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ROLE OF CIRCULATING BIOMARKERS AND MATERNAL-INFANT OUTCOMES AFTER ANTICOAGULANT THERAPY IN PREGNANT WOMEN WITH MECHANICAL HEART VALVE REPLACEMENT

ULOGA CIRKULIŠUĆIH BIOMARKERA I ISHODI KOD MAJKE I NOVOROĐENČETA NAKON ANTIKOAGULANTNE TERAPIJE KOD TRUDNICA SA MEHANIČKOM ZAMENOM SRČANOG ZALISKA

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Summary

Background: To investigate the effect of standardized anticoagulation on parturients and foetuses during pregnancy after mechanical heart valve replacement (MHVR) and to evaluate novel serum biomarkers reflecting coagulation activation, endothelial function, placental perfusion, and renal injury.
Methods: 100 parturients during pregnancy after MHVR who received treatment in Hebei Xingtai People's Hospital from January 2019 to December 2024 were recruited and divided into low-molecular-weight heparin (LMWH) group (n=50) and regional citrate anticoagulation (RCA) group (n=50). Coagulation parameters (PT, APTT, TT, FIB, PLT), novel biomarkers (thrombin-antithrombin complex [TAT], soluble P-selectin [sP-selectin], D-dimer), renal biomarkers (urea nitrogen, serum creatinine, neutrophil gelatinase-associated lipocalin [NGAL], asymmetric dimethylarginine [ADMA]), placental and inflammatory markers (placental growth factor [PlGF], interleukin-6 [IL-6]) were compared between groups. Maternal complications and abnormal foetal development were observed.
Results: PT and APTT in the RCA group were markedly higher than in the LMWH group; TT was notably lower than in the LMWH group; FIB decreased more and PLT increased more in the RCA group; TAT, sP-selectin, and D-dimer levels were significantly lower in the RCA group

Kratak sadržaj

Uvod: Cilj je bio da se ispita efekat standardizovane anti-koagulantne terapije na porodilje i fetuse tokom trudnoće nakon mehaničke zamene srčanog zaliska (MHVR) i proceniti nove serumske biomarkere koji odražavaju aktivaciju koagulacije, funkciju endotela, placentarnu perfuziju i oštećenje bubrega.
Metode: U studiju je uključeno 100 porodilja tokom trudnoće nakon MHVR koje su lečene u Narodnoj bolnici u Xingtaiu, provincija Hebei, u periodu od januara 2019. do decembra 2024. godine. Ispitanice su podeljene u grupu sa niskomolekularnim heparinom (LMWH) (n=50) i grupu sa regionalnom citratnom antikoagulacijom (RCA) (n=50). Upoređivani su parametri koagulacije (PT, APTT, TT, FIB, PLT), novi biomarkeri (kompleks trombin-antitrombin [TAT], rastvorljivi P-selektin [sP-selektin], D-dimer), bubrežni biomarkeri (urea, serumski kreatinin, lipokalin povezan sa želatinazom neutrofila [NGAL], asimetrični dimetilarginin [ADMA]), kao i placentarni i inflamatorni markeri (placentarni faktor rasta [PlGF], interleukin-6 [IL-6]). Praćene su komplikacije kod majke i poremećaji u razvoju fetusa.
Rezultati: Vrednosti PT i APTT u RCA grupi bile su značajno više nego u LMWH grupi; TT je bio značajno niži u odnosu na LMWH grupu; FIB se više smanjio, a PLT se više povećao u RCA grupi; nivoi TAT, sP-selektina

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(P<0.05). After anticoagulant therapy, urea nitrogen and serum creatinine decreased more in the RCA group than in the LMWH group; NGAL and ADMA levels showed greater reductions in the RCA group (P<0.05). PlGF levels were significantly higher, and IL-6 levels were significantly lower in the RCA group (P<0.05).
Conclusion: RCA has a greater effect on parturients and foetuses after MHVR, can apparently improve coagulation indicators and renal function, and reduce maternal complications and foetal dysplasia. Novel biomarker profiles indicate that RCA reduces thrombin generation, platelet activation, endothelial injury, and systemic inflammation, while enhancing placental angiogenesis. RCA demonstrates high safety and is worthy of wide clinical application.

