Combining *KRAS* gene status with preoperative D-dimer levels as a predictive marker of venous thromboembolism risk in patients with resectable colorectal cancer: A prospective cohort study

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Abstract. Colorectal cancer (CRC), one of the most prevalent types of cancer, is accompanied by a notably high incidence of thrombotic complications. The present study aimed to elucidate the association between *KRAS* mutations and hypercoagulability in operable CRC. The prognostic value of preoperative D-dimer levels was also investigated, thus providing novel insights into the development of therapeutic strategies to enhance patient survival and diminish morbidity. Therefore, a prospective analysis of 333 CRC cases post-surgery at Yan'an Hospital Affiliated to Kunming Medical University, between May 2019 and October 2022 was performed. Data on demographics, tumor characteristics and D-dimer levels were compiled from the electronic health records. Venous thromboembolism (VTE) was diagnosed by doppler or computed tomography angiography, with D-dimer thresholds set at 550 and 1,650 μg/l. *KRAS* mutations at codons 12 and 13 were assessed in a subset of 56 cases. Subsequently, the factors affecting the hypercoagulable state in these patients were prospectively analyzed, focusing on the pivotal role of *KRAS*. The results showed that *KRAS* mutations were associated with elevated preoperative D-dimer levels, with 1,076 μg/l compared with 485 μg/l in the wild-type cohort, indicative of a hypercoagulable state. Increased D-dimer levels were also associated with vascular invasion, distant metastases and a heightened risk of postoperative VTE. Furthermore, multivariate analyses identified *KRAS* mutations, distant metastases and vascular invasion as independent predictors of elevated D-dimer levels, with relative risk values of 2.912, 1.884 and 1.525, respectively. Conversely, sex, age, tumor location, differentiation grade, Ki67 index and tumor stage could not significantly affect D-dimer levels, thus indicating a complex interplay between tumor genetics and coagulation dysfunction in CRC. The current study suggested that the *KRAS* mutation status, distant metastasis and vascular invasion could be considered as independent risk factors of blood hypercoagulability in patients with CRC, potentially serving as prognostic factors for VTE risk.

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Introduction

Colorectal cancer (CRC), the second leading cause of cancer-related deaths globally, has the third highest incidence among all types of cancer (1). Its association with thrombosis dates back to 1865, when Armand Trousseau identified superficial thrombophlebitis as an early indicator of underlying malignancy (2). Among all common types of cancer, CRC is characterized by significantly high incidence of thrombotic lesions (3). A hypercoagulable state, characterized by vascular endothelial cell damage, abnormalities in fibrinolytic and anticoagulant systems, and the presence of indicative markers, such as elevated D-dimer levels, is closely associated with the clinical progression and prognosis of patients with CRC (4).

Several factors, such as tumor size, depth and degree of differentiation, can affect the hypercoagulable state (5). Additionally, it has been reported that the presence of tumors can activate the immune system, which in turn releases several inflammatory factors that exacerbate inflammation and further enhance the risk of blood hypercoagulability (6). Combination chemotherapy and targeted therapies are the most significant treatment strategies for CRC. However, emerging evidence has suggested that they can significantly increase the likelihood of developing arteriovenous thromboembolism (7-9). *KRAS* mutations, found in ~30-50% of colorectal tumors, are involved in the aforementioned process (5,10-12). This mutation was associated with an increased risk of venous thromboembolism (VTE) in patients with metastatic CRC (13) and affected the secretion dynamics of D-dimers, thus promoting tumor metastasis and cancer cell proliferation (14).

However, the intrinsic procoagulant nature of cancer cells largely stems from their unique genetic profile. Particularly, previous studies demonstrated that mutations in oncogenes, such as *KRAS*, and the loss of p53 could upregulate tissue factor (TF), thus affecting the coagulation system (15,16). Elevated TF not only increased VTE risk, but it was also associated with tumor progression and metastasis.

The present study delved into the multifaceted factors affecting the hypercoagulable state in patients with CRC, further highlighting the pivotal role of *KRAS*. Therefore, the current study aimed to elucidate the association between this coagulation anomaly and clinical pathologies, thus providing novel insights for improving therapeutic outcomes and patient survival, and reducing mortality rates. The present study focused on patients with resectable CRC and analyzed preoperative D-dimer blood specimens to assess coagulation status.

