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Original Article

Immune checkpoint inhibitors combined with chemotherapy enhance coagulation activation and elevate venous thromboembolism risk in non-small cell lung cancer

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Abstract: Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related mortality. The combination of immune checkpoint inhibitors (ICIs) with chemotherapy has become a standard first-line treatment, yet their effects on coagulation and thrombosis risk are not fully defined. The retrospective cohort study analyzed 218 NSCLC patients receiving either ICI plus chemotherapy (n=102) or chemotherapy alone (n=116). We compared objective response rate (ORR), disease control rate (DCR), key coagulation biomarkers (D-dimer, fibrinogen, fibrin degradation products [FDP], and plasmin-α_2 antiplasmin complex [PAP]), and venous thromboembolism (VTE) incidence between groups. Compared to chemotherapy alone, combination therapy had significantly higher ORR (50.98% vs. 31.03%, P=0.003) and DCR (84.31% vs. 72.41%, P=0.034). Following treatment, the combination group also showed significantly greater elevations in coagulation biomarkers: D-dimer (1.12±0.48 vs. 1.84±0.41 mg/L), fibrinogen (4.26±1.08 vs. 3.78±0.94 g/L), FDP (6.27±2.48 vs. 5.18±2.13 μg/mL), and PAP (1.28±0.46 vs. 1.02±0.41 μg/mL; all P<0.001). Moreover, VTE incidence was notably higher in the combination group (16.67% vs. 7.76%, P=0.043). While ICI-chemotherapy offers superior antitumor efficacy, it is associated with greater coagulation activation and an increased VTE risk compared to chemotherapy alone in NSCLC patients.

Keywords: Non-small cell lung cancer, immune checkpoint inhibitors, venous thromboembolism, coagulation biomarkers, chemotherapy

Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. It accounts for about 85% of all lung cancer cases and is a major cause of cancer-related deaths worldwide [1-3]. The global burden of NSCLC is still increasing. It is estimated that about 2.2 million new lung cancer cases are diagnosed each year [2, 4]. This shows that we urgently need more effective treatments. Platinum-based chemotherapy (PTx) has long been the standard treatment for advanced NSCLC, providing a survival benefit. However, its efficacy is often limited by disease progression and the development of drug resistance over time [5-7].

The treatment paradigm for cancer has been transformed by immune checkpoint inhibitors (ICIs). These agents target regulatory pathways, mainly the programmed cell death protein 1 (PD-1)/programmed cell death-ligand 1 (PD-L1) axis, to enhance and restore anti-tumor T-cell activity [8, 9]. The combination of ICIs with PTx has shown superiority over chemotherapy alone in multiple major clinical trials [10, 11]. Therefore, it is recognized as a first-line treatment option for many patients with advanced NSCLC without actionable driver mutations [7, 8]. While this paradigm shift has focused on survival and tumor response, there is a need to further explore the impact of these potent combination therapies on systems beyond just the tumor and the immune system [12].

Cancer inherently increases the risk of venous thromboembolism (VTE), a significant complica-

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tion affecting many cancer patients [13-15]. The prothrombotic state is a complex process driven by three primary factors: the procoagulant activity of tumor cells, the host proinflammatory response to the tumor, and physical compression of blood vessels [13, 14, 16]. Elevated levels of coagulation and fibrinolysis biomarkers - such as D-dimer, fibrin degradation products (FDP), fibrinogen, and plasmin-antiplasmin complexes (PIC) - are frequently observed in cancer patients. These levels correlate with disease stage, prognosis, and VTE risk [15, 17, 18]. Chemotherapy itself contributes to this risk by directly injuring endothelial cells, increasing the release of procoagulant microparticles, and reducing endogenous anticoagulant activity [19, 20]. The impact of anticancer treatment on coagulation is therefore a critical consideration in the comprehensive management of patients.

