reported in individuals with prosthetic heart valves, with prevalence rates varying from 26 to 95% depending on the diagnostic criteria [36]. Data on bioprosthetic heart valves are limited, but existing studies have reported that subclinical MIH occurs in about 5% of bioprosthetic valves, where the valves are functional and degeneration has not yet developed [34]. Turbulent flow is considered to play a significant role in the mechanisms underlying hemolysis, which is why older-generation valves, which are disadvantageous in terms of turbulent flow, have been associated with more severe hemolysis and anemia. In newer valve designs, this issue has largely been resolved, and significant erythrocyte destruction leading to heart failure and hemolytic anemia only occurs if there is substantial paravalvular leakage. The "cage-top" model of mechanical valves, valve position, valve size, mechanical valve mean gradient, and valve brand have been identified as factors that influence the severity of MIH in studies. Additionally, there are studies in the literature indicating that left ventricular systolic function can also affect the severity of hemolysis [37]. In our study, the presence of a mechanical valve implanted in the mitral position was found to be associated with greater hemolysis severity [38, 39]. Furthermore, in line with other studies in the literature, we found that the group with higher hemolysis severity had a significantly higher RDW value, which is an indicator of differences in erythrocyte size/volume in the circulation. However, this can also be seen as a result of nutritional deficiencies or other causes of hemolysis, which can develop as a consequence of MIH. Therefore, using RDW as a parameter in the follow-up of MIH severity may not be appropriate [36, 40].

Although prosthesis-related factors such as valve model and size, post-procedural gradients, paravalvular leak, prosthesis–patient mismatch, and anticoagulation intensity are clinically relevant, these parameters were not consistently available in this pilot retrospective cohort, and the limited sample size precluded reliable multivariable adjustment. Future prospective studies with systematic collection of prosthesis-specific data are warranted.

Intravascular hemolysis, which constitutes a significant portion of prosthetic valve-related MIH, results in the release of free hemoglobin (Hb), heme, and erythrocyte arginase into the plasma. These molecules are thought to play a key role in several pathophysiological mechanisms by directly inactivating nitric oxide (NO) and reducing the substrate for NO synthesis. In vitro and in vivo studies have demonstrated that these molecules decrease NO reserve and bioactivity. Symptoms and clinical findings such as dysphagia, erectile dysfunction, abdominal pain, thromboembolic

events, and fatigue in patients with chronic hemolytic anemia have been evaluated in relation to the reduction in NO reserve. In patients with paroxysmal nocturnal hemoglobinuria (PNH), inhibition of phosphodiesterase-5, which breaks down cyclic guanosine monophosphate, responsible for intracellular effects in the NO signaling pathway, has been shown to reduce erectile dysfunction associated with chronic hemolysis. This strengthens the view that NO reserve plays a critical role in pathophysiology. Another important aspect in diseases characterized by chronic hemolysis is the frequent observation of elevated pulmonary artery pressures and pulmonary hypertension, which has been linked to reduced nitric oxide bioavailability. The incidence of PHT assessed by echocardiographic methods in patients with chronic hemolysis due to PNH has been reported to be 50%, and it has been shown that PHT in these patients is largely due to pulmonary embolism. This incidence rate has been reported as 10–75% in β-thalassemia patients and 30% in patients with sickle cell disease (OHA). Although thrombotic lesions are the primary mechanism in OHA patients, it is thought that the reduction in NO reserve and the increase in endothelin-1 levels via free hemoglobin and heme released from intravascular hemolysis also play a role in the pathophysiological process of PHT. In interventional studies testing treatment approaches to reduce intravascular hemolysis and the effects of molecules released as a result of hemolysis, promising results have been obtained, indicating a strong relationship between intravascular hemolysis and PHT [32, 41, 42]. In all three diseases, PHT appears as an important determinant of prognosis and mortality [43–45]. In PNH patients with low levels of complement inhibitors, eculizumab antibody therapy, which prevents the activation of complement 5, has been shown to normalize both hemolysis severity and pulmonary artery pressures, supporting an observed association between hemolysis and the development of pulmonary hypertension and other adverse clinical findings, potentially mediated by reduced nitric oxide bioavailability. Furthermore, in chronic hemolytic diseases, in addition to the decrease in NO reserve due to hemolysis, endothelial dysfunction, pro-inflammatory effects, platelet activation, accompanying predisposing genetic factors (hereditary elliptocytosis, OHA, etc.), and increased cardiac output are thought to also contribute to the development of PHT. In our study, the positive correlation between follow-up LDH levels, new PHT development, and the increase in sPAB follow-up values is consistent with previous studies on the relationship between intravascular hemolysis and PHT.