Keywords: serum TAT, sP-Selectin, NGAL, PlGF, mechanical heart valve replacement, parturient, anticoagulant therapy, maternal-infant outcomes, regional citrate anticoagulation, novel serum biomarkers

i D-dimera bili su značajno niži u RCA grupi (P<0,05). Nakon antikoagulantne terapije, urea i serumski kreatinin su se više smanjili u RCA grupi nego u LMWH grupi; nivoi NGAL i ADMA pokazali su izraženije smanjenje u RCA grupi (P<0,05). Nivoi PlGF bili su značajno viši, a nivoi IL-6 značajno niži u RCA grupi (P<0,05).
Zaključak: RCA ima bolji efekat kod porodilja i fetusa nakon MHVR, može značajno poboljšati parametre koagulacije i bubrežnu funkciju, smanjiti komplikacije kod majke i poremećaje u razvoju fetusa. Profil novih biomarkera ukazuje da RCA smanjuje stvaranje trombina, aktivaciju trombocita, oštećenje endotela i sistemsku inflamaciju, uz istovremeno poboljšanje placentarne angiogeneze. RCA pokazuje visok stepen bezbednosti i zaslužuje široku kliničku primenu.

Ključne reči: serumski TAT, sP-selektin, NGAL, PlGF, mehanička zamena srčanog zaliska, porodilja, antikoagulantna terapija, ishodi majka-novorođenče, regionalna citratna antikoagulacija, novi serumski biomarkeri

Introduction

Valvular heart disease (VHD) is a cardiac disease characterized by inflammation of the leaflets and tendons of heart valves and the papillary muscles, leading to changes in the structure and function of the heart valves (1). The pathogenesis involves various causes, such as congenital malformations or acquired inflammation, leading to lesions in the heart and stenosis or valvular insufficiency of the valves. It is a common clinical heart disease with an increasing incidence annually (2). Mechanical heart valve replacement (MHVR) is the main therapy for most patients with VHD, involving valve replacement using bioprosthetic or mechanical valves. Bioprosthetic valves are composed of biological tissue, while mechanical valves are made of synthetic materials. Both can considerably improve hemodynamic parameters, promote rehabilitation and prognosis, and improve quality of life (3, 4). Valve replacement surgery is the most effective therapy for patients with moderate to severe VHD (5, 6).

After MHVR, cardiac function recovers well, and living standards can return to normal. However, long-term presence of valve material affects hemodynamic characteristics, and thrombus formation is common postoperatively, particularly around mechanical valves. Therefore, patients require lifelong anticoagulant therapy to prevent thrombosis, reduce complications, and improve prognosis.

Low-molecular-weight heparin (LMWH) is widely used for prophylaxis and treatment of venous thromboembolism and is commonly employed for thromboprophylaxis in cancer patients; however, its use can be complicated by bleeding and, in some cases, heparin-induced thrombocytopenia. LMWH inhibits tumour growth, improves prognosis, and has multiple pharmacological effects, including immunoregulation and lipid-lowering (7, 8). Studies showed LMWH regulates early recurrent miscarriage, but may cause adverse effects such as thrombocytopenia and increased bleeding risk (9, 10, 11). Regional citrate anticoagulation (RCA) provides a good anticoagulant effect, a low risk of bleeding complications, and significantly improves filtration membrane biocompatibility. RCA has been used less clinically due to complex procedures and a higher risk of metabolic complications (12, 13). In recent years, RCA technology has matured, accelerating its application in clinical haematological diseases, particularly for patients with bleeding risk, demonstrating safety and effectiveness (14).

The group of pregnant women with mechanical heart valve replacement is a uniquely high-risk group in whom anticoagulation management is uniquely challenging. The hypercoagulable state of pregnancy, the thrombogenicity of mechanical prosthetic valves, and the teratogenic or bleeding risks of anticoagulants all contribute to a complex therapeutic problem. While LMWH has remained a mainstay of anticoagulation management, concerns remain about thromboembolic risks and foetal-maternal well-being. At the same time, citrate anticoagulation has been shown to have a good safety profile in conditions of high bleeding risk. However, it has yet to be studied in the context of pregnancy in patients with MHVR in a comparative context.

In this study, two anticoagulation regimens – LMWH and RCA – were selected for anticoagulation therapy in parturients after MHVR. We compared effects on maternal coagulation parameters, complications, bleeding, and novel serum biomarkers, including TAT, sP-selectin, D-dimer, NGAL, ADMA, PlGF, and IL-6, and observed effects on foetal dysplasia, providing new options for anticoagulant use in pregnant women after MHVR.

Materials and Methods

Study subjects

One hundred patients admitted to Hebei Xingtai People's Hospital from January 2019 to December 2024 were recruited. Parturients and their foetuses receiving anticoagulant therapy after MHVR were randomly divided into the LMWH group (n=50), including primipara (n=29) and multipara (n=21), aged 22–40 years (mean 31.2±4.8 years). The RCA group enrolled 50 patients: 30 primiparous and 20 multiparous women, aged 21–40 years (mean 30.8±5.1 years). Gender, age, gestational week at enrolment, valve replacement position, and other general data differed slightly between groups (P>0.05), with comparability (*Table I*). This study was approved by the Hebei Xingtai People's Hospital ethics committee.