Materials and methods

Case selection. In the present prospective study, an analysis of 492 CRC cases managed surgically at the Department of Gastrointestinal Surgery, Yan'an Hospital Affiliated to Kunming Medical University (Kunming, China) between May 2019 and October 2022 was performed. Postoperative histopathological confirmation was performed via paraffin-embedded tissue samples. Pathological classification adhered to the 8th edition of the tumor-node-metastasis staging system according to the Union for International Cancer Control/American Joint Committee on Cancer guidelines. A dedicated team of pathologists from the Department of Pathology, Yan'an Hospital Affiliated to Kunming Medical University (Kunming, China) rigorously reviewed and verified the pathological data and staging. Only cases with comprehensive clinicopathological records were considered eligible. The present study was approved by the Ethics Committee of Yan'an Hospital, Affiliated to Kunming Medical University (approval no. 2018-049-02; Kunming, China). Written informed consent was obtained from all participants prior to their enrollment in the current study. The criteria for patient selection were systematically outlined (Fig. 1). The inclusion criteria were as follows: i) Patients with postoperative pathological diagnosis of CRC; and ii) eligible for and completion of surgery. The exclusion criteria were as follows: i) Patients with a history of VTE or arteriovenous thrombosis within the previous year; ii) with antecedent cardiovascular or cerebrovascular diseases, liver or renal insufficiency, or severe infections; iii) with a previous diagnosis of other malignancies; iv) undergoing an alternative surgical intervention within the past year; v) with history of neoadjuvant chemotherapy; and vi) those without complete clinicopathological data.

Data collection and access. To perform a comprehensive evaluation of the clinicopathological parameters, patient data were systematically accessed and reviewed through the electronic medical record system of the Yan'an Hospital Affiliated to Kunming Medical University. The collected data included an array of variables, including demographic details (age and sex), primary disease diagnosis, anatomical location of the tumor, presence of distant metastases, histological grade of tumor differentiation, evidence of vascular invasion and clinical staging. Additionally, the preoperative D-dimer levels, as well as perioperative venous doppler ultrasound findings, specific to the lower extremities, were also recorded. The proliferative index was quantified via measuring Ki67 labeling. The aforementioned ensemble of data provided the multifaceted profile of each case, thus enabling a robust analysis of clinicopathological associations.

VTE and D-dimer definitions. VTE was characterized by the presence of lower limb deep vein thrombosis, verified by color doppler ultrasound imaging or pulmonary embolism, ascertained by computed tomography angiography of the pulmonary arteries. A preoperative serum concentration of >550 μg/l, was considered to indicate increased D-dimer levels. Furthermore, D-dimer levels of >1,650 μg/l were considered as significantly elevated.

Determination of D-dimer concentration. To determine D-dimer concentration using enzyme-linked immunosorbent assay (ELISA), patient blood specimens were collected and centrifuged at 2,500 g for 15 min at 4˚C to separate plasma. Plasma samples were then analyzed using a D-dimer ELISA kit (cat. no. ab315310; Abcam), following the manufacturer's protocol. Each well of the microplate provided in the kit was pre-coated with a D-dimer specific antibody (Mouse Anti-D-dimer Monoclonal Antibody; cat. no. RAB0648; 5 μg/ml; Sigma-Aldrich; Merck KGaA). A total of 100 μl of standard or sample was added per well and incubated for 2 h at 37˚C. After the incubation, wells were washed four times with 300 μl of wash buffer (0.05% Tween-20 in PBS, Sigma-Aldrich; Merck KGaA) to remove unbound substances. Subsequently, 100 μl of a biotinylated detection antibody (specific to D-dimer; 1:1,000; cat. no. ab64255; Abcam) was added to each well and incubated for 1 h at 37˚C. Following a repeat of the washing step, 100 μl of horseradish peroxidase (HRP)-conjugated streptavidin (1:2,000; Bio-Rad Laboratories, Inc.) was added and incubated for 45 min at room temperature. Wells were washed again, and 100 μl of Tetramethylbenzidine (TMB) substrate solution (Thermo Fisher Scientific, Inc.) was added to each well. The reaction was allowed to develop in the dark for 30 min at room temperature and stopped with 50 μl of stop solution (1M H_2SO_4; Sigma-Aldrich; Merck KGaA). The optical density of each well was measured at 450 nm using a microplate reader. All measurements were performed in duplicates to ensure reproducibility and accuracy.