Pulmonary hypertension was assessed echocardiographically at predefined follow-up intervals; however, mPAP estimation based on the Chemla formula has known limitations in the absence of right heart catheterization, including limited accuracy and susceptibility to measurement variability. Therefore, causal inferences should be interpreted with caution in the context of this observational design.

However, the presence of uncontrolled factors in the study population, the timing of surgical treatment for VHD, and the inability to evaluate the optimal prosthetic valve size selected for each patient make it difficult to establish a cause-and-effect relationship.

Valve diseases can lead to pulmonary hypertension (PHT) if they are severe and intervention is delayed. In particular, in mitral valve pathologies, the impairment of forward blood flow and the pooling of blood in the pulmonary veins and left atrium results in elevated pulmonary capillary wedge pressure (PCWP), leading to the development of Group 2 PHT. If there is a delay in valve intervention or if the valve size selected for implantation is too small (PPM; “prosthesis-patient mismatch”), PHT may become persistent in the postoperative period, and persistent PHT (pPHT) is a significant cause of mortality and morbidity [46]. Studies on patients undergoing mitral valve replacement (MVR) and repair have shown that the development of pPHT is less frequent in non-rheumatic primary mitral valve pathologies [46–48]. In our study, in the group undergoing MVR, postoperative transthoracic echocardiography (TTE) follow-up revealed that patients with a higher mean gradient, transvalvular-paravalvular mitral regurgitation, and relatively small valve sizes (≤28 mm) had more severe hemolysis. Additionally, when comparing surgical groups, patients undergoing MVR due to mostly rheumatic causes had significantly higher fLDH values compared to the aortic valve replacement (AVR) group. There were also differences observed between the AVR and MVR groups in follow-up sPAB values, correlating with tLDH values. Therefore, in patients undergoing valve replacement, the severity of chronic hemolysis, in addition to valve function and the timing of the procedure, may be associated with the persistence of pPHT. The correlation we observed between PHT parameters and hemolysis severity is consistent with this hypothesis. To evaluate the effect of left heart pathologies, which are the most important cause of all forms of PHT, on this correlation, an analysis was conducted on patients without systolic dysfunction, and significant correlations were found between follow-up sPAB and ΔsPAB measurements and tLDH values. In our study, due to the significantly higher severity of hemolysis and the trend of increased sPAB in the group undergoing double valve replacement (AVR + MVR), this group was compared with the group undergoing single valve replacement, and no differences were found between the groups, except for baseline and follow-up left ventricular ejection fraction (LVEF). Although the number of patients with clinical symptoms of congestive heart failure to (CHF) in the study group was

low, this finding may be related to the impact of left ventricular systolic dysfunction on pPHT and hemolysis severity.

