

# Post-Cardiopulmonary Bypass Alterations in Liver Delta Ratio: A Retrospective Clinical Study

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## Abstract

**Aim:** To investigate the effect of cardiopulmonary bypass (CPB) on the liver using data obtained from differences in liver-related blood parameters in blood samples taken before and after coronary artery bypass grafting surgery.

**Methods:** This retrospective study included a total of 88 patients who underwent coronary artery bypass graft surgery. A difference analysis was performed for AST, ALT, LDH, CRP, RBC, Hct, albumin, CRP, lactate, K, and bilirubin levels. Kruskal-Wallis and correlation analyses were used to evaluate the relationships between the differences in the data.

**Results:** When preoperative and postoperative values were compared, significant differences were found between AST difference ALT, AST difference LDH, ALT difference LDH, ALT difference CRP, LDH difference CRP, RBC difference Hct, and Albumin difference CRP ( $p < 0.05$ ). GGT, albumin, Hct, RBC, and potassium levels decreased significantly ( $p < 0.05$ ).

**Conclusions:** Significant increases in AST, ALT, LDH, total and direct bilirubin, and CRP levels were observed in the postoperative period, while significant decreases were observed in albumin, Hct, and RBC counts. The increase in AST levels was found to be related to the total perfusion time, while the increase in LDH was sensitive to the cross-clamp time.

**Keywords:** Liver parameters; Cardiopulmonary bypass; Cardiopulmonary bypass and hematological parameters; De Ritis

## 1. Introduction

Intraoperative factors contributing to the development of liver dysfunction after cardiac surgery include cardiopulmonary bypass (CPB), cross-clamping, and surgical procedure duration. Open heart surgery threatens the liver's homeostatic environment due to the physiological, immunological, and metabolic requirements of CPB.<sup>1</sup> Reduced perfusion, hemolysis, and obstruction play a role in the development of this threat. As a result, increases in transaminases such as aspartate aminotransferase (AST) and alanine transaminase (ALT), as well as liver function biomarkers, may be observed.<sup>2</sup> Transaminases such as AST and ALT are routine parameters that can be easily evaluated in clinical practice.<sup>3</sup> The predictive value of transaminases on morbidity and mortality has been studied more in patients with chronic heart failure than in those with acute myocardial infarction (AMI) or coronary artery disease. Organ perfusion in AMI patients is related to the severity of myocardial damage and its effect on cardiac contractility.

According to data obtained from studies conducted on patients with chronic heart failure, elevated liver enzymes have been reported to be associated with chronic hypoxia-induced liver damage. However, liver damage is generally only observable after

AMI. The AST/ALT (De-Ritis) ratio may serve as a biomarker for ischemic end-organ damage during the acute phase of AMI.<sup>4</sup> Plasma AST and ALT levels are within normal limits in healthy individuals. These parameters may vary depending on coronary heart disease, chronic hepatitis, renal dysfunction, and certain medications. It has been reported that it is more appropriate to evaluate AST and ALT together as a single parameter rather than evaluating them separately.<sup>5</sup>

The aim of our retrospective study is to investigate the effect of cardiopulmonary bypass on the liver using data obtained from differences in liver-related blood parameters in blood samples taken before and after coronary artery bypass grafting surgery.

## 2. Materials and Methods

### Study design and patient selection

This study was conducted using a retrospective and cross-sectional design. Data were obtained from the archived medical records of patients who underwent elective isolated CABG procedures

at the Cardiovascular Surgery Clinic of Harran University Hospital between 2024 (January)-2025 (May). A total of 88 patients who underwent coronary artery bypass grafting (CABG) surgery were included in the study. Ethical approval for the study was obtained from the Harran University Clinical Research Ethics Committee (Decision No: HRÜ/25.13.57, Date: 14.07.2025). The study was carried out in accordance with the principles of the Declaration of Helsinki.

#### Participant selection

The study included patients aged 18–80 years who had undergone CABG surgery, had no active liver disease, hematological disease, or malignancy in the preoperative period, and had complete biochemical data.

#### Data collection and evaluated parameters

Biochemical parameters of patients were evaluated based on laboratory data obtained at two different time points: the preoperative period, which includes the period after anesthesia and before bypass, and the postoperative period, which includes data obtained within 24–48 hours after the end of CPB. The parameters examined were AST, ALT, gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), lactate, Albumin, C-reactive protein (CRP), Red blood cells (RBC), Hematocrit [Hct (%)], and Potassium (K) levels.

#### Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA) and Python 3.10 statistical packages. The Wilcoxon Signed-Rank Test, which is appropriate for comparing two nonparametric and dependent groups, was used. For parameters with significant differences detected in comparisons between the three time points, post-hoc pairwise comparisons were performed with Bonferroni correction. The Kruskal–Wallis test was applied for intergroup comparisons. The relationships between parameters were evaluated using the Spearman correlation coefficient, and a  $p < 0.05$  value was accepted as the statistical significance threshold in all analyses. Additionally, multiple linear regression analyses were performed to examine the relationship between postoperative changes in liver function parameters (ALT, AST, GGT, LDH, Albumin, Total Bilirubin) and erythrocyte levels (RBC) with patient-specific clinical parameters. The difference between postoperative and preoperative values (Post – Pre) was defined as the dependent variable for each parameter; age, gender, cross-clamp time, and total perfusion time were included in the model as independent variables. The overall significance of the regression models was assessed using the F test, and the level of explanatory power was reported using R-squared ( $R^2$ ). Significant factors were indicated by the  $\beta$  coefficient and p-value.

### 3. Results

Descriptive statistics of the demographic characteristics of the patients included in the study and some parameters related to surgery are presented in Table 1.

The mean age of the 88 patients included in the study was  $61.22 \pm 8.07$  years, and the median age was 61.00 (39.00–77.00). The mean height was  $166 \text{ cm} \pm 0.8$ , and the median height was 165 cm (150–185). The average body weight of the patients was  $79.51 \pm 13.85$  kg, with a median of 80.00 (55.00–114.00) kg. The average body surface area (BSA) was  $1.87 \pm 0.18 \text{ m}^2$ , with a median of 1.89 (1.50–2.21)  $\text{m}^2$ .

The mean flow rate applied during cardiopulmonary bypass related to surgery was  $4497.30 \pm 422.64 \text{ mL/min}$ , with a median value of 4535.00 (3670.00–5340.00) mL/min. The mean cross-clamp time was  $48.67 \pm 10.46$  minutes, with a median of 48.00 (30.00–78.00) minutes; the mean total perfusion time was  $76.17 \pm 10.25$

minutes, with a median of 76.00 (56.00–100.00) minutes.

Of the 88 patients included in the study, 64.8% were male ( $n=57$ ) and 35.2% were female ( $n=31$ ). The majority of patients underwent four-vessel coronary artery bypass grafting (CABGX4) ( $n=40$ , 45.5%), followed by CABGX3 ( $n=26$ ), CABGX5 ( $n=13$ ), CABGX2 ( $n=6$ ), and CABGX1 ( $n=3$ ). When comorbidities were evaluated, the most common group was those with the DM + HT combination (28 patients, 31.8%). This was followed by DM alone (14 patients, 15.9%), DM + HT + CAD (12 patients, 13.6%), and HT alone (8 patients, 9.1%). There were 6 patients (6.8%) with DM + CAD, 3 patients (3.4%) with HT + CAD, and 1 patient (1.1%) with CAD alone. The number of patients without any comorbidities was 16 (18.2%).

The Wilcoxon Signed-Rank Test, which is suitable for testing the difference between two nonparametric and dependent groups, was used. The mean, standard deviation, and median values of the biochemical and hematological parameters measured in the preoperative and postoperative periods, along with the significance results of the Wilcoxon test, are presented in Table 2.

**Table 1**

Descriptive Statistics of Demographic Characteristics and Surgery-Related Parameters

Parameter	Mean $\pm$ SD	Median (Min–Max)
Age (year)	$61.22 \pm 8.07$	61.00 (39.00–77.00)
Height (cm)	$166 \pm 0.8$	165 (150–185)
Weight (kg)	$79.51 \pm 13.85$	80.00 (55.00–114.00)
BSA	$1.87 \pm 0.18$	1.89 (1.50–2.21)
Flow (ml/min)	$4497.30 \pm 422.64$	4535.00 (3670.00–5340.00)
Cros Time (min)	$48.67 \pm 10.46$	48.00 (30.00–78.00)
Total Time (min)	$76.17 \pm 10.25$	76.00 (56.00–100.00)
Gender	Category	Number of Patients
Operation Type	(Male/Female)	57/31
	CABGX1	3
	CABGX2	6
	CABGX3	26
	CABGX4	40
	CABGX5	13
Comorbidity	No	16
	DM	14
	HT	8
	CAD	1
	DM + HT	28
	DM + CAD	6
	HT + CAD	3
	DM + HT + CAD	12