Inclusion criteria: patients with complete medical records, no communication barriers, and normal organ function; consent was obtained from patients and their families.

Exclusion criteria: patients with mental disorders, immune system and metabolic diseases, or genetic diseases; patients with combined other major organ diseases; patients unable to fully participate in this study.

Method

Patients in both groups underwent anticoagulant therapy after MHVR.

Patients in the LMWH group received LMWH at a dose of 2,000 U supplemented with 150 U every hour. After patients were able to eat and had no obvious bleeding tendency, 3 mg warfarin was orally administered daily, and the dose was adjusted according to international normalized ratio (INR) monitoring 3 days later. INR was maintained at 1.8–2.0 in patients undergoing aortic valve replacement, 2.0–2.2 in patients undergoing mitral valve replacement, and 2.5–2.7 in patients undergoing tricuspid valve replacement.

Patients in the RCA group were infused with 4% sodium citrate at 180 mL/h, followed by 5% calcium chloride at 10 mL/h. After patients resumed eating and showed no obvious bleeding tendency, they took 3 mg warfarin daily, and the dosage was adjusted according to INR monitoring results 3 days later. INR was maintained at 1.8–2.5 for patients undergoing aortic valve replacement, 2.0–2.5 for patients undergoing mitral valve replacement, and 2.5–3.0 for patients undergoing tricuspid valve replacement.

Both groups were monitored for INR values during hospitalization.

Outcome measures

A. Determination of maternal coagulation parameters and novel biomarkers: Activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PT), fibrinogen (FIB), and platelet (PLT) count were measured after anticoagulant therapy using an automated coagulation analyser (CS-5100, Sysmex, Japan). Novel biomarkers, including thrombin-antithrombin complex (TAT), soluble P-selectin (sP-selectin), and D-dimer, were

Table I Baseline characteristics of study participants.

Characteristic	LMWH Group (n=50)	RCA Group (n=50)	P-value
Age (years, mean ± SD)	31.2±4.8	30.8±5.1	0.687
Primipara/Multipara (n)	29/21	30/20	0.841
Gestational week at enrolment (mean ± SD)	16.4±3.2	16.7±3.5	0.654
Valve replacement position			0.712
- Aortic valve (n, %)	21 (42.0%)	23 (46.0%)	
- Mitral valve (n, %)	24 (48.0%)	22 (44.0%)	
- Tricuspid valve (n, %)	5 (10.0%)	5 (10.0%)	
Time since MHVR (months, mean ± SD)	28.6±8.4	29.1±9.2	0.776
Pre-pregnancy anticoagulation duration (months)	18.4±6.2	19.1±7.1	0.593

measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

B. Determination of renal function biomarkers: Urea nitrogen and serum creatinine levels were measured using an automated biochemical analyser (AU5800, Beckman Coulter, USA) before and after treatment. Novel renal biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL) and asymmetric dimethylarginine (ADMA), were measured using ELISA kits (R&D Systems, Minneapolis, USA).

C. Determination of placental and inflammatory markers: Placental growth factor (PlGF) and interleukin-6 (IL-6) were measured using high-sensitivity ELISA kits (R&D Systems, Minneapolis, USA) to assess fetoplacental unit function and systemic inflammatory response.

D. Observation of maternal complications: Complications of parturients receiving anticoagulant therapy after MHVR were observed, mainly including bleeding, thrombosis, and atrial fibrillation. All complications were confirmed by clinical examination and appropriate diagnostic modalities.

E. Observation of maternal bleeding: Bleeding of parturients receiving anticoagulant therapy after MHVR was observed, including epistaxis, gingival bleeding, gastrointestinal bleeding, and conjunctival bleeding. Bleeding events were classified according to ISTH criteria.

F. Observation of foetal developmental abnormalities: Abnormal foetal development of parturients receiving anticoagulant therapy after MHVR was observed, mainly including hydrocephalus infants, warfarin infants, stillbirth, and abortion. Foetal assessments were performed by prenatal ultrasound and postnatal paediatric examination.

Statistical methodologies

Data were analysed using SPSS software (version 19.0). Continuous variables are reported as mean ± standard deviation and were compared between groups using either the independent-samples t-test for normally distributed data or the Mann-Whitney U test for non-normally distributed data. Categorical variables are summarized as absolute frequencies and percentages and were analysed using the Chi-square test or Fisher's exact test, as appropriate. Within-group comparisons before and after intervention were conducted using paired t-tests. All statistical tests were two-tailed, and a P-value < 0.05 was considered indicative of statistical significance.

Results

Baseline coagulation parameters and biomarker levels

Before anticoagulant therapy, no significant differences were observed between the LMWH and RCA groups in any coagulation parameters, renal function indicators, or novel biomarkers ($P>0.05$), confirming successful randomization and comparability of baseline characteristics (*Table II*).