KRAS gene test. *KRAS* mutations at codons 12 and 13 were detected using High Resolution Melting (HRM) and Intplex analyses. For HRM, a reaction with 20 ng DNA, HOT qPCR Mix (Qiagen GmbH), Plus HRM Master Mix (Roche Molecular Systems, Inc.) and specific primers was prepared. The thermocycling conditions used were as follows: Initial denaturation at 95˚C for 10 min and 50 cycles at 95˚C for 15 sec and 60˚C for 1 min, followed by a melting curve at 60-95˚C. The analysis was carried out with HRM v1.1 software (Roche Molecular Systems, Inc.). The Intplex assay was applied to amplify a 300-bp *KRAS* region, with two adjacent segments (60-100 bp) representing the mutant and wild-type sequences. This approach, employing low Tm primers and a 3'-phosphate modified blocker, is used to

specifically distinguish mutant from wild-type alleles. PCR was performed in a 96-well real-time PCR format. To analyze full exons, G12 and G13 sites and particular introns of *KRAS*, the corresponding reagents for PCR and sequencing were purchased from Shanghai Sidi Biomedical Technology. Due to resource constraints, only a subset of 56 cases was selected for *KRAS* genotyping. Mutations in codons 12 and 13 of *KRAS* are commonly observed in cancerous tissues. The DNA sequences of codons 12 and 13 of *KRAS* were as follows: For codon 12, wild-type (normal) sequence, 5'-CAAGAGGTAGTCTTCCTG AAGGAG-3'; and for codon 13, wild-type (normal) sequence, 5'-GAAGACTGATAATATGCCTGCCAC-3'. The mutated sequence for both codons varied depending on the specific mutation. For example, a common mutation in codon 12 is the substitution of glycine (G) for aspartic acid (D), resulting in the sequence: 5'-CAAGAGGGAGTCTTCCTGAAGGAG-3'. Consistently, a common mutation in codon 13 is the G for D substitution, resulting in the sequence: 5'-GAAGAGTGATAA TATGCCTGCCAC-3'.

Statistical analysis. Statistical analyses were performed utilizing SPSS v.26.0 software (IBM Corp.). Continuous variables, assumed to be normally distributed, are expressed as the mean ± standard deviation. Categorical variables are expressed as percentages. For the assessment of categorical data, univariate analysis was performed using Chi-square test. Multivariate analyses were carried out using a modified Poisson regression approach. Linear correlation analyses were performed using Pearson's test. A two-tailed P-value of <0.05 was considered to indicate a statistically significant difference.

Results

Baseline patient characteristics. The study included a total of 333 patients, stratified into 198 males and 135 females, with an average age of 63.1±11.2 years. A total of 192 and 141 patients with colon cancer and rectal cancer, respectively, were enrolled. Among the 56 cases examined, 19 carried *KRAS* mutations and the remaining 37 the wild-type gene. Histopathological grading delineated 47 patients as having highly differentiated adenocarcinomas, whereas 286 presented with moderately to poorly differentiated tumors. Vascular invasion was histologically confirmed in 142 patients, while it was absent in 191. Distant metastasis was recorded in 17/333 patients. The Ki67 proliferation index was ≥70% in 208 patients, while in the remaining 125 the Ki67 index was lower. A total of 151 patients were of I/II and 182 of III/IV tumor stages. Preoperative plasma D-dimer levels averaged at 686±547 μg/l, reaching a peak value of 3,680 μg/l (data not shown). Based on D-dimer levels, 184 patients (55.3%) were categorized in the normal, 119 (35.7%) in the elevated and 30 (9.0%) in the significantly elevated D-dimer groups (Tables I and II). VTE was clinically confirmed in nine patients during hospitalization, exclusively postoperatively. Each case was diagnosed via lower limb venous ultrasound and all patients showed improvement following anticoagulant therapy and supportive care.

Association of KRAS mutation with coagulation parameters. Analysis of coagulation profiles with respect to *KRAS* mutational status revealed a marked disparity in D-dimer levels. Patients carrying *KRAS* mutation exhibited increased mean D-dimer levels (1,076±621 μg/l), which were significantly higher compared with those with the wild-type genotype (485±264 μg/l). Furthermore, the incidence of raised D-dimer levels was more prevalent among patients in the mutant *KRAS* cohort (62.3%) compared with the wild-type *KRAS* population (37.7%). This finding highlighted a potential association between *KRAS* genetic aberrations and hypercoagulable state.

