European Review for Medical and Pharmacological Sciences 2022; 26: 8508-8522

Predictive model for deep venous thrombosis caused by closed lower limb fracture after thromboprophylactic treatment

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Abstract. – **OBJECTIVE: Currently, there are still no convincing clinical models predicting closed lower extremity fracture-associated deep vein thrombosis in patients treated through thromboprophylactic methods. We aimed at using two retrospective cohorts to develop and externally verify a clinical prediction model for deep vein thrombosis in patients treated with anticoagulants after suffering closed lower extremity fractures.**

PATIENTS AND METHODS: We evaluated the patients' pre- and post-operatively, to accurately determine the predictive power of the biomarkers and clinical risk factors. Two retrospective cohorts were used for the development and external verification of a pre-operative clinical prediction model (development: n = 2,253; verification: n = 833) and post-operative clinical prediction model (development: n = 1,422; verification: n = 449), respectively.

RESULTS: The C-indices were used to show the predicted incidence of objective thrombosis at the pre- and post-operative stage, which were then compared with the observed incidence of thrombosis in both cohorts. Biomarkers and clinical indicators were included in pre- and post-operative nomograms, which were adequately calibrated in both cohorts. The cross-validated C-indices of the pre- and post-operative clinical prediction models in the verification cohort were 0.706 (95% CI, 0.67-0.74) and 0.875 (95% CI, 0.84-0.91), respectively.

CONCLUSIONS: We present our findings of novel pre- and post-operative nomograms for the prediction of deep venous thrombosis in patients who received thromboprophylaxis after suffering closed lower extremity fractures.

Key Words:
Deep venous thrombosis, Closed lower extremity fractures, Clinical prediction models, Thromboprophylaxis.

Introduction

Deep vein thrombosis (DVT) is a complicated and problematic type of venous thromboembolism (VTE), affecting millions of people around the world[1]. Some DVTs can develop into the serious complication of pulmonary embolism. The conservative estimate of people dying from VTE is higher than 100,000 each year, and at least 350,000 people are diagnosed with DVT annually in The United States[2]. In the last three decades, however, there has been a large literature regarding thromboprophylaxis and therapy of DVT. The incidence of VTE did not significantly change as the standardized use of thromboprophylactic drugs and clinical physical therapy counteracted the negative effect of increasing prevalence of active cancer, trauma, and surgery[3]. However, the growing surge in trauma and surgery may suggest that the concurrent efforts of venous thromboprophylaxis are insufficient[3].

Previous studies[4-7] identified numerous risk factors of DVT[4], such as age, sex[5], season[6], trauma, surgery, active cancer[7], and so on. According to these risk factors, several studies[7-9] have focused on developing a clinical prediction model

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or the identification of one or more predictors with high sensitivity and specificity for DVT. However, it is hard to find a single clinical model to predict DVT in all patients, because there are many risk factors, especially those that are uncontrollable (blood type[10], seasonal variation[6]). This makes it challenging to study all the concurrent risk factors.

Previous studies have shown that approximately 40% of VTEs are provoked by strong risk factors[11], such as cancer[7], surgery[9,12], trauma or fracture[13], and immobility, therefore studies currently aim to find one model to predict DVT associated with these factors. Upon focusing on predicting DVT in patients with closed lower extremity fractures, we found it difficult to determine a clinical prediction model with high accuracy and specificity. The risk assessment and prediction tool and Caprini score[14] have been used to predict the risk of developing VTE in surgical patients; however, these scoring systems do not subdivide lower extremity fractures, the Caprini score is too complicated for clinicians to apply[14], and they cannot predict the incidence of DVT in patients undergoing thromboprophylaxis treatment. These scores were also not developed for fractures or orthopedic surgery (internal fixation and joint replacement surgery) associated venous thromboembolism only. This means that it is easier to under-estimate or over-estimate the associated risk factors, as these scores incorporate numerous risk factors[15].

There is currently no convincing closed lower extremity fracture-associated DVT clinical prediction model. The predictive ability of biomarkers, such as D-dimer, pre-operative waiting time, and surgery still reduce the decision-making ability of clinicians to balance thromboprophylaxis and bleeding risk. We aimed to use two retrospective cohorts to develop and externally verify a clinical prediction model for DVT in patients treated with anticoagulants after suffering closed lower extremity fractures. We evaluated the patients pre- and post-operatively, to accurately determine the predictive power of the biomarkers and clinical risk factors.

Patients and Methods

We divided the clinical prediction models of surgical patients with closed lower extremity fractures into a pre-operative DVT prediction model (pre-op-CPM) and a post-operative DVT prediction model (post-op-CPM). We used two independent cohorts from a single centre to initially develop our model, by collecting pre- and post-operative clinical data from all of patients who suffered lower extremity closed fractures between 1 January, 2014 and 31 December, 2021. Data from 1 January, 2019 to 31 December, 2021 were part of data form prospective study (Registration number: ChiCTR1800017754, Chinese Clinical Trial Registry, ChiCTR), which was used for external validation. Data from 1 January, 2014 to 31 December, 2018 about retrospective cohorts were used for model development.

Eligibility criteria included patients who suffered lower extremity closed fractures, were treated within 30 days, and underwent double whole leg ultrasound scanning (DWLUS) at least once at pre- and post-operation[16]. Thromboprophylaxis therapies were not recorded as included patients followed the same guideline[17]: 1) For fractures around the hip and knee joints, and femoral shaft fractures, patients always received LMWH according to body weight and intermittent pneumatic compression device (IPCD) for thrombotic prophylaxis, when their hemodynamics were confirmed to be stable by monitoring blood pressure in upper extremities and heart rate[18]. To reduce the risk of bleeding in patients on anticoagulation medication, all thromboprophylaxis therapy was suspended for 12 hours before surgery until 12 hours after surgery. 2) For tibia or fibula shaft fractures and fractures around the ankle, patients received the same clinical treatment as mentioned at point 1. 3) When patients were diagnosed with DVT by DWLUS, they received LMWH, which was only suspended approximately 12 hours before and after surgery[19]. 4) In our center, the type of low molecular weight heparin (LMWH) is enoxaparin. The dose of enoxaparin is 4,100 KD. For thromboprophylaxis and treatment, patients were administered 4,100 KD enoxaparin by subcutaneous injection every day.