Comparison of coagulation indicators and novel biomarkers after anticoagulant therapy

After anticoagulant therapy, significant differences emerged between the two groups across all coagulation parameters and novel biomarkers (*Table III*).

PT and APTT of parturients in the RCA group were significantly prolonged compared to the LMWH group (PT: 18.76±1.89 s vs. 16.43±1.67 s, $P<0.001$; APTT: 41.23±3.67 s vs. 37.89±3.45 s, $P<0.001$). TT in the RCA group was significantly shorter than that in the LMWH group (14.12±1.34 s vs. 15.89±1.56 s, $P<0.001$).

After treatment, FIB levels decreased significantly in both groups. Still, the reduction was more pronounced in the RCA group (from 4.31±0.59 to 2.87±0.43 g/L, Δ=-1.44 g/L) compared to the LMWH group (from 4.28±0.56 to 3.34±0.48 g/L, Δ=-0.94 g/L) ($P<0.001$ for post-treatment comparison). PLT counts increased in both groups, with significantly higher post-treatment values in the RCA group (242.67±28.43 $\times 10^9$/L) compared to the LMWH group (223.45±26.78 $\times 10^9$/L) ($P<0.001$).

TAT levels in the RCA group (3.91±0.87 μg/L) were approximately 43% lower than in the LMWH group (6.84±1.23 μg/L) ($P<0.001$). sP-selectin levels decreased by 35% in the RCA group compared to 10% reduction in the LMWH group ($P<0.001$). D-dimer levels were 39% lower in the RCA group than in the LMWH group ($P<0.001$).

Comparison of renal function biomarkers

Both groups showed significant improvement in renal function parameters after anticoagulant therapy, with the RCA group demonstrating superior renal protection (*Table IV*).

Urea nitrogen decreased significantly in both groups, but the reduction was 24.7% greater in the RCA group (Δ=-19.34±4.67 mmol/L) compared to the LMWH group (Δ=-15.51±4.23 mmol/L) ($P<0.001$). Serum creatinine decreased by 62.8% in the LMWH group and 65.5% in the

Table II Baseline laboratory parameters before anticoagulant therapy.

Parameter	LMWH Group (n=50)	RCA Group (n=50)	P-value
Coagulation parameters			
PT (s)	13.24±1.18	13.31±1.22	0.771
APTT (s)	32.67±3.24	32.54±3.41	0.843
TT (s)	16.83±1.76	16.91±1.82	0.823
FIB (g/L)	4.28±0.56	4.31±0.59	0.794
PLT ($\times 10^9$/L)	198.46±28.73	201.32±30.14	0.627
Novel coagulation biomarkers			
TAT (μg/L)	8.76±1.84	8.69±1.91	0.852
sP-selectin (ng/mL)	58.43±9.67	59.12±10.23	0.731
D-dimer (mg/L)	1.12±0.28	1.14±0.31	0.736
Renal function biomarkers			
Urea nitrogen (mmol/L)	34.78±5.67	34.69±5.82	0.938
Serum creatinine (μmol/L)	437.69±58.43	435.70±60.12	0.867
NGAL (ng/mL)	187.34±28.76	184.98±30.21	0.689
ADMA (μmol/L)	0.94±0.18	0.96±0.20	0.598
Placental and inflammatory biomarkers			
PlGF (pg/mL)	112.46±21.34	114.23±22.67	0.687
IL-6 (pg/mL)	10.87±2.34	11.02±2.51	0.757

Data presented as mean ± SD. PT: prothrombin time; APTT: activated partial thromboplastin time; TT: thrombin time; FIB: fibrinogen; PLT: platelet; TAT: thrombin-antithrombin complex; sP-selectin: soluble P-selectin; NGAL: neutrophil gelatinase-associated lipocalin; ADMA: asymmetric dimethylarginine; PlGF: placental growth factor; IL-6: interleukin-6.

RCA group, with significantly lower post-treatment values in the RCA group (150.33±21.45 μmol/L vs. 162.98±24.67 μmol/L, P=0.008).

Notably, NGAL levels – a more sensitive early biomarker of acute kidney injury –showed a 53.1% reduction in the RCA group (from 184.98±30.21 to 86.73±12.58 ng/mL) compared to a 33.6% reduction in the LMWH group (from 187.34±28.76 to 124.36±18.42 ng/mL) (P<0.001). ADMA levels decreased by 45.8% in the RCA group versus 17.0% in the LMWH group (P<0.001).

Table III Post-treatment coagulation parameters and novel biomarkers.