Association between D-dimer levels, vascular invasion and distant metastasis in patients with CRC. Increased D-dimer levels were associated with the incidence of vascular invasion and distant metastasis in patients with CRC. The mean D-dimer concentration was found to be significantly higher in patients with intravascular tumor invasion (926±584 μg/l) compared with those without (537±246 μg/l). A cohort of 333 patients was stratified based on D-dimer levels into the normal (184 patients), elevated (119 patients) and significantly elevated (30 patients) D-dimer groups (Fig. 2). A positive association between enhanced D-dimer levels and the incidence of vascular invasion, as well as distant metastasis was also recorded. The aforementioned trend showed an increase in the

Table I. Baseline patient characteristics.

Variable	Number of patients (n=333, %)
Sex	
Female	135 (40.5)
Male	198 (59.5)
Age	63.1 (51.9-74.3)
Distant metastasis	
Present	17 (5.1)
Absent	316 (94.9)
Primary tumor site	
Colon	192 (57.7)
Rectum	141 (42.3)
Differentiation degree	
High	47 (14.1)
Moderate/Poor	286 (85.9)
Vascular invasion	
Present	142 (42.6)
Absent	191 (57.4)
Ki67	
<70%	125 (37.5)
≥70%	208 (62.5)
D-dimer (μg/l)	
≤550	184 (55.3)
>550	119 (35.7)
≥1,650	30 (9.0)
Tumor stage	
I/II	151 (45.3)
III/IV	182 (54.7)