Similar to the development of pulmonary hypertension (PHT) in intravascular hemolysis, the reduction in nitric oxide (NO) reserve, the development of oxidative stress, endothelial damage, increased inflammation and immune response, increased lipoprotein oxidation, and procoagulant effects are also thought to contribute to pathways involved in the development of atherosclerosis. An ex-vivo study suggested that the increase in heme following hemorrhage and hemolysis could induce endoplasmic reticulum stress and heme-associated cell death, potentially leading to the development of pathologies, including atherosclerosis. The study emphasized that the most important mechanism for counteracting the harmful effects of heme in the body is the synthesis of heme oxygenase-1 (HO-1), which can be induced [49]. Similar to our study, there is a lack of sufficient high-level clinical evidence in the literature regarding the outcomes of subclinical intravascular hemolysis in patients with prosthetic heart valves. However, the relationship between the severity of intravascular hemolysis, endothelial dysfunction, atherosclerosis, and major cardiovascular events (MACE) remains an important area of research in both past and current studies. Many observational studies conducted on diseases characterized by intravascular hemolysis have shown that the severity of hemolysis is associated with poor clinical outcomes in cardiovascular diseases [23–27]. A study conducted in Sweden demonstrated that autoimmune hemolytic anemia significantly increased the risk of coronary artery disease (CAD) [28]. These findings are consistent with the hypothesis that the severity of subclinical intravascular hemolysis could be associated with a higher frequency of MACE over the long term in our study. On the other hand, a cohort study in the African-American population reported that carrying the known predisposing factor for intravascular hemolysis/hemolytic anemia, OHA, did not increase the frequency of CAD and MACE [50]. In another study conducted on mice, it was found that subjects with OHA disease developed arterial thrombosis later than healthy subjects despite induced carotid artery thrombosis [51]. In the same study, chronic inhibition of HO-1 in OHA mice led to an increase in atherosclerotic lesion areas and a significant shortening of the time before carotid artery thrombosis, suggesting that HO-1 may serve as a protective factor against atherosclerosis. The results of the study paradoxically suggest that OHA carriers may have a protective effect against atherosclerosis and thrombosis. However, it may be more accurate to interpret this as a result of the body's protective defense mechanisms, primarily the increased activity and gene expression of the HO-1 enzyme, against the hemolytic risk posed by OHA, which has anti-atherosclerotic and anti-thrombotic effects. Therefore, although the degree of the relationship between hemolysis severity and MACE remains uncertain due to methodological limitations and the small number of events in our study, we believe it could be clinically significant.

The major limitations of our study evaluating the long-term clinical outcomes of subclinical intravascular hemolysis in patients with prosthetic heart valves are as follows: First, it is a single-center, retrospective study with a relatively small sample size, meaning that our study cannot provide definitive explanations for the mechanisms between hemolysis severity and clinical cardiovascular events or the development of pulmonary hypertension. Additionally, since our patient selection method does not ensure randomization, there could be potential bias. Second, the classification of hemolysis diagnosis and severity was based solely on follow-up LDH levels; a retrospective study model is not ideal for excluding other conditions that might influence LDH levels (see Table 3). In a prospective study, other laboratory findings that support the diagnosis of intravascular hemolysis (MIH) besides LDH would provide more precise results for interpreting the presence and severity of hemolysis. Lastly, in the analysis of pulmonary hypertension and atherosclerosis progression, only echocardiographic methods and MACE evaluations were used. No invasive or non-invasive imaging or stress testing was performed to assess severe coronary artery disease (CAD), and right heart catheterization, which is necessary for the definitive assessment of PHT, was not performed.

The low event frequency in our study population may have also influenced the results. The lower mean age at the end of follow-up (57.5 ± 12.5 years) and the relatively lower prevalence of CAD risk factors compared to the general population may have contributed to the low frequency of events. Furthermore, it should be noted that our study population consisted of patients who underwent surgery and are under regular follow-up in our hospital. Since we were unable to access patients who were followed up at other centers or those who had died or experienced cardiovascular events, the findings should be interpreted with the potential for selection bias in mind.

Conclusion

This study suggests that subclinical intravascular hemolysis in patients with prosthetic heart valves may be associated with the development of cardiovascular diseases and pulmonary hypertension. In routine clinical practice, evaluating the severity of hemolysis through LDH levels could serve as a prognostic factor in this patient group and may contribute to the appropriate management and treatment of these patients. To fully understand the clinical significance

of subclinical intravascular hemolysis related to prosthetic heart valves in our clinical practice, further research, including large-scale prospective randomized controlled clinical trials, is needed.

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Data availability The data underlying this study are available from the corresponding author upon reasonable request. Due to ethical and institutional restrictions, the data are not publicly available.

Declarations

Conflict of interest The named authors have no conflict of interest, financial or otherwise.

Consent publication The authors confirm that written consent for submission and publication of this case report, including imaging and associated text, has been obtained from the patient's family in line with COPE guidance.

Ethics approval This study was approved by the Ankara University Faculty of Medicine Ethics Committee on July 27, 2023 (Approval No: I07-447–23).

Clinical trial registration N/A

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