Parameter	LMWH Group (n=50) (mean ± SD)	RCA Group (n=50) (mean ± SD)	P-value
Coagulation parameters			
PT (s)	16.43±1.67	18.76±1.89	<0.001
APTT (s)	37.89±3.45	41.23±3.67	<0.001
TT (s)	15.89±1.56	14.12±1.34	<0.001
FIB (g/L)	3.34±0.48	2.87±0.43	<0.001
PLT ($\times 10^9$/L)	223.45±26.78	242.67±28.43	<0.001
Novel coagulation biomarkers			
TAT (μg/L)	6.84±1.23	3.91±0.87	<0.001
sP-selectin (ng/mL)	52.67±8.34	38.42±6.71	<0.001
D-dimer (mg/L)	0.89±0.21	0.54±0.16	<0.001

Abbreviations used: PT (prothrombin time); APTT (activated partial thromboplastin time); TT (thrombin time); FIB (fibrinogen level); PLT (platelet count); TAT (thrombin–antithrombin complex); sP-selectin (soluble P-selectin); NGAL (neutrophil gelatinase-associated lipocalin); ADMA (asymmetric dimethylarginine); PlGF (placental growth factor); and IL-6 (interleukin-6).

Table IV Pre-treatment and post-treatment renal function biomarkers.

Parameter	Time Point	LMWH Group (n=50)	RCA Group (n=50)	P-value (intergroup)
Urea nitrogen (mmol/L)	Pre-treatment	34.78±5.67	34.69±5.82	0.938
	Post-treatment	19.27±3.45*	15.35±2.89*	<0.001
	D Change	-15.51±4.23	-19.34±4.67	<0.001
Serum creatinine (mmol/L)	Pre-treatment	437.69±58.43	435.70±60.12	0.867
	Post-treatment	162.98±24.67*	150.33±21.45*	0.008
	D Change	-274.71±45.67	-285.37±48.92	0.264
NGAL (ng/mL)	Pre-treatment	187.34±28.76	184.98±30.21	0.689
	Post-treatment	124.36±18.42*	86.73±12.58*	<0.001
	D Change	-62.98±15.67	-98.25±18.34	<0.001
ADMA (mmol/L)	Pre-treatment	0.94±0.18	0.96±0.20	0.598
	Post-treatment	0.78±0.14*	0.52±0.09*	<0.001
	D Change	-0.16±0.08	-0.44±0.12	<0.001

Data presented as mean ± SD. *P<0.05 vs. pre-treatment within group.

Table V Pre-treatment and post-treatment placental and inflammatory biomarkers.

Parameter	Time Point	LMWH Group (n=50)	RCA Group (n=50)	P-value (intergroup)
PlGF (pg/mL)	Pre-treatment	112.46±21.34	114.23±22.67	0.687
	Post-treatment	156.42±23.67*	218.35±31.84*	<0.001
	D Change	+43.96±15.34	+104.12±24.67	<0.001
IL-6 (pg/mL)	Pre-treatment	10.87±2.34	11.02±2.51	0.757
	Post-treatment	8.73±1.86*	5.21±1.34*	<0.001
	Δ Change	-2.14±1.23	-5.81±1.67	<0.001

Data presented as mean ± SD. *P<0.05 vs. pre-treatment within group.

Table VI Maternal complications during pregnancy.

Complication	LMWH Group (n=50)	RCA Group (n=50)	P-value
Bleeding events (n, %)	8 (16.0%)	3 (6.0%)	0.201
Thrombosis (n, %)	5 (10.0%)	1 (2.0%)	0.204
Atrial fibrillation (n, %)	11 (22.0%)	7 (14.0%)	0.432
Total complications (n, %)	**24 (48.0%)**	**11 (22.0%)**	**0.006**

Comparison of placental and inflammatory biomarkers

RCA therapy was associated with significantly improved placental angiogenesis markers and reduced systemic inflammation compared to LMWH therapy (*Table V*).

PlGF levels increased by 39.1% in the LMWH group (from 112.46±21.34 to 156.42±23.67 pg/mL) and by 91.2% in the RCA group (from 114.23±22.67 to 218.35±31.84 pg/mL) (P< 0.001). The absolute increase in PlGF was more than double in the RCA group compared to the LMWH group (Δ +104.12±24.67 pg/mL vs. Δ +43.96±15.34 pg/mL, P<0.001).

IL-6 levels decreased by 19.7% in the LMWH group and by 52.7% in the RCA group (P<0.001). Post-treatment IL-6 levels in the RCA group (5.21±1.34 pg/mL) were approximately 40% lower than in the LMWH group (8.73±1.86 pg/mL).

Comparison of maternal complications

The incidence of maternal complications was significantly lower in the RCA group than in the LMWH group (*Table VI*).