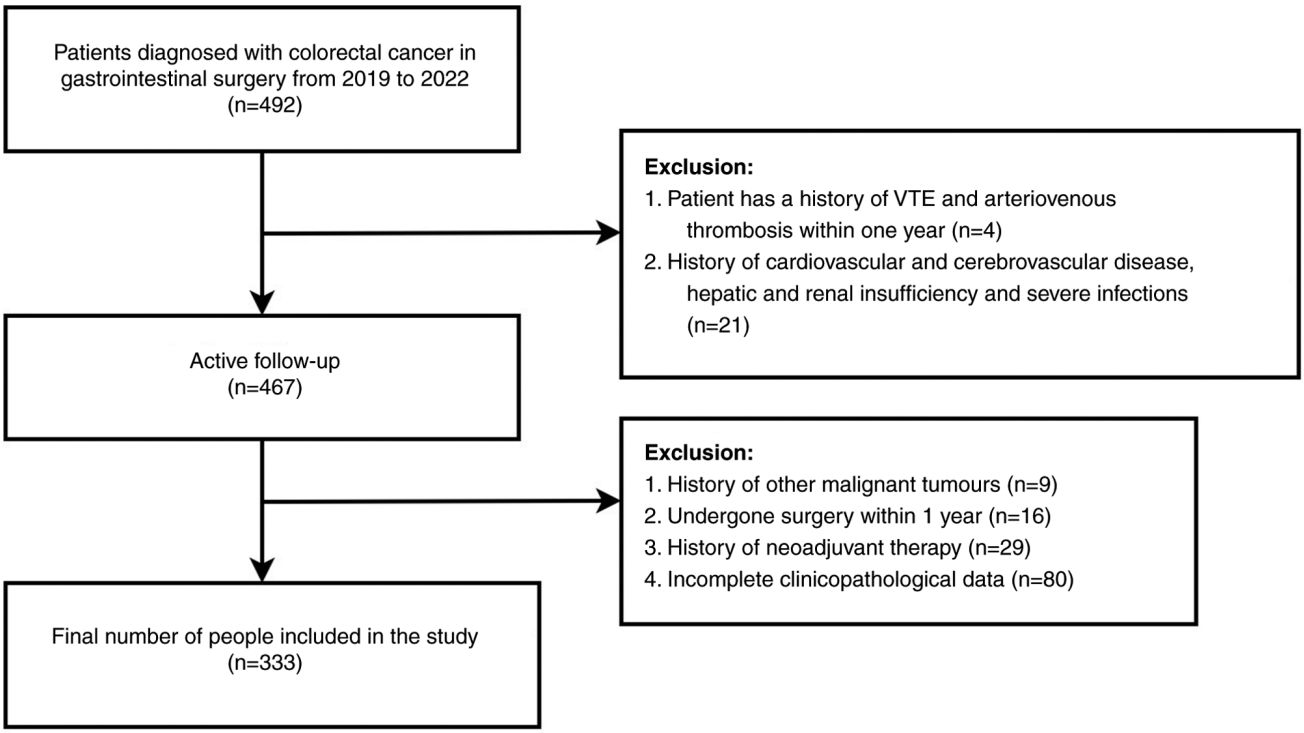

Figure 1. Flowchart for screening cases of resectable colorectal cancer.

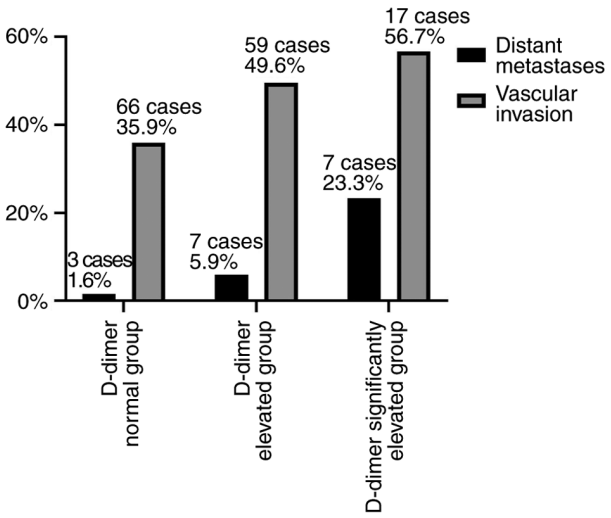

Figure 2. Elevated D-dimer levels were positively associated with the incidence of vascular invasion and distant metastases.

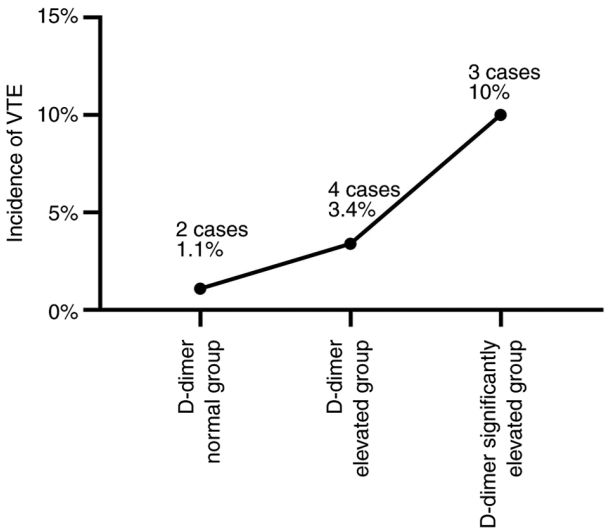

Figure 3. Elevated D-dimer levels were positively associated with the incidence of VTE. VTE, venous thromboembolism.

proportion of both vascular invasion and distant metastasis with escalating D-dimer levels, with intergroup differences reaching statistical significance.

Association between D-dimer levels and VTE incidence. An analysis of the association between D-dimer levels and the occurrence of VTE revealed a stratified patient distribution based on D-dimer values. The cohort was categorized into the following three groups: The normal D-dimer group (184 cases); the elevated D-dimer group (119 cases); and the significantly elevated D-dimer group (30 cases). Within these cohorts, a total of nine VTE incidents were recorded. The incidence of VTE increased proportionally with increasing D-dimer levels, thus supporting the significant association between D-dimer concentration and the occurrence of VTE ($r=0.712$; $P<0.05$; Fig. 3).

Single determinants of preoperative D-dimer levels. The assessment of preoperative D-dimer levels in patients with CRC revealed a significant association with several clinicopathological features. One-way analysis indicated that the presence of mutations in *KRAS*, distant metastasis and vascular invasion were notably associated with increased

Table II. One-way analysis of preoperative D-dimer levels.