The exclusion criteria for the pre-operative cohort are presented in **Supplementary Table I**. The primary outcomes were independently assessed venous thromboembolism at the pre-operative and post-operative stage objectively confirmed by double DWLUS[20], which are routine procedure in our center. The pulmonary embolism was collected from final medical records and diagnosed according to CT angiography.

We collected candidate predictors in the development cohorts according to relevant previous univariate or multivariate studies[11], including sex[4], age[4], fracture site[21,22], coronary atherosclero-

Table I. Distribution characteristics of study cohorts for establishing pre-operative clinical predictive model.

Risk factors	Development group (n = 2,253)	Verification group (n = 833)	*p*/SMD [95% CI]	Chi-square, df
Sex, n (%)			0.66	0.19, 1
Male	1,126 (49.98)	409 (49.10)		
Female	1,127 (50.02)	424 (50.90)		
Age, n (%)				
Young	584 (25.92)	219 (26.29)	0.84	0.04, 1
Middle-aged	538 (23.88)	188 (22.57)	0.45	0.58, 1
Young old	498 (22.10)	214 (25.69)	0.04	4.41, 1
Old old	586 (26.01)	203 (24.37)	0.35	0.86, 1
Very old	49 (2.17)	8 (0.96)	0.02	NA
Deep venous thrombosis, n (%)*			<0.0001	26.28, 1
With	475 (21.08)	249 (29.89)		
Without	1,778 (78.92)	584 (70.11)		
Fractures around the hip joint, n (%)			0.97	0.0017, 1
With	1,191 (52.86)	441 (52.94)		
Without	1,062 (47.14)	392 (47.06)		
Fractures around the knee joint, n (%)			0.67	0.18, 1
With	1,822 (19.13)	165 (19.81)		
Without	1,822 (80.87)	668 (80.19)		
Fractures around the ankle, n (%)			0.27	1.19, 1
With	358 (15.89)	146 (17.53)		
Without	1,895 (84.11)	687 (82.47)		
Femoral shaft fracture, n (%)			0.41	NA
With	95 (4.22)	29 (3.48)		
Without	2,158 (95.78)	804 (96.52)		
Tibial or fibula shaft fracture, n (%)			0.94	0.005, 1
With	174 (7.72)	65 (7.80)		
Without	2,079 (92.28)	768 (92.20)		
Coronary atherosclerosis, n (%)			0.01	6.28, 1
With	461 (20.46)	137 (16.45)		
Without	1,792 (79.54)	696 (83.55)		
Hypertension, n (%)			0.02	5.66, 1
With	475 (21.08)	209 (25.09)		
Without	1,778 (78.92)	624 (74.91)		
Arrhythmias, n (%)			0.67	0.19, 1
With	242 (10.74)	94 (11.28)		
Without	2,011 (89.26)	739 (88.72)		
Multiple fractures, n (%)			0.01	6.29, 1
With	162 (7.19)	39 (4.68)		
Without	2,091 (92.81)	794 (95.32)		
Remote cerebral infarction, n (%)*			<0.0001	20.82, 1
With	133 (5.90)	89 (10.68)		
Without	2,120 (94.10)	744 (89.32)		
Type 2 diabetes, n (%)			0.1	2.79, 1
With	199 (8.83)	90 (10.80)		
Without	2,054 (91.17)	743 (89.20)		
Thoracic injures, n (%)			0.66	NA
With	46 (2.04)	14 (1.68)		
Without	2,207 (97.96)	819 (98.32)		

Continued

Table I. Distribution characteristics of study cohorts for establishing pre-operative clinical predictive model.

Risk factors	Development group (n = 2,253)	Verification group (n = 833)	*p*/SMD [95% CI]	Chi-square, df
Hepatitis, n (%)			>0.9999	NA
With	68 (3.02)	25 (3.00)		
Without	2,185 (96.98)	808 (97.00)		
No complications, n (%)			0.41	0.67, 1
With	1,077 (47.80)	412 (49.46)		
Without	1,176 (52.20)	421 (50.54)		
Pre-operative HGB, IQR (Min-Max)	124 (19.76-188)	126 (63-177)	0.15 [0.23, 0.07]	NA
Pre-operative HCT, IQR (Min-Max)	37.5 (5.71-53.6)	37.8 (20.5-120)	0.15 [0.23, 0.07]	NA
Pre-operative DD, IQR (Min-Max)	4.6 (0.13-274.32)	2.895 (0.05-164.1)	0.27 [0.19, 0.35]	NA
Pre-operative Fg, IQR (Min-Max)	3 (0.84-320)	2.94 (0.65-273.9)	0.20 [0.28, 0.12]	NA
Injury to operation, days, IQR (Min-Max), days	5 (1-28)	4 (1-19)	0.59 [0.50, 0.67]	NA
Hospitalization to operation, IQR (Min-Max), days	4 (0-23)	4 (1-19)	0.19 [0.11, 0.27]	NA
Hospitalization to discharge, IQR (Min-Max), days	9 (0-29)	7 (3-29)	0.47 [0.39, 0.55]	NA
Injury to hospitalization, IQR (Min-Max), days	0 (0-24)	1 (1-1)	NA	NA
Injury to pre-operative ultrasonography, IQR (Min-Max), days	2 (0-26)	2 (0-16)	0.25 [0.17, 0.33]	NA
Hospitalization to pre-operative ultrasonography, IQR (Min-Max), days	1 (0-14)	2 (0-16)	0.51 [0.59, 0.43]	NA
Pre-operative ultrasonography to operation, IQR (Min-Max), days*	2 (0-20)	1 (0-12)	0.66 [0.57, 0.74]	NA

Development group: the development cohort of pre-operative clinical predictive model (data collected from 1 January, 2014 to 31 December, 2018); Verification group: validation cohort of pre-operative clinical predictive model (data collected from 1 January, 2019 to 31 December, 2021); SMD: standardized mean difference; NA: not available. Continuous data presented as median (IQR) with maximum value and minimum value; categorical data presented as n (%). The distribution of variable was considered a potential similarity between the development group and the verification group when $p > 0.01$ or SMD < 0.6.(36). *Distribution of variable showed significant divergence between study cohorts.

sis[23], hypertension[23], cardiac arrhthmias[23], multiple fractures[12], remote cerebral infarction[24], type 2 diabetes[12], thoracic injures[25], hepatitis, and no complications. Laboratory testing[7,26] before surgery included hemoglobin (HGB, g/L), red blood cell specific volume (HCT, g/L), D-dimer (DD, mg/L), and fibrinogen (Fg, g/L). We also recorded duration from injury to operation, hospitalization to operation, hospitalization to discharge, injury to hospitalisation[23], injury to pre-operative ultrasonography, hospitalizations to pre-operative ultrasonography, and pre-operative ultrasonography to operation.