CABG: Coronary artery bypass graft, CAD: Coronary artery disease, DM: Diabetes Mellitus, HT: Hypertension, Max: Maximum, Min: Minimum, min: minutes, SD: Standard Deviation,

When preoperative and postoperative values were compared; AST level from  $32.53 \pm 32.07$  to  $63.02 \pm 42.85$ , ALT level from  $24.90 \pm 18.63$  to  $43.67 \pm 48.15$ , LDH level from  $343.45 \pm 126.36$  to  $535.25 \pm 209.27$  ( $p=0.0000$ ), total bilirubin level from  $0.65 \pm 0.33$  to  $1.32 \pm 5.64$  ( $p=0.0208$ ), direct bilirubin level from  $0.20 \pm 0.11$  to  $0.28 \pm 0.17$  and CRP level increased significantly from  $1.99 \pm 2.45$  to  $25.89 \pm 16.59$  ( $p=0.0000$ ).

**Table 2**

Descriptive Statistics of Parameters Measured During the Preoperative and Postoperative Periods and Results of the Wilcoxon Signed-Rank Test

Parameter	Pre-op Mean±SD	Post-op Mean±SD	Pre-Median (Min-Maks)	Post-Median (Min-Maks)	Wilcoxon p-value
AST (U/L)	32.53±32.07	63.02± 42.85	24.00 (7.00-267.00)	47.50 (13.00-182.00)	0.0000
ALT (U/L)	24.90±18.63	43.67± 48.15	19.00 (6.00-109.00)	28.00 (5.00-264.00)	0.0000
GGT (IU/L)	30.39±25.50	24.76± 20.72	24.50 (3.00-159.00)	21.00 (2.00-178.00)	0.0062
T.Bil. (mg/dL)	0.65±0.33	1.32±5.64	0.60 (0.20-2.10)	0.66 (0.24-53.50)	0.0208
D.Bil. (mg/dL)	0.20±0.11	0.28±0.17	0.20 (0.05-0.60)	0.23 (0.08-1.19)	0.0000
LDH (U/L)	343.45±126.36	535.25± 209.27	328.00 (172.00-778.00)	494.00 (274.00-1142.00)	0.0000
Lactate (U/L)	2.06±1.01	2.03±1.06	1.90 (1.00-5.30)	1.80 (1.00-5.40)	0.0625
Albumin (g/dl)	4.15±0.40	3.22±0.42	4.20 (2.60-5.10)	3.20 (2.40-4.60)	0.0000
CRP (mg/L)	1.99±2.45	25.89±16.59	0.79 (0.09-10.10)	25.20 (4.39-65.00)	0.0000
RBC (million/mm <sup>3</sup> )	4.96±0.45	3.37±0.51	5.01 (3.85-6.00)	3.38 (2.13-4.83)	0.0000
Hct (%)	39.61±4.15	28.53±4.20	39.90 (28.70-47.90)	28.50 (18.90-38.90)	0.0000
K (mEq/L)	4.26±0.41	4.24±0.45	4.25 (3.50-5.40)	4.20 (3.00-5.60)	0.0004

ALT: Alanine aminotransferase, AST: Aspartate Aminotransferase, CRP: C-reactive protein, D.Bil.: Direct bilirubin, GGT: Gamma-glutamyl transferase, Hct: Hematocrit, K: Potassium, LDH: Lactate dehydrogenase, Pre-op: Preoperative, post-op: Postoperative, RBC: Red blood cells, T.Bil.: Total Bilirubin

**Table 3**

Kruskal-Wallis Test Results of the Difference Values of Preoperative and Postoperative Biochemical Parameters According to Cross-Clamp Time, Total Perfusion Time and Comorbidity Status