In the LMWH group, 24 patients (48.0%) developed complications, including 8 bleeding events (16.0%), 5 thrombotic events (10.0%), and 11 cases of atrial fibrillation (22.0%). In the RCA group, only 11 patients (22.0%) developed complications, including 3 bleeding events (6.0%), 1 thrombotic event (2.0%), and 7 cases of atrial fibrillation (14.0%). The overall complication rate was 54.2% lower in the RCA group than in the LMWH group (P=0.006).

Comparison of maternal bleeding patterns

Among patients with bleeding complications, the distribution of bleeding types differed between groups (*Table VII*).

In the LMWH group, gastrointestinal bleeding was the most frequent bleeding complication

Table VII Distribution of bleeding types in patients with bleeding complications.

Bleeding Type	LMWH Group (n=8)	RCA Group (n=3)
Anaemia (n, %)	1 (12.5%)	0 (0%)
Epistaxis (n, %)	2 (25.0%)	1 (33.3%)
Gingival bleeding (n, %)	2 (25.0%)	1 (33.3%)
Gastrointestinal bleeding (n, %)	3 (37.5%)	1 (33.3%)
Conjunctival bleeding (n, %)	0 (0%)	0 (0%)

Table VIII Foetal developmental abnormalities.

Abnormality Type	LMWH Group (n=50)	RCA Group (n=50)	P-value
Abortion (n, %)	3 (6.0%)	2 (4.0%)	1.000
Hydrocephalus (n, %)	2 (4.0%)	1 (2.0%)	1.000
Warfarin infant (n, %)	1 (2.0%)	0 (0%)	1.000
Stillbirth (n, %)	3 (6.0%)	1 (2.0%)	0.618
Total abnormalities (n, %)	9 (18.0%)	4 (8.0%)	0.045

(37.5%), followed by epistaxis (25.0%) and gingival bleeding (25.0%). Anaemia occurred in one patient (12.5%). In the RCA group, epistaxis, gingival bleeding, and gastrointestinal bleeding each occurred in one patient (33.3% each), with no cases of anaemia. Due to the small number of bleeding events (8 in the LMWH group and 3 in the RCA group), a formal statistical comparison of bleeding subtypes was not performed; the table therefore presents absolute counts and percentages for descriptive purposes only.

Comparison of foetal development abnormalities

Foetal outcomes were significantly better in the RCA group than in the LMWH group (*Table VIII*).

In the LMWH group, 9 foetuses (18.0%) developed abnormalities, including 3 abortions (6.0%), 2 cases of hydrocephalus (4.0%), 1 warfarin infant (2.0%), and 3 stillbirths (6.0%). In the RCA group, only 4 foetuses (8.0%) developed abnormalities, including 2 abortions (4.0%), 1 hydrocephalus case (2.0%), and 1 stillbirth (2.0%), with no warfarin infants. The total foetal abnormality rate was 55.6% lower in the RCA group than in the LMWH group (P=0.045).

Among abnormal foetal development cases, abortion was the most common outcome in both groups (33.3% in the LMWH group, 50.0% in the RCA group).

Summary of key outcome measures

Table IX provides a comprehensive summary of all key outcome measures comparing the RCA and LMWH groups.

Discussion

MHVR, a convenient and effective surgical method, is widely used for heart valve disease. However, long-term clinical studies reveal hidden risks; postoperative patients are prone to bleeding, and mechanical material placement easily causes thromboembolism, seriously affecting quality of life and threatening patient safety (15). The mechanical valve surface lacks endothelial cell coverage, triggering coagulation mechanisms, forming thrombus around the valve, affecting valve leaflet opening and closing, causing valve dysfunction, and, in severe cases, leading to heart failure or sudden death. Therefore, MHVR patients require lifelong

Table IX Summary of key outcome measures between RCA and LMWH groups.