The post-operative clinical prediction model included the same candidate variables, as well as fixation method, anesthesia method, post-operative laboratory tests results, operation time (min), duration of tourniquet (min), volume of blood transfusion (U), intraoperative blood loss (mL), volume of intraoperative liquid (mL), duration from operation to post-operative ultrasonography (days), and duration from injury to post-operative ultrasonography (days).

Age was divided into five grades accounting to The World Health Organization: young (18-44 years), middle-age (44-59 years), young old (60-74 years), old (75-89 years), and very old (>90 years). Lower extremity fractures were divided into five subgroups: fracture around the hip joint (pelvic fractures, acetabular fractures, femoral neck fractures, femoral tuberosity fractures); fracture around the knee joint (femoral condyle frac-

tures, patella fractures, tibial plateau fractures, fibular head/neck fractures); fracture around the ankle joint (fractures in ankle joint, talus fracture, calcaneal fracture); femoral shaft fracture; tibial or fibula shaft fracture.

Laboratory test results were collected during the first blood test after hospitalization and after surgery. The diagnosis of coronary atherosclerosis, hypertension, and cardiac arrhythmias were collected in medical records. Type 2 diabetes was diagnosed by blood glucose determination. Multiple fractures and thoracic injures were diagnosed by x-ray or computed tomography. No-complication was defined as a patient who did not have any chronic or acute diseases. Fracture fixation method included intramedullary fixation (such as an intramedullary nail or joint replacement) and extramedullary fixation (such as a steel plate and screws). The anesthesia method included general anesthesia and others.

Missing Data

We only analyzed complete data for the development and verifications of the model. Missing data was excluded prior to the statistical analysis.

Statistical Analysis

Statistical analyses were performed with IBM SPSS version 25 (IBM Corp., Armonk, NY, USA). Nomograms, receiver operating characteristic (ROC) curve graphs, and decision curves were drafted with R version 4.0.1. The distributional differences of each variable were assessed between the development and verification groups at pre- and post-operation, calculating standardized mean differences (SMD; >0.6 was considered a potential middle-difference between the two cohorts) for continuous data and using Chi-square test or Fisher's exact probability for categorical data. $p < 0.01$ was considered statistically significant.

We first identified potential correlation between venous thromboembolism and potential predictors, with inclusion variables in the development cohort using univariate logistic regression analysis ($p < 0.1$ was considered a potential correlation). Then, to reduce the model, the included variables were selected as predictors using stepwise binary logistic regression analysis with forward selection (likelihood ratio - LR). $p < 0.05$ was considered to be a significant correlation. The selected predictors were used to model the cause-specific risk of VTE, and nomograms were drafted using the R program (The R Foundation for Statistical Computing, Vienna, Austria).

To account for over-optimism and perform internal validation of the model, the C-indices were calibrated with cross validation (1,000 bootstrapping). To help clinicians easily identifying the usefulness of these models in predicting deep venous thrombosis, we presented decision-curve comparisons with a treat-all or treat-none strategy. This study followed the principle of transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD)[27].

Results

We collected results from 2,803 and 903 patients for the development and verification cohorts, respectively. From these, patients were excluded according to exclusion criteria as follows: pre-operative development cohort, n = 432; post-operative development cohort, n = 572; pre-operative verification cohort, n = 47; post-operative verification cohort, n = 246. We finally included 2,253 and 1,422 patients in the development pre- and post-operation cohort, and 833 and 449 patients in the verification pre-operative and post-operative models, respectively. The detailed numbers of patients excluded, included, with missing data, and the rate of missing data are presented in **Supplementary Tables I** and **II**.

Sample Characteristics and Outcomes

Most relevant variables presented similar distribution in the pre-operative development and verification cohorts (Table I); however, the percentage of patients who had a history of cerebral infraction was significantly higher in the validation cohort (n = 89, 10.68%) than in the development cohort (n = 133, 5.7%; $p < 0.0001$). The duration from pre-operative DWLUS to surgery was significantly longer in the development cohort (IQR = 2, range: 0-20) compared with the validation cohort (IQR = 1, range: 0-12) (SMD: 0.66). A higher proportion of patients had DVT in the validation cohort (n = 249, 29.89%) than the development cohort (n = 475, 21.08%; $p < 0.0001$).

The distribution of relevant variables was compared between the post-operative development cohort and the post-operative validation cohort, with most potential risk factors presenting similar distribution according to *p*-values and SMDs (Table II). However, a significantly higher percentage of patients in the validation cohort (n

Table II. Distribution of relevant variables in the post-operative development cohort and in the post-operative validation cohort.