ICIs introduce a novel immunologic dimension. By activating T-cells, they can induce the expression of tissue factor and the release of potent inflammatory cytokines like tumor necrosis factor-alpha and interleukin-1 beta. These factors are known to activate vascular endothelial cells and disrupt hemostatic balance [21]. Additionally, other immune-related effects, such as vasculitis, may damage blood vessels and cause thrombosis [11, 13]. Emerging preclinical and clinical reports suggest a potential association between ICI therapy and an increased incidence of thromboembolic events [12, 16]. Consequently, a critical unanswered question arises: does combining ICIs with standard chemotherapy exacerbate the prothrombotic risk associated with chemotherapy alone? Furthermore, does the pattern of hemostatic disruption differ between these regimens? At present, it remains unclear whether changes in key coagulation and fibrinolysis biomarker levels differ significantly between patients receiving ICI-chemotherapy combination and those receiving chemotherapy alone.

The innovation of this study lies in its direct, systematic comparison of coagulation profile changes between these two important treatments within a NSCLC cohort. It evaluates a panel of pertinent coagulation and fibrinolysis biomarkers and assesses the incidence of clinical VTE. The clinical significance is substantial: identifying an elevated thrombotic risk associated with this combination treatment can inform risk stratification, guide vigilant monitoring, and prompt consideration of preventive measures for patients undergoing this powerful yet effective treatment, ultimately aiming to optimize total patient outcomes.

Materials and methods

Case selection

A retrospective analysis was conducted on 218 patients with NSCLC admitted to Nantong First People's Hospital from January 2020 to December 2022. Inclusion criteria: (1) Age 45-85 years; (2) Treatment with either ICI plus PTx or PTx alone, as determined by a multidisciplinary tumor board based on comprehensive clinical assessments, current guidelines, and individual factors such as PD-L1 expression, Eastern Cooperative Oncology Group (ECOG) performance status, comorbidities, and after detailed patient counseling; (3) Histologically confirmed NSCLC according to World Health Organization classification [22]; (4) ECOG performance status score 0-2; (5) Complete medical records and follow-up data available. Exclusion criteria: (1) History of severe cardiovascular disease within the past 6 months, including myocardial infarction, stroke, or New York Heart Association class III/IV heart failure; (2) Active autoimmune disease requiring immunosuppression; (3) Life expectancy <3 months; (4) Ongoing anticoagulation therapy or history of VTE within 3 months prior to enrollment; (5) Platelet count $<100\times10^9$/L at study entry or known coagulation disorder.

For sample size determination, G*Power 3.1 was employed. The calculation was based on the following parameters: a medium effect size (d=0.5), a significance level of α=0.05 (two-tailed), and a statistical power of 90%. The analysis indicated a minimum requirement of 86 participants per group to detect a significant difference between groups with the specified power. The primary statistical test planned for group comparisons was a two-sided, two-sample t-test assuming equal variance.

Ethical statement

This research obtained ethical approval from the Institutional Review Board of Nantong First People's Hospital. It was carried out in compliance with the ethical guidelines of the