Outcome Category	Parameter	LMWH Group (n=50)	RCA Group (n=50)	Absolute Difference	Relative Difference	P-value
Coagulation	PT (s)	16.43±1.67	18.76±1.89	+2.33 s	+14.2%	<0.001
	APTT (s)	37.89±3.45	41.23±3.67	+3.34 s	+8.8%	<0.001
	TT (s)	15.89±1.56	14.12±1.34	-1.77 s	-11.1%	<0.001
	FIB (g/L)	3.34±0.48	2.87±0.43	-0.47 g/L	-14.1%	<0.001
	PLT ($\times 10^9$/L)	223.45±26.78	242.67±28.43	+19.22	+8.6%	<0.001
Novel coagulation biomarkers	TAT (mg/L)	6.84±1.23	3.91±0.87	-2.93 mg/L	-42.8%	<0.001
	sP-selectin (ng/mL)	52.67±8.34	38.42±6.71	-14.25 ng/mL	-27.1%	<0.001
	D-dimer (mg/L)	0.89±0.21	0.54±0.16	-0.35 mg/L	-39.3%	<0.001
Renal function	Urea nitrogen (mmol/L)	19.27±3.45	15.35±2.89	-3.92 mmol/L	-20.3%	<0.001
	Serum creatinine (mmol/L)	162.98±24.67	150.33±21.45	-12.65 mmol/L	-7.8%	0.008
	NGAL (ng/mL)	124.36±18.42	86.73±12.58	-37.63 ng/mL	-30.3%	<0.001
	ADMA (mmol/L)	0.78±0.14	0.52±0.09	-0.26 mmol/L	-33.3%	<0.001
Placental/ inflammatory	PlGF (pg/mL)	156.42±23.67	218.35±31.84	+61.93 pg/mL	+39.6%	<0.001
	IL-6 (pg/mL)	8.73±1.86	5.21±1.34	-3.52 pg/mL	-40.3%	<0.001
Maternal complications	Total complications	24 (48.0%)	11 (22.0%)	-27.0%	-54.2%	0.006
Foetal abnormalities	Total abnormalities	9 (18.0%)	4 (8.0%)	-10.0%	-55.6%	0.045

Data presented as mean ± SD or n (%). Positive absolute difference indicates a higher value in the RCA group; negative absolute difference indicates a lower value in the RCA group.

anticoagulation therapy (16, 17). Haemorrhage is a common postoperative complication from improper warfarin anticoagulant therapy. Additionally, patients are prone to lower extremity venous thrombosis, atrial fibrillation, nasal bleeding, gastrointestinal bleeding, anaemia, and mucosal bleeding (18, 19).

Clinically used anticoagulants are mainly heparin. Heparin's anticoagulant mechanism involves thrombin inhibition: heparin binds to thrombin in blood, forming complexes that inhibit microthrombus formation and balance the coagulation system (20). Heparin also inhibits thrombin and fibrin-induced inflammatory responses, inhibits histone-induced cytotoxic effects, repairs vascular endothelium, and performs immunoregulation (21). LMWH is a commonly used clinical anticoagulant. Compared with heparin, LMWH has more ideal antithrombotic effects, a longer pharmacodynamic profile, greater

efficacy, significantly improved immune function, improved placental blood flow by reducing blood viscosity, and inhibition of tissue factor and tumour necrosis factor-α (22).

Citric acid generates calcium citrate by complexing free calcium in plasma, reducing free calcium levels in extracorporeal circulation, blocking prothrombin conversion to thrombin, and performing in vitro anticoagulation. After entering the body, complexed calcium citrate dissociates into calcium ions and bicarbonate, losing its anticoagulant effect. Therefore, RCA exerts local anticoagulant effects in vitro but none in vivo. Citrate also forms complexes with chelated calcium and magnesium, reduces platelet count, improves the biocompatibility of extracorporeal circulation, relieves the inflammatory response, reduces the risk of cardiovascular disease, and ensures patient safety. Thus, RCA is safer and more effective for critically ill patients with bleeding risk, reducing bleeding risk and complication probability. Studies revealed that RCA has a favourable effect on continuous renal replacement therapy in critically ill patients with bleeding risk, with no statistical differences in biochemical, coagulation, or blood routine indicators compared with heparin therapy, and no bleeding occurred (23).

The novel biomarkers evaluated in this study provide mechanistic insights into RCA's superior clinical outcomes. Thrombin-antithrombin complex is a sensitive marker of real-time thrombin generation and coagulation activation, and elevated levels indicate a pro-thrombotic state (24). Significantly lower TAT levels in the RCA group confirm more effective suppression of thrombin generation despite RCA's predominantly in vitro mechanism. Soluble P-selectin (sP-selectin) reflects platelet activation (25); lower levels in the RCA group indicate superior platelet quiescence beyond simple platelet count changes. D-dimer, a fibrin degradation product, quantifies thrombotic burden (26); lower levels in the RCA group demonstrate reduced subclinical thrombotic activity.

Renal biomarker findings are particularly noteworthy. Neutrophil gelatinase-associated lipocalin (NGAL) is an early biomarker of acute kidney injury, more sensitive than serum creatinine (27). Significantly greater NGAL reduction in the RCA group indicates superior renal protection beyond creatinine changes. Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is a marker of endothelial dysfunction (28). Greater ADMA reduction in the RCA group suggests citrate improves not only coagulation but also endothelial integrity.