Risk factors	Development group (n = 1,422)	Verification group (n = 449)	p/SMD [95% CI]	Chi-square, df
Sex, n (%)			0.79	0.07, 1
Male	691 (48.59)	215 (47.88)		
Female	731 (51.41)	234 (52.12)		
Age, n (%)				
Young	347 (24.40)	103 (22.94)	0.53	0.40, 1
Middle age	333 (23.42)	101 (22.49)	0.69	0.16, 1
Young old	320 (22.50)	115 (25.61)	0.17	1.8, 1
Old old	384 (27.00)	126 (28.06)	0.66	0.19, 1
Very old	38 (2.67)	4 (0.89)	0.03	NA
Post-thrombosis, n (%)			0.83	0.05, 1
With	701 (49.30)	224 (49.89)		
Without	721 (50.70)	225 (50.11)		
Pre-thrombosis, n (%)			0.79	0.07, 1
With	437 (30.73)	141 (31.40)		
Without	985 (69.27)	308 (68.60)		
Fractures around the hip joint, n (%)			0.04	4.42, 1
With	868 (61.04)	249 (55.46)		
Without	554 (38.96)	200 (44.54)		
Fractures around the knee joint, n (%)			0.16	1.9, 1
With	232 (16.32)	86 (19.15)		
Without	1,190 (83.68)	363 (80.85)		
Fractures around the ankle, n (%)			0.08	3.11, 1
With	173 (12.17)	69 (15.37)		
Without	1,249 (87.83)	380 (84.63)		
Femoral shaft fracture, n (%)			0.66	NA
With	47 (3.31)	17 (3.79)		
Without	1,375 (96.69)	432 (96.21)		
Tibial or fibula shaft fracture, n (%)			0.9	0.001, 1
With	102 (7.17)	32 (7.13)		
Without	1,320 (92.83)	417 (92.87)		
Coronary atherosclerosis, n (%)			0.1	2.72, 1
With	308 (21.66)	81 (18.04)		
Without	1,114 (78.34)	368 (81.96)		
Hypertension, n (%)			0.04	4.14, 1
With	311 (21.87)	119 (26.50)		
Without	1,111 (78.13)	330 (73.50)		
Arrhythmias, n (%)			0.85	0.03, 1
With	157 (11.04)	51 (11.36)		
Without	1,265 (88.96)	398 (88.64)		
Multiple fractures, n (%)			0.0012	NA
With	112 (7.88)	16 (3.56)		
Without	1,310 (92.12)	433 (96.44)		

Continued

Table II. Distribution of relevant variables in the post-operative development cohort and in the post-operative validation cohort.

Risk factors	Development group (n = 1,422)	Verification group (n = 449)	p/SMD [95% CI]	Chi-square, df
Remote cerebral infarction, n (%)*			<0.0001	17.10, 1
With	87 (6.12)	54 (12.03)		
Without	1,335 (93.88)	395 (87.97)		
Type 2 diabetes, n (%)			0.71	0.13, 1
With	125 (8.79)	42 (9.35)		
Without	1,297 (91.21)	407 (90.65)		
Thoracic injures, n (%)			>0.9999	NA
With	27 (1.90)	8 (1.78)		
Without	1,395 (98.10)	441 (98.22)		
Hepatitis, n (%)			0.73	NA
With	36 (2.53)	13 (2.90)		
Without	1,386 (97.47)	436 (97.10)		
No complications, n (%)			0.4	0.72, 1
With	661 (46.48)	219 (48.78)		
Without	761 (53.52)	230 (51.22)		
Fixed method, n (%)			0.49	0.48, 1
Intramedullary fixation	743 (52.25)	243 (54.12)		
Extramedullary fixation	679 (47.75)	206 (45.88)		
Anesthesia method, n (%)*			0.01	NA
General anesthesia	1,402 (98.59)	449 (100)		
Others	20 (1.41)	0 (0.00)		
Pre-operation HGB, IQR (Min-Max)	123 (61-188)	126 (68-172)	0.13 [0.24, 0.03]	NA
Pre-operation HCT, IQR (Min-Max)	37.1 (17.7-53.6)	37.7 (20.5-120)	0.16 [0.27, 0.06]	NA
Pre-operation DD, IQR (Min-Max)	4.98 (0.18-274.32)	3.1 (0.16-164.1)	0.24 [0.14, 0.35]	NA
Pre-operation Fg, IQR (Min-Max)	3.03 (0.84-93.36)	2.94 (0.65-273.9)	0.33 [0.43, 0.22]	NA
Post-operation HGB, IQR (Min-Max)	108 (7-176)	112 (67-158)	0.07 [0.18, 0.03]	NA
Post-operation HCT, IQR (Min-Max)	32.5 (3.4-343.7)	33.1 (20.7-45.6)	0.03 [0.14, 0.07]	NA
Post-operation DD, IQR (Min-Max)	3.8 (0.14-59.25)	1.21 (0.19-127)	0.47 [0.36, 0.57]	NA
Post-operation Fg, IQR (Min-Max)	3.88 (1.50-76.51)	4.17 (1.60-260)	0.21 [0.31, 0.10]	NA
Operation time, IQR (Min-Max), min	100 (20-625)	110 (40-700)	0.09 [0.20, 0.02]	NA
Duration of tourniquet, IQR (Min-Max), min	0 (0-240)	0 (0-300)	0.09 [0.20, 0.01]	NA
Volume of blood transfusion, IQR (Min-Max), ml	0 (0-12)	0 (0-9)	0.24 [0.13, 0.34]	NA
Intraoperative blood loss, IQR (Min-Max), ml	200 (0-5,600)	150 (0-1,800)	0.14 [0.04, 0.25]	NA
Volume of intraoperative liquid, IQR (Min-Max), ml	1,600 (0-5,700)	1600 (600-6,200)	0.10 [0.20, 0.01]	NA
Hospitalization to discharge, IQR (Min-Max), days	9 (3-27)	7 (3-25)	0.52 [0.41, 0.63]	NA
Injury to hospitalization, IQR (Min-Max), days	0 (0-20)	1 (1-1)	Not estimable	NA

Table II. Distribution of relevant variables in the post-operative development cohort and in the post-operative validation cohort.

Risk factors	Development group (n = 1,422)	Verification group (n = 449)	p/SMD [95% CI]	Chi-square, df
Injury to operation, IQR (Min-Max), days*	5 (0-27)	3 (1-19)	0.68 [0.57, 0.78]	NA
Hospitalization to operation, IQR (Min-Max), days	4 (0-23)	3 (0-14)	0.32 [0.21, 0.42]	NA
Operation to post-operative ultrasonography, IQR (Min-Max), days	3 (0-11)	3 (0-8)	0.11 [0.00, 0.21]	NA
Injury to pre-operative ultrasonography, IQR (Min-Max), days	2 (0-22)	2 (0-16)	0.30 [0.19, 0.40]	NA
Injury to post-operative ultrasonography, IQR (Min-Max), days*	8 (2-29)	6 (2-25)	0.66 [0.56, 0.77]	NA

Development group: development cohort of post-operative clinical predictive model (data collected from 1 January, 2014 to 31 December, 2018); Verification group: validation cohort of post-operative clinical predictive model (data collected from 1 January, 2019 to 31 December, 2021); SMD: standardized mean difference; NA: not available. Continuous data presented as median (IQR) with maximum value and minimum value; categorical data presented as n (%). The distribution of variables was considered a potential similarity between the development group and the verification group when $p > 0.01$ or SMD < 0.6[36]. *Distribution of variable showed significant divergence between study cohorts.