Variable	D-dimer (n%)		P-value
	Normal group n=184	Elevated group n=149	
Sex			0.077
Female	73 (40.1)	62 (41.6)	
Male	111 (59.9)	87 (58.4)	
Age			0.362
<65 years old	90 (48.9)	83 (55.7)	
≥65 years old	94 (51.1)	66 (44.3)	
Gene type			0.001
KRAS mutant	4 (12.1)	15 (62.3)	
KRAS wild	29 (87.9)	8 (37.7)	
Distant metastasis			0.001
Present	3 (1.6)	14 (9.4)	
Absent	181 (98.4)	135 (90.6)	
Primary tumor site			0.723
Colon	115 (62.5)	77 (51.7)	
Rectum	69 (37.5)	72 (48.3)	
Differentiation degree			0.684
High	26 (14.1)	21 (14.1)	
Moderate/Poor	158 (85.9)	128 (85.9)	
Vascular invasion			0.007
Present	66 (35.9)	76 (51.0)	
Absent	118 (64.1)	73 (49.0)	
Ki67			0.213
<70%	74 (40.2)	51 (34.2)	
≥70%	110 (59.8)	98 (65.8)	
Tumor stage			0.781
I/II	74 (40.2)	77 (51.7)	
III/IV	110 (59.8)	72 (48.3)	

D-dimer levels (P<0.05). Conversely, other factors, such as sex, age, the primary location of the tumor, the degree of cellular differentiation, Ki67 index and overall tumor stage were not significantly associated with enhanced D-dimer levels (Table II).

Multifactorial determinants of preoperative D-dimer levels. Subsequently, a multivariate regression analysis was performed to elucidate the factors involved in the increased preoperative D-dimer levels. Several clinicopathological variables were identified as independent predictors. Therefore, *KRAS* mutations were strongly associated with increased D-dimer levels, yielding a relative risk (RR) of 2.912, with a 95% confidence interval (CI) ranging from 2.320 to 3.674 (P<0.001). Similarly, distant metastasis also had a notable effect on D-dimer overexpression (RR=1.884; 95% CI, 1.415-2.632; P<0.001). Furthermore, vascular invasion was also identified as an independent factor, which was associated with higher D-dimer levels; however, to a lesser extent (RR=1.525; 95% CI, 1.146-2.328; P<0.05). Conversely, there was no significant association between sex, age, primary tumor site, degree of differentiation, Ki67 index and tumor stage, and D-dimer levels, since no statistical significance was obtained (Fig. 4; Table III).

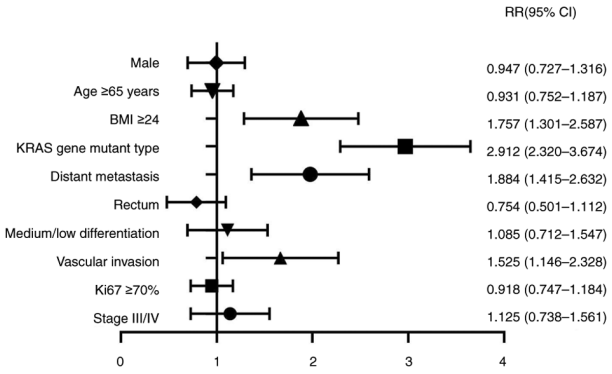

Figure 4. Forest plot of multifactorial regression analysis of preoperative D-dimer. *KRAS* genotype, distant metastasis and vascular invasion were identified as independent risk factors. BMI, body mass index; RR, relative risk; CI, confidence interval.

Table III. Multifactorial analysis of preoperative D-dimer levels.

Variable	RR value (95% CI)	P-value
Sex		
Female	0.947 (0.727-1.316)	0.729
Male		
Age		
<65 years old	0.931 (0.752-1.187)	0.754
≥65 years old		
Gene type		
KRAS wild	2.912 (2.320-3.674)	<0.001
KRAS mutant		
Distant metastasis		
Absent	1.884 (1.415-2.632)	<0.001
Present		
Primary tumor site		
Colon	0.754 (0.501-1.112)	0.694
Rectum		
Differentiation degree		
High	1.085 (0.712-1.547)	0.732
Moderate/Poor		
Vascular invasion		
Absent	1.525 (1.146-2.328)	0.012
Present		
Ki67		
<70%	0.918 (0.747-1.184)	0.587
≥70%		
Tumor stage		
I/II	1.125 (0.738-1.561)	0.612
III/IV		

RR, relative risk; CI, confidence interval.