Parameter	Cross Time H	Cross Time p-value	Total Time H	Total Time p-value	Comorbidity H	Comorbidity p-value
AST (U/L)	0.981	0.6124	7.465	0.0239*	3.358	0.4998
ALT (U/L)	0.335	0.8457	1.350	0.5091	2.376	0.6669
GGT (IU/L)	0.702	0.7039	5.034	0.0807	2.739	0.6025
T.Bil (mg/dL)	1.834	0.3997	0.981	0.6122	3.932	0.4153
DBil (mg/dL)	2.986	0.2247	0.784	0.6758	4.192	0.3806
LDH (U/L)	0.812	0.6659	2.188	0.3359	2.147	0.7097
Lactate (U/L)	1.356	0.5074	3.245	0.1970	3.672	0.4527
Albumin (g/dl)	0.631	0.7287	1.040	0.5948	3.083	0.5443
CRP (mg/L)	0.894	0.6404	1.904	0.3855	3.588	0.4648
RBC (million/mm <sup>3</sup> )	2.702	0.2592	1.415	0.4919	1.310	0.9339
Hct (%)	3.277	0.1946	0.460	0.7945	0.857	0.9710
K (mEq/L)	2.617	0.2701	2.216	0.3295	3.040	0.5512

ALT: Alanine aminotransferase, AST: Aspartate Aminotransferase, CRP: C-reactive protein, D.Bil.: Direct bilirubin, GGT: Gamma-glutamyl transferase, Hct: Hematocrit, K: Potassium, LDH: Lactate dehydrogenase, Pre-op: Preoperative, post-op: Postoperative, RBC: Red blood cells, T.Bil.: Total Bilirubin

In contrast, GGT level decreased from 30.39 ± 25.50 to 24.76 ± 20.72 (p=0.0062), albumin level from 4.15 ± 0.40 to 3.22 ± 0.42, Hct (%) value from 39.61 ± 4.15 to 28.53 ± 4.20, RBC level from 4.96 ± 0.45 to 3.37 ± 0.51 (p=0.0000) and potassium level decreased significantly from 4.26 ± 0.41 to 4.24 ± 0.45 (p=0.0004). The change in lactate level (2.06 ± 1.01-2.03 ± 1.06) was not found to be statistically significant (p=0.0625) (p>0.05).

Biochemical parameters created based on the differences between the values of preoperative and postoperative biochemical parameters were compared according to surgical times (cross-clamp and total perfusion time) and comorbidity conditions (Table3).

In the Kruskal-Wallis analysis, a significant change was only observed in terms of the difference between preoperative and postoperative AST and total perfusion time (p=0.0239). No significant difference was observed in the other effects (p>0.05).

Within the scope of the study, the Spearman correlation coefficients between biochemical and clinical parameters are shown in figure 1.

Correlations found to be significant at the p<0.05 level are presented in Table 4.

According to Spearman correlation analysis, significant relationships were found between some biochemical and hematological parameters. Strong positive correlations were observed between the AST difference and the ALT difference (r=0.541, p<0.001) and the LDH difference (r=0.544, p<0.001). The ALT difference also showed a significant positive correlation with the LDH difference (r=0.516, p<0.001). Additionally, there are weak but significant positive correlations between the ALT difference and the CRP difference (r=0.240, p=0.024) and between the LDH difference and the CRP difference (r=0.241, p=0.024). A very strong correlation was deter-

mined between the RBC difference and the hematocrit (Hct) difference ( $r=0.899$ ,  $p<0.001$ ). On the other hand, a negative significant relationship was found between the albumin difference and the CRP difference ( $r=-0.422$ ,  $p<0.001$ ).

**Table 4**

Significant Spearman Correlations Between Preoperative and Postoperative Difference Values

Correlation between Parameters	Correlation Coefficient (r)	p-value
AST- ALT difference	0.541	<0.001
AST- LDH difference	0.544	<0.001
ALT- LDH difference	0.516	<0.001
ALT- CRP difference	0.240	0.024
LDH- CRP difference	0.241	0.024
RBC- Hct difference	0.899	<0.001
Albumin- CRP difference	-0.422	< 0.001

ALT: Alanine aminotransferase, AST: Aspartate Aminotransferase, CRP: C-reactive protein, Hct: Hematocrit, LDH: Lactate dehydrogenase, RBC: Red blood cells,