= 54, 12.03%) than in the development cohort (n = 87, 6.12%; $p < 0.0001$), and a higher proportion of patients, underwent general anesthesia (n = 449, 100% in the validation cohort *vs.* n = 1,402, 98.59% in the development cohort, $p = 0.01$). The duration from injury to surgery (development cohort *vs.* validation cohort: IQR = 5, [range: 0-27] *vs.* IQR = 3 [range: 1-19], SMD = 0.68) and from injury to post-operative DWLUS (development cohort *vs.* validation cohort: IQR = 8, [range: 2-29] *vs.* IQR = 6 [range: 2-25], SMD = 0.66) in the development cohort were significantly longer than in the validation cohort. The morbidities of pulmonary embolism (PE) and fatal PE in development cohort were 2.25% and 0.21%, while in the verification cohort 2% and 0.22%, respectively. There were no significant divergences between cohorts in morbidities of PE ($p = 0.85$) and fatal PE ($p > 0.999$). Details of PE were presented in **Supplementary Table III**.

Predictors Selection and Model Development

Univariable logistic regression modelling of risk factors of venous thrombosis in lower extremity fractures at the pre-operative stage identified 19 potential clinical predictors and biomarkers (**Supplementary Table IV**). Of these, the pre-specified multivariate logistic modelling of variable selection process selected seven variables that evolved into the established pre-operative clinical predictive model and nomogram: pre-operation HCT; duration from injury to operation; duration from hospitalization to pre-operative ultrasonography; age; fractures around the knee joint; femoral shaft fracture; and no complications (**Supplementary Table IV**).

26 clinical potential predictors and biomarkers were selected using univariable logistic regression modelling of cause-specifical venous thrombosis at the post-operative stage (**Supplementary Table V**). Of these, six were selected by using pre-specified multivariate logistic analysis to build the post-operative clinical predictive model and post-operative nomogram: age; fractures around the ankle; femoral shaft fractures; tibia or fibula shaft fractures; post-operation D-dimer; and pre-operative thrombosis (**Supplementary Table V**).

The C-index of the pre-operative clinical predictive model in the development cohort was 0.69 (95% CI: 0.67-0.72) and was corrected to 0.689 by 1,000 bootstrap replications (**Supplementary Figure 1A**). In the verification cohort, the C-index of the pre-operative clinical predictive model was 0.706 (95% CI: 0.67-0.74) that was corrected to 0.705 (**Supplementary Figure 1B**). This showed that the predicted incidence of venous thrombosis in the verification cohort matched the observed incidence. The discriminations of this model were simplified through presentation by ROC curves in the development (Figure 1A, red line) and verification cohorts (Figure 1A, green line). This model did not under-estimate or over-estimate venous thrombosis in the development (**Supplementary Figure 1A**) and verification cohorts (**Supplementary Figure 1B**) after sufficient calibration. The pre-op-

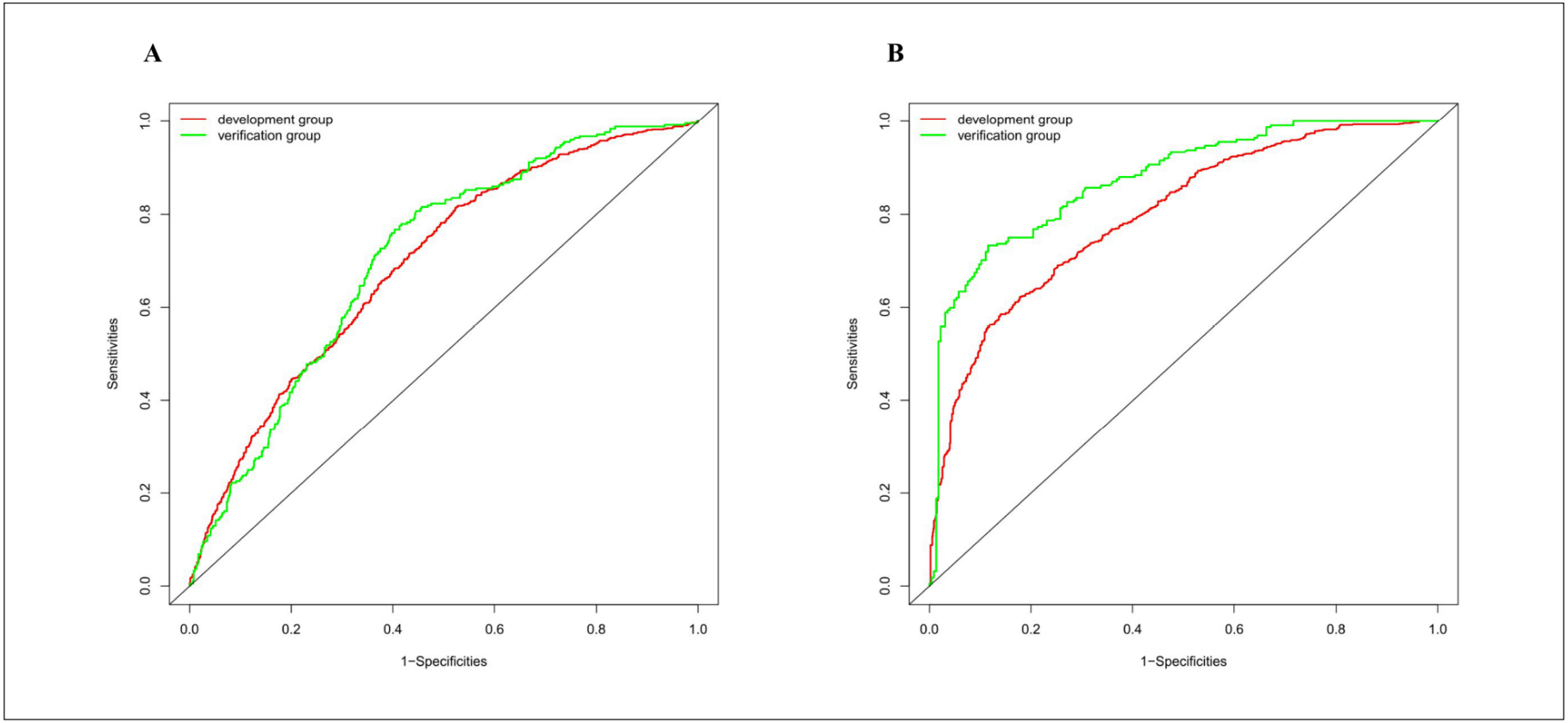

Figure 1. The cross-validated ROC curve of the prediction model in development group (red line) and verification group (green line). **A**, The cross-validated ROC curve of the pre-operation prediction model. **B**, The cross-validated ROC curve of the pre-operation prediction model.