Discussion

The association between malignancies and thromboembolic events is a well-established paradigm within oncology, which was also highlighted by the results of the present study, thus suggesting that the survival of patients with cancer, also suffering from thrombotic episodes, was notably reduced. Tumor-associated thrombosis, also known as cancer-associated thrombosis (CAT), can exacerbate patient morbidity, diminish survival rates and stand as a prominent cause of mortality among cancer cohorts (5). The disparity in the incidence of VTE among patients with different types of cancer can be attributed to their distinct interactions with the coagulation cascade, with several types of tumors promoting hypercoagulability to varying degrees (17). Hypercoagulability not only promotes VTE pathogenesis, but is also a harbinger of such vascular complications.

The current study meticulously explored the clinical conundrum of CAT via examining hypercoagulability, secondary to tumor pathology. The results revealed that the mean preoperative D-dimer levels were increased, exceeding the normal threshold by ~30%. Notably, a subset of patients was presented with D-dimer levels three times higher than the upper normal limit, underscoring the extent of the existing hypercoagulability. Nevertheless, the incidence of perioperative VTE was a modest 2.8%, a rate that differed from previous studies (18,19). The discrepancy in the prevalence of VTE could be attributed to the design of the present study, which focused on perioperative parameters, thus potentially mitigating the long-term recording of VTE incidents.

Previous studies indicated a temporal scope of VTE between six months prior the definitive clinical diagnosis of CRC and patient mortality, showing a considerable duration (20-22). However, the research design was structured to exclusively record VTE events in the perioperative phase, thus providing a diminished incidence rate. The present study aimed to delineate the effect of the neoplasm on the hypercoagulable state inherent in patients with CRC. Although longitudinal surveillance can mitigate the risk of missing VTE events, confounding effects of subsequent therapeutic interventions, such as chemotherapy, radiotherapy, immunotherapy and procedures involving deep vein access, cannot be ruled out. Fluorouracil-based chemotherapy has become the standard treatment approach for CRC (9). A previous study, including a large sample size, showed that postoperative chemotherapy for CRC predisposed to VTE, with a 1-year cumulative incidence of 13.7%. In addition, the administration of bevacizumab, a targeted therapy agent, could increase the likelihood of arterial and VTE (7,8).

In light of these considerations, it was hypothesized that the preoperative D-dimer levels in patients with resectable CRC could serve as a robust surrogate for assessing hypercoagulable state. The rationale for selecting this biomarker lies in its capacity to reflect thrombotic predisposition directly attributable to malignancy, independent of confounding variables introduced by postoperative treatments. Additionally, *KRAS*-driven tumors can induce a hypercoagulable state via upregulating prothrombotic factors and promoting vascular dysfunction, thus promoting the formation of blood clots. In turn, the aforementioned clots can then be broken down, thus releasing D-dimer fragments into the bloodstream (19). Therefore, elevated D-dimer levels serve as a biomarker reflecting both the presence of *KRAS* mutations and the associated prothrombotic environment in patients with cancer. By employing this approach, it was endeavored to provide a more isolated assessment of the contribution of cancer to coagulopathy, thereby yielding insights that are clinically relevant to the preoperative evaluation of thrombotic risk in patients with CRC. Focusing on preoperative D-dimer levels in patients with resectable CRC aimed to distill the effect of the tumor itself, without considering confounding therapeutic interventions.

D-dimer, a byproduct of fibrin degradation, serves as a versatile biomarker in various pathological conditions, including inflammation, malignancy and thrombosis (23). In CRC, enhanced D-dimer levels are indicative of the concomitant inflammatory response and prothrombotic milieu induced by malignancy progression (24). Herein, the stratified analysis revealed an association between enhanced hypercoagulability and *KRAS* mutations, distant metastasis and vascular invasion. However, no significant

association with other demographic or tumor characteristics was obtained.

An intriguing facet of the current study was the association between *KRAS* mutations and hypercoagulability. The presence of mutations could enhance the risk of hypercoagulability, thus further verifying the findings from other cohort studies and shedding light on possible underlying molecular mechanisms (13). The interplay between *KRAS* mutations and hypercoagulability involves the effect of the oncogene in modulating key coagulation factors, such as TF expression, via intricate pathways, including the MEK/MAPK and PI3K pathways (25-27).