**Table 5**

Multiple linear regression analyses for liver function tests and RBC change (Post-Pre differences)

Parameter	R-squared	Model p-value	Significant Variable(s)
ALT Increase	0.062	0.250	None
AST Increase	0.096	0.076	Gender (p = 0.040)
GGT Increase	0.132	0.018	Total time (p = 0.031)
Albumin Change	0.129	0.021	Gender (p = 0.055), Cross time (p = 0.064)
LDH Increase	0.161	0.0053	Cross time (p = 0.004)
Total Bilirubin Change	0.031	0.626	None
RBC Change	0.062	0.251	None

ALT: Alanine aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase, RBC: Red blood cells,

In the regression analysis performed, weak but significant positive correlations were found between the change in ALT and the change in CRP ( $r=0.240$ ,  $p=0.024$ ) and between the change in LDH and the change in CRP ( $r=0.241$ ,  $p=0.024$ ). A very strong correlation was observed between changes in RBC and changes in hematocrit (Hct) ( $r=0.899$ ,  $p<0.001$ ).

Multiple linear regression analyses were performed to evaluate the relationships between changes in liver function parameters (ALT, AST, GGT, Albumin, LDH, Total Bilirubin) and erythrocyte levels (RBC) in the patients included in the study and age, gender, cross-clamp time, and total cardiopulmonary bypass time. The pre- and post-operative difference (post-pre) for each biomarker was included in the model as a dependent variable. Summary data regarding the regression analyses are presented in Table 5. The explanatory levels ( $R^2$ ) of the model, overall significance (model p-value), and significant variables are summarized below.

No statistically significant relationship was found between the increase in ALT levels and any independent variable ( $R^2=0.062$ ,  $p=0.250$ ). The increase in AST levels was found to be significantly associated with female gender ( $\beta=23.14$ ,  $p=0.040$ ). For the increase in GGT levels, total bypass time was found to be a significant nega-

tive predictor ( $\beta=-0.50$ ,  $p=0.031$ ). The decrease observed in albumin levels was marginally more significant in females ( $p=0.055$ ) and showed a marginally significant negative correlation with cross-clamp time ( $p=0.064$ ). The increase in LDH levels was positively and significantly associated with cross-clamp time ( $\beta=13.03$ ,  $p=0.004$ ). No significant relationship was found between the change in total bilirubin levels and any independent variable in the model ( $R^2=0.031$ ,  $p=0.626$ ). Similarly, the change in RBC levels did not show a significant variation based on age, gender, and surgical duration ( $R^2=0.062$ ,  $p=0.251$ ).

#### 4. Discussion

Recent studies on liver function tests during the preoperative and postoperative periods of CPB have focused on the AST/ALT (De Ritis) ratio. In our study, we examined the differences between these parameters rather than their ratios.

In their study, Amaç et al. found it appropriate to use the AST/ALT ratio as a marker for postoperative outcomes in cardiac surgery by applying different cardioplegia solutions. They reported that the AST/ALT ratio was lower in the Del Nido cardioplegia group compared to the blood cardioplegia group in the postoperative period after CPB.<sup>6</sup> A study examining the relationship between the AST/ALT ratio and cardiovascular disease mortality in peritoneal dialysis patients revealed that peritoneal dialysis patients with high AST/ALT ratios are at serious risk for cardiovascular disease mortality.<sup>7</sup> In our study, when preoperative and postoperative values were compared, a significant increase in AST and ALT levels was observed. Additionally, when the parameters were compared in terms of total perfusion time, a statistically significant difference was found between the AST difference and total perfusion time.

AST, ALT and LDH levels have been suggested to predict all-cause mortality in heart failure.<sup>8</sup> Furat et al divided a total of 60 patients scheduled for CABG surgery into three groups, each receiving one of a different myocardial protection strategy. Preoperative and postoperative blood samples were taken. When the groups were compared, no significant difference was observed in perioperative LDH levels, but an increase in postoperative LDH levels was reported.<sup>9</sup> In parallel with this study, in our own study, LDH levels were significantly increased in the postoperative period; in addition, a significant positive correlation was found between the AST difference and the LDH difference and the ALT difference and the LDH difference. The increase in LDH level was positively and significantly correlated with cross-clamp duration, supporting ischemia-induced cellular damage.