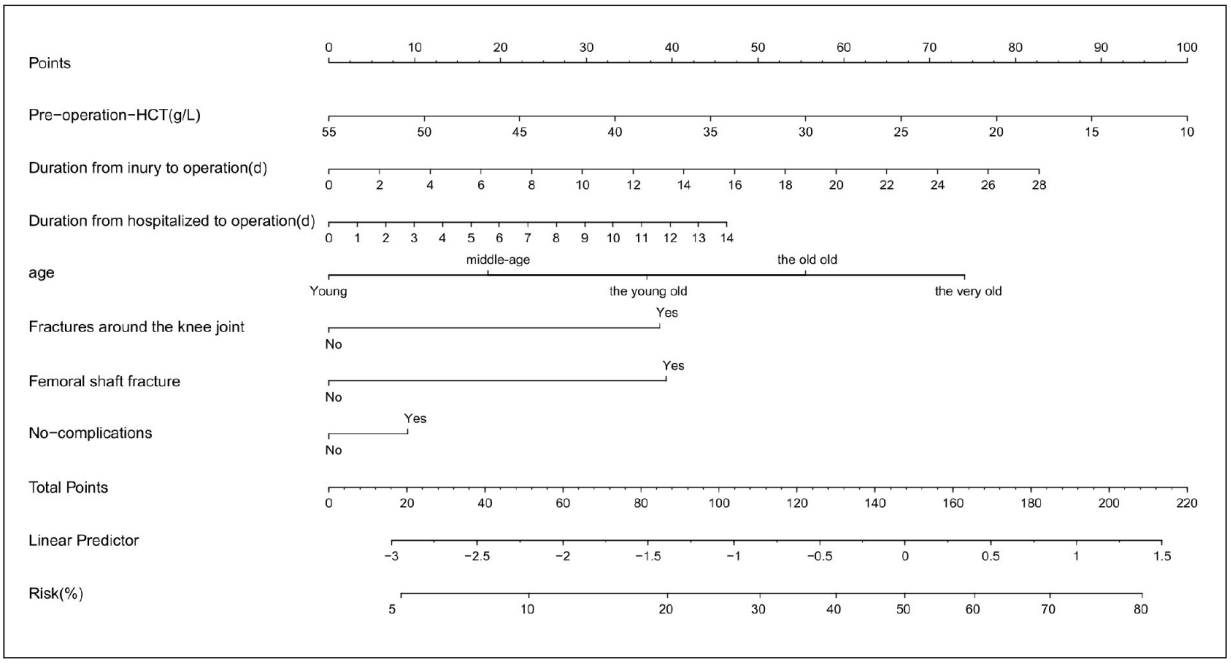

Figure 2. Nomogram for predicting the risk of venous thromboembolism in closed low limb fracture patients at pre-operative stage. Age was divided into five levels: young (<44 years); middle-age (44-59 years); young old (60-74 years); old (75-89 years); very old (>90 years old). Pre-operation-HCT, red blood cell specific volume (g/L) obtained from the first blood testing after injury.

erative clinical predictive model was simplified through presentation in a nomogram (Figure 2).

We further established the distribution of other included factors in patients with and without complications (**Supplementary Table VI**). Most patients with complications were older than the young old level, while the age of patients without complications mainly distributed in young and middle-age levels (71.49%). Additionally, more patients in the complication population suffered fractures around the knee joint than those without complications (24.32% *vs.* 14.37%). There were no significant divergences between the complication population and patients without complications in pre-operative HCT (mean, 38.05 *vs.* 35.96), femoral shaft fracture (4.72% *vs.* 3.91%), injury to operation (mean, 5.82 *vs.* 6.4), hospitalized to operation (mean, 1.71 *vs.* 1.76).

For the post-operative clinical predictive model, the C-indexes in the development and validation cohorts were 0.798 (95% CI: 0.78-0.82) and 0.875 (95% CI: 0.84-0.91), respectively, which were adequately calibrated and corrected to 0.795 and 0.875, respectively (**Supplementary Figure 2 A-B**). The calibration curves showed that this nomogram had greater predictive probability in both cohorts (**Supplementary Figure 2 A-B**). The discriminations of this model in both cohorts were visualized by ROC curves (Figure 1B). This post-operative clinical predictive model was simplified through presentation in a nomogram (Figure 3).

The decision-curve analysis demonstrated that the pre-operative and post-operative nomogram present outstanding clinical utility for prediction of venous thrombosis in patients with closed lower extremity fractures in the development (Figure 4A, C) and verification cohort (Figure 4B, D), respectively.

Discussion

We developed and externally validated a novel clinical prediction model for specific-associated DVT in two independent retrospective cohorts. The decision-curve analysis, representing the pre- and post-operative nomograms, have satisfactory clinical utility in both cohorts, and can, therefore, help clinicians make more appropriate decisions to balance thromboembolism and bleeding events during surgery in patients with closed lower extremity fractures.