In the intricate association between malignancies and hemostatic processes, CRC cells are involved in inducing a hypercoagulable state, a phenomenon characterized by an increased tendency of blood to clot. This disorder in coagulation can be broadly classified into three distinct, but interrelated, mechanisms.

Firstly, tumor cells directly express procoagulant substances. A significant factor in this category is TF, also known as factor III, which stands at the forefront of the coagulation cascade under both physiological and pathological conditions (28). When TF forms a complex with activated factor VII, it catalyzes the activation of IX and X factors, thus promoting the activation of the exogenous coagulation pathway. Aberrant expression of TF is a hallmark of numerous solid tumors and hematologic malignancies, with its expression levels commonly serving as a barometer for tumor malignancy (29). Interestingly, a previous study demonstrated that TF expression was modulated by genetic alterations within cancer cells, including the activation of proto-oncogenes, such as *KRAS* and *MET*, or the inactivation of tumor suppressor genes, including *TP53* and *PTEN* (30).

The second mechanism involves the procoagulant effects triggered by tumor cell-derived extracellular vesicles (EVs) (31,32). These vesicles not only reflect the surface proteins of their cells of origin, but can also assimilate proteins from other cells via fusion. In the context of thrombotic disorders, an increase in both the concentration and procoagulant activities of EVs has been associated with their pivotal role in the pathogenesis of thrombosis (33).

The third and final mechanism, by which CRC cells induce hypercoagulability, is through interactions between tumor and host cells. It has been reported that the interface of p-selectin proteins on platelet membranes and podoplanin proteins on particular tumor cells can facilitate the formation of tumor cell-platelet aggregates (34,35). In turn, the aforementioned aggregates can activate platelets, thus resulting in a hypercoagulable state. Furthermore, these platelet aggregates impart a form of ‘cloak’ over the tumor cells, which acts as a shield against the immune surveillance of circulating natural killer cells. This process not only aids in evading immunological detection, but also augments the possibility of hematogenous tumor metastasis (36).

In addition, cancer itself is a slow-moving inflammatory response. Therefore, it has been suggested that elevated C-reactive protein, IL-6, IL-8 and TNF-α levels are associated with an increased risk of VTE during systemic inflammatory response. Subsequent platelet activation could enhance the pre-thrombotic state, thus leading to the development of VTE (37-39). Identifying and elucidating inflammatory markers, associated with VTE, could provide novel targets for future therapy.

The aforementioned mechanisms, when considered collectively, could depict a multifaceted scheme through which CRC insidiously co-opts the body's coagulation system to its advantage, thus promoting both its survival and propagation. The implications of these findings are enormous, affecting therapeutic strategies and requiring a multifaceted approach to target the coagulation cascade at various junctures in the management of CRC. The results of the present study further emphasized the prognostic properties of CAT. Therefore, it was suggested that the *KRAS* mutational status could serve as a valuable marker to assess the risk of hypercoagulability and, by extension, could predict worse prognosis in patients with CRC.

Despite the robust findings, the present study has some limitations. Firstly, the current investigation was constrained only to the effect of a single gene, namely *KRAS,* on coagulation in patients with CRC. It is widely accepted that several oncogenes and tumor suppressor genes, through their aberrant expression or mutations, can regulate the production of coagulation factors by tumor cells (34,36,40-43). Therefore, comprehensive multigene studies are needed to unravel the differential effects of various genotypes on coagulation, thus potentially improving anticoagulant and anticancer therapeutic strategies. Additionally, the focus of the study on the perioperative period, without long-term follow-up, precluded further insights into the long-term effect of *KRAS* mutations on the survival of patients with CRC. Lastly, due to financial constraints, only a limited number of cases were assessed for *KRAS* mutations, introducing a degree of selection bias.

In summary, the results of the current study supported the association between cancer and thrombosis. Exploring the genetic factors associated with hypercoagulability holds promise for enhancing the management of patients with CRC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

DX made substantial contributions to the concept and design. CL and YH made substantial contributions to the acquisition of data and the analysis and interpretation of the data. JT were involved in drafting the article or revising it critically for important intellectual content. All authors read and approved the final the manuscript. DX and JT confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study involving human subjects was reviewed and approved by the Medical Ethics Committee of Yan'an Hospital of Kunming Medical University (approval no. 2018-049-02; Kunming, China). Patients provided written informed consent to participate in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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