A meta-analysis of 29,401 patients undergoing cardiac surgery by Heo et al. found that preoperative and postoperative biomarkers in open heart surgery, particularly CRP, were associated with an increased risk of all-cause mortality and major adverse cardiovascular events.<sup>10</sup> However, some data have reported that CPB does not affect CRP levels.<sup>11</sup> In our study, when the values before and after CPB were compared, a significant increase in CRP values was observed. In addition, there are weak but significant positive correlations between the ALT difference and the CRP difference and the LDH difference and the CRP difference.

In a study in which the mean CPB duration was determined in patients undergoing heart valve surgery and patient groups were formed according to longer and shorter durations, it was explained that GGT levels were higher in the group with longer perfusion time;<sup>12</sup> however, in our study, total bypass time was found to be a significant negative predictor of an increase in GGT levels. In other words, as the bypass duration increases, the GGT increase decreases.

In a study examining the effect of CPB on hemogram parameters, it was observed that the Hct value decreased compared to the pre-operative period.<sup>13</sup> Similarly to this study, in our work, when pre-operative and postoperative values were compared, a significant decrease was observed in hematocrit and RBC levels. A very strong correlation was found between the RBC difference and the Hct difference.

Due to insufficient tissue oxygenation or circulatory disorders, a shift to anaerobic respiration may occur, leading to elevated lactate levels. In the study conducted by Bayanmelek et al., no significant differences were found between the groups in terms of pre- and post-CPB lactate values; however, the lactate values at the 24th hour in the intensive care unit were found to be significant.<sup>14</sup> In our study, however, the change in lactate levels before and after CPB was not found to be statistically significant.

Albumin deficiency (hypoalbuminemia) may occur due to liver disease.<sup>15</sup> In a study that followed more than 100,000 people for 8.5 years, low serum albumin levels were reported to independently predict myocardial infarction, ischemic heart disease, and ischemic stroke.<sup>16</sup> However, in our study, it was observed that significant changes in albumin levels, particularly in female patients, were associated with intraoperative factors such as total bypass and cross-clamp times, indicating that gender-related physiological differences should be taken into account. Furthermore, a significant negative correlation was found between albumin changes and CRP changes, suggesting that albumin levels decrease as the level of inflammation increases.

In a study conducted to evaluate changes in liver function tests and the risk factors associated with these changes following CABG surgery performed using the CPB technique, it was reported that preoperative total bilirubin and direct bilirubin levels reached their highest values on the second postoperative day.<sup>17</sup> Similarly, in our study, total bilirubin and direct bilirubin levels increased significantly in the postoperative period.

#### Limitations

Among the limitations of this study is that some patients had diabetes and hypertension in addition to coronary artery disease. Another limitation is that the study was single-center and retrospective in design, and this center may not be representative of national rates. Therefore, multicenter and larger-scale studies are needed.

## 5. Conclusions

This study revealed that significant changes occurred in liver function parameters after CPB. In particular, a marked postoperative increase was observed in AST, ALT, LDH, total and direct bilirubin, and CRP levels, while albumin, hematocrit, and erythrocyte counts showed a significant decrease. The increase in AST levels was found to be associated with total perfusion time, whereas the rise in LDH was determined to be sensitive to cross-clamp duration. These findings highlight the importance of optimizing surgical times during CPB procedures, closely monitoring liver functions, and carefully evaluating preventive measures against possible complications. We also believe that delta changes in liver function tests are predictive of mortality and morbidity in cardiovascular diseases.

#### Statement of ethics

Ethical approval for the study was obtained from the Harran University Clinical Research Ethics Committee (Decision No: HRÜ/25.13.57, Date: 14.07.2025).

#### Author Contributions

YH: Study conception and design; first draft of the manuscript, and all authors edited, re-viewed, and approved the final version of the

manuscript. MZB: First draft of the manuscript, and all authors edited, reviewed, and approved the final version of the manuscript. KE: Study conduction, the analysis and interpretation of the results.

#### Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

#### Conflict of interest statement

The authors declare that they have no conflict of interest.

#### Availability of data and materials

The data and materials of this study are available upon reasonable request.

#### genAI

No artificial intelligence-based tools or generative AI technologies were used in this study. The entire content of the manuscript was originally prepared, reviewed, and approved by both authors.

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