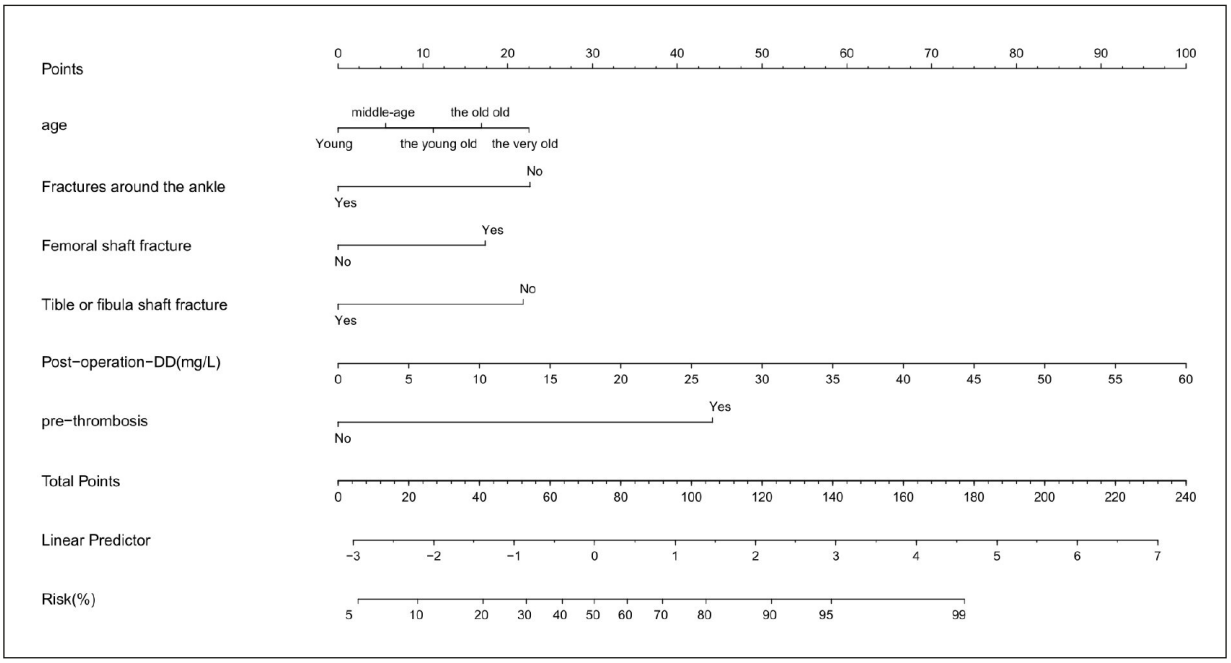

Figure 3. Nomogram for predicting the risk of venous thromboembolism in closed low limb fracture patients at post-operative stage. Age was divided into five levels: young (<44 years); middle-age (44-59 years); young old (60-74 years); old (75-89 years); very old (>90 years old). Post-operation-DD, concentration of D-dimer (mg/L) obtained from the first blood testing after surgery. Pre-thrombosis, deep venous thrombosis at the pre-operative stage.

Our study presented some novel findings regarding predictors of DVT in patients with closed lower extremity fractures treated prophylactically. In our study, age, which has been identified as a risk factor in several studies[9,12,23,28] of multiple or single factors of thrombosis, is a persistent predictor of thromboembolism throughout the treatment stages of closed lower extremity fractures and is more weighted at the pre-operative stage than at the post-operative stage. Femoral shaft fractures and fractures around the knee joint were identified as predictors of thromboembolism at the pre-operative stage, which positively correlated with incidents of thromboembolism. Furthermore, femoral shaft fractures were also identified as positive predictors at the post-operative stage in closed lower extremity fractures. The duration from injury to operation and from hospitalization to operation may represent immobility time that is a transient risk factor (strong risk factor: bedridden > 3 days; weak risk factor: travel > 4 hours)[11] and has been identified as a factor predisposing patients to thrombosis[29].

It is worth noting that red blood cell specific volume (HCT) was initially, to the best of our knowledge, included in clinical prediction models of DVT. HCT normally indicates there is enough oxygen carried by red blood cells within the circulatory system[30]. Therefore, we affirmed that a low level of HCT may represent a degree of tissue hypoxia, which can harm the venous wall, induce an inflammatory reaction, activate leukocytes, and then produce neutrophil extracellular traps that promote thrombosis[31]. In the pre-operative nomogram, our results singled out patients with no complications more prone to thrombus formation. A previous study[23] suggests that the nonspecific ST-segment and T-wave on electrocardiograms are positively related to increasing incidence of DVT at the pre-operative stage. Another study[24] shows that hypertension and diabetes are also risk factors for VTE. Park et al[28] also identified cardiovascular disease and chronic lung disease as positive risk factors for DVT. Therefore, to determine if the divergences of distribution of other included factors caused this unexpected result, we further established the distribution of other included factors in patients with and without complications (**Supplementary Table VI**). Most patients with complications were older than the young old level, while the age of patients without complications mainly distributed in young and middle-age levels. Additionally, more patients in the complications'