Placental growth factor (PlGF) is critical for placental vascular development (29). Significantly higher PlGF levels in the RCA group provide mechanistic evidence that improved maternal coagulation translates to better placental perfusion, explaining reduced foetal dysplasia and abortion rates. Interleukin-6 (IL-6), a systemic inflammatory marker (30), was significantly lower in the RCA group, confirming citrate's anti-inflammatory effects via calcium chelation and complement inhibition – particularly relevant in pregnancy, a proinflammatory state. To interpret these data in clinically relevant terms, the absolute risk reduction for total maternal complications was 26% (48% with LMWH vs. 22% with RCA), corresponding to an NNT of 4. For total foetal abnormalities, the absolute risk reduction was 10% (18% vs. 8%), yielding an NNT of 10. This data suggests that these relative risk reductions provide clinically significant absolute benefits for this at-risk population.

However, an apparent paradox exists: the INR targets used in the RCA group were marginally higher than those in the LMWH group. Warfarin is a teratogen, especially when used in the first trimester, as it inhibits vitamin K-dependent carboxylation. However, foetal outcome was better in the RCA group. One possible explanation for the better foetal outcome is that the beneficial effects of improved maternal haemostasis, decreased thrombin generation, decreased systemic inflammation, and enhanced placental perfusion, as reflected by increased PlGF levels and decreased IL-6, might outweigh the adverse effects of the marginally increased warfarin dose. However, as the study was not randomized for warfarin dose, the exact effect of warfarin dose needs to be evaluated in future prospective studies (31).

In this study, anticoagulant therapy was performed for parturients after MHVR using LMWH and RCA regimens. Results showed that PT and APTT in the RCA group were considerably superior to those in the LMWH group, whereas TT was markedly inferior. FIB values decreased in both groups, with a greater decrease in the RCA group; PLT values increased in both groups, with a greater increase in the RCA group. Novel biomarkers showed significantly lower TAT, sP-selectin, and D-dimer levels in the RCA group ($P<0.05$). After anticoagulant therapy, maternal urea nitrogen and serum creatinine decreased more in the RCA group; NGAL and ADMA demonstrated greater reductions in the RCA group ($P<0.05$). PlGF was significantly higher and IL-6 significantly lower in the RCA group ($P<0.05$). These findings reveal that RCA more effectively improves postoperative coagulation and renal function while enhancing placental angiogenesis and reducing inflammation.

A possible pharmacological rationale for the superior renal biomarker profile of the RCA could be related to the different elimination mechanisms of the anticoagulation strategies used. Low-molecular-weight heparins are primarily excreted

renally and have been shown to accumulate and/or alter their pharmacodynamics in patients with renal dysfunction, potentially influencing renal hemodynamic and injury biomarkers. In contrast, citrate acts locally in the extracorporeal circuit and, after systemic metabolism to bicarbonate, primarily in the liver and muscle, with little systemic anticoagulant effects and no requirement for renal elimination of anticoagulant activity. These differences in pharmacokinetics could explain the larger reduction in NGAL and ADMA levels in the RCA group (32, 33).

The incidence of various complications in the RCA group was markedly lower than in the LMWH group, with only 3 parturients experiencing bleeding complications. Gastrointestinal bleeding incidence was highest in parturients receiving MHVR. The probability of foetal dysplasia in the RCA group was markedly lower than in the LMWH group, with only 4 cases ($P<0.05$). Abortion was most likely in cases of abnormal foetal development.

Limitations

This study has several limitations. First, it was conducted at a single tertiary-care centre, which may limit generalizability to other settings and populations. Second, the study was not blinded; investigators and treating clinicians were aware of the anticoagulation modality, which may introduce performance or detection bias. Third, although the overall sample size (n=100) was adequate to detect differences in several primary laboratory outcomes, subgroup counts for some clinical events (e.g., specific bleeding types) were small, precluding formal statistical comparisons. Fourth, our follow-up was limited to the pregnancy and perinatal period; longer-term developmental outcomes in the offspring were not assessed and warrant future study. Finally, multiple related biomarkers were assessed, which raises the possibility of Type-I error; while the consistency of the findings across coagulation, renal, and placental markers lends biological plausibility, future studies should pre-specify primary endpoints and apply multiplicity adjustments where appropriate (34, 35). We acknowledge that multiple hypothesis testing increases the risk of Type-I error. To mitigate this risk, we used consistency across related coagulation, renal, and placental biomarkers to support the biological plausibility of our findings.

Conclusion

In conclusion, RCA therapy has a better effect on parturients and foetuses after MHVR, can considerably improve maternal coagulation parameters and renal function, has a low probability of maternal complications, and results in less bleeding. Novel biomarker profiles demonstrate that RCA significantly reduces thrombin generation (TAT), platelet activation (sP-selectin), thrombotic burden (D-dimer), renal tubular injury (NGAL), and endothelial dysfunction (ADMA), while enhancing placental angiogenesis (PlGF) and reducing systemic inflammation (IL-6). RCA therapy also reduces foetal dysplasia, promotes normal foetal birth and development, has a better anticoagulant effect, is highly safe, and is worthy of active clinical application.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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