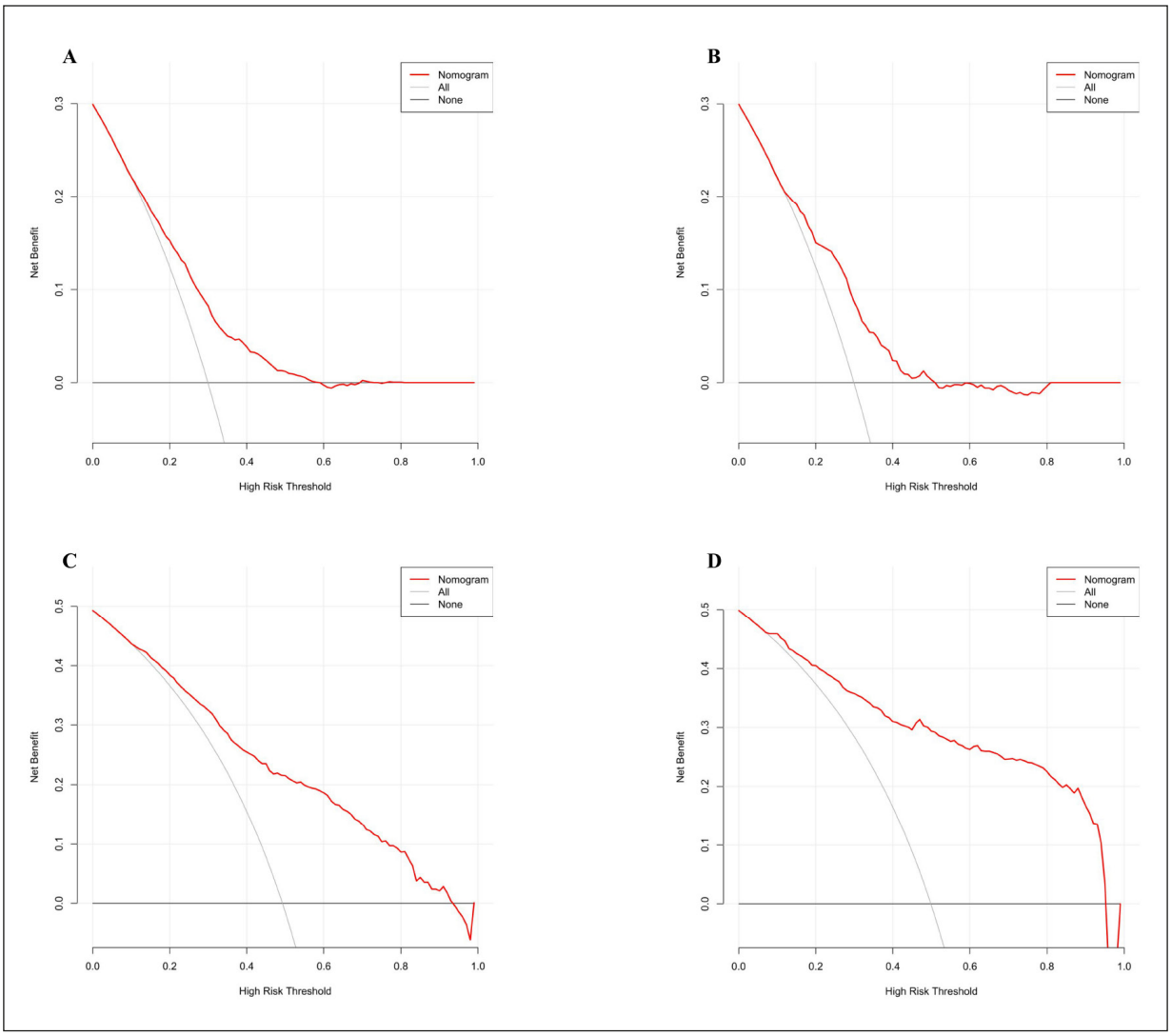

Figure 4. The decision-curve analysis for predicting deep venous thrombosis at preoperative stage [development group (**A**) and verification group (**B**)] and postoperative stage [development group (**C**) and verification group (**D**)]. The X-axis represents the predicted thrombosis, and the Y-axis means the net clinical benefit.

population suffered from fractures around the knee joint compared to those without complications. Therefore, we believe that relatively more patients with fractures around the knee joint without complications caused a higher incidence of DVT. In addition, previous studies[32,33] determined that approximately 20% of VTE was accounted for strong risk factors such as major trauma, surgery, and immobility, while most VTEs were provoked by weak risk factors or no apparent risk factors. It is possible that the unexpected higher incidence of DVT in patients without complications was caused by no apparent risk factors or other potential unincluded factors.

In the post-operative stage, in comparison with other types of closed lower extremity fractures, fractures around the ankle or in the tibia or fibula seemed to be protective factors. If patients suffered a DVT at the pre-operative stage, they have a higher risk of a recurrent DVT at the post-operative stage. Iorio et al[34] evaluated the risk of recurrent DVT after provoked or unprovoked DVT by meta-analysis. They showed that 3.3% of patients provoked a DVT by transient risk factors and underwent recurrent DVT after stopping anticoagulation after a year. DVTs provoked by non-surgical factors caused a higher risk of recurrent DVTs compared to those provoked by surgical factors.

The concentration of D-dimer at the post-operative stage was included as a positive predictor our prediction model (Figure 3, **Supplementary Table V**). D-dimer levels indicate the degradation of products of fibrin. Any cause of increased fibrin formation, such as DVT, age, cancer, infection, and inflammation, can induce an increased concentration of D-dimer. This means that a D-dimer test only can exclude DVT if the concentration is normal[11]. Therefore, it should be noted that anticoagulant therapy can elicit a false negative D-dimer result[35,36]. This means that, if patients receive different methods of anticoagulation or different doses of drugs, the sensitivity of D-dimer tests would decrease. Therefore, the concentration of D-dimer should be externally verified.

Limitations

There were several limitations of our study. First, we had a high rate of missing data (development post-operative CPM *vs.* validation post-operative CPM: 28.86% *vs.* 23.03%) and a retrospective cohort for model development, which may have caused potential selection bias. Second, our study did not include body mass index and injury of energy, which may have significant predictive ability[9]. This means we may have missed some significant predictive factors in our nomograms. Third, we did not test the interaction and correlation between involved predictors of the nomograms; therefore, further risk stratification with those predictors is difficult. Fourth, the development and validation cohorts were from a single center during a different period. Therefore, the results of our nomograms should be tested further in other cohorts from different centers. Last, our study did not subgroup analysis of thromboprophylaxis as all of patients with lower limbs fracture were injected by LMWH in our center.

Conclusions

We presented a novel clinical predictive model for predicting deep venous thrombosis caused by closed lower limb fracture after thromboprophylactic treatment, which have externally validated in independent retrospective cohort. Pre- and post-operation nomograms showed good discriminations in verification cohorts. And patients used our nomograms may benefit form thromboprophylaxis.

Conflict of Interest

The authors declare that they have no conflict of interest to declare.

Ethics Approval

We confirmed that the included prospective study was approved by the appropriate Ethics Committee of Honghui Hospital Affiliated to Xi'an Jiaotong University and the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Acknowledgments

We gratefully acknowledge the assistance of Dr. Chen Fen and Dr. Shuhao Li collecting 361 clinical data.

Funding

This work supported by Social Development Foundation of Shaanxi Province under grant (2017ZDXM-SF-009 and 2022SF-394).

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Authors' Contributions

Jian Xing contributed to write the manuscript, preform research and organize data; Huiya Fu and Zhe Song contributed to check the manuscript and analyze data; Qian Wang contributed to collect patient's basic information; Teng Ma contributed to collect clinical data; Ming Li contributed to perform statistical analysis; Yan Zhuang, Zhong Li and Yangjun Zhu contributed to sort data; Wei Tang, Sangui Wang and Na Yang contributed to collect data from laboratory; Pengfei Wang contributed to the work equally, was responsible to statistics, design research and should be regard as co- corresponding authors; Kun Zhang contributed to design research and review manuscript.

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Data Availability Statement

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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