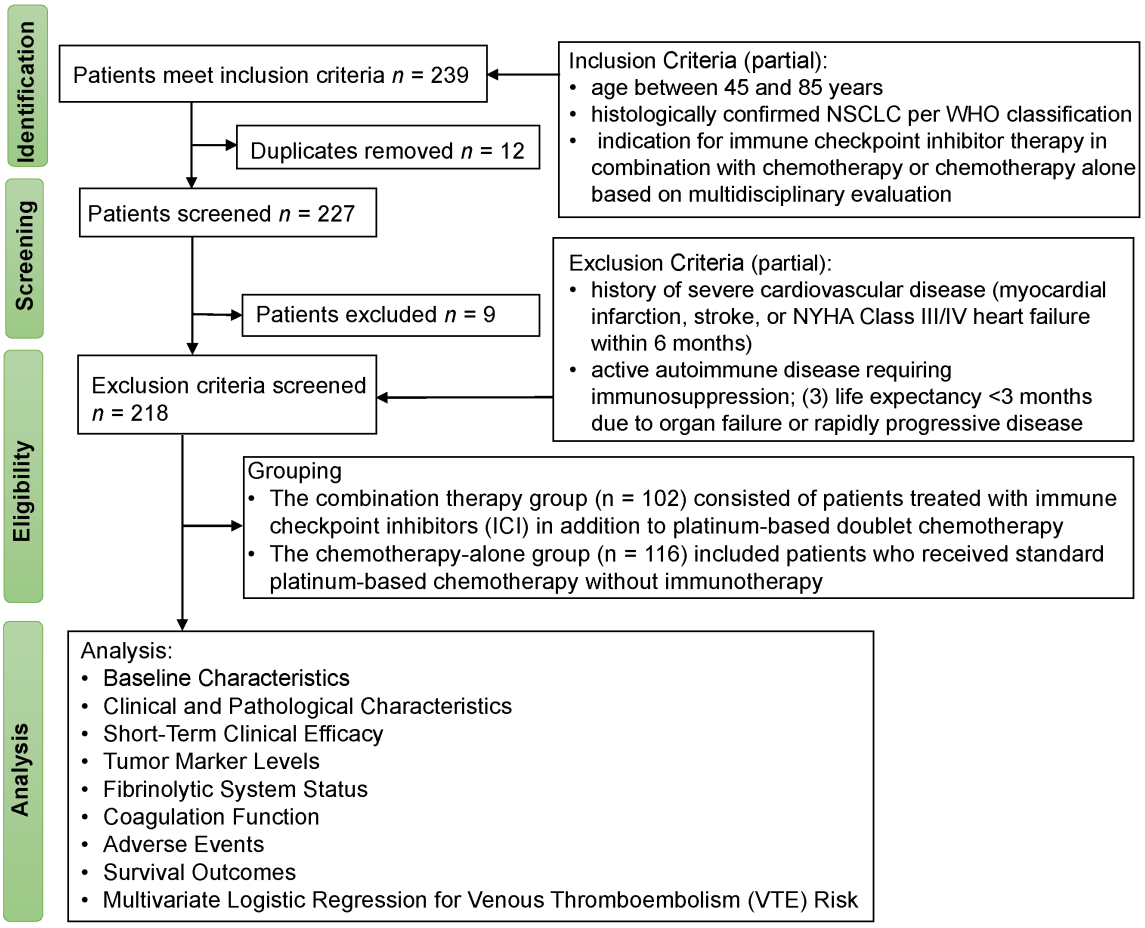

Figure 1. Flowchart of patient selection and group allocation. NSCLC: non-small cell lung cancer; WHO: World Health Organization; NYHA: New York Heart Association.

Declaration of Helsinki. As this was a retrospective analysis involving only de-identified patient information, the Ethics Committee granted a waiver of informed consent. This decision was based on the absence of any foreseeable risk to patient welfare.

Grouping and treatment protocols

Grouping: This was a retrospective cohort study. Participants were categorized into two groups according to the treatment regimen they received. The combination therapy group (n=102) consisted of patients treated with ICI plus PTx. The chemotherapy-alone group (n=116) comprised patients who received standard PTx without immunotherapy (**Figure 1**).

Treatment: In the chemotherapy-alone group, patients with adenocarcinoma or large cell lung carcinoma received pemetrexed (500 mg/m^2; Guosi Mei (Wuhan) Pharmaceutical Co., Ltd., Hubei, China; National Drug Approval No. H20213204) combined with cisplatin (75 mg/m^2; Qilu Pharmaceutical Co., Ltd., Shandong, China; National Drug Approval No. H37021358), administered intravenously every three weeks for a total of 6 weeks. Those diagnosed with squamous cell carcinoma were treated with paclitaxel (135-175 mg/m^2; Jiangsu Honma Pharmaceutical Co., Ltd., Jiangsu, China; National Drug Approval No. H20067345) and cisplatin (75 mg/m^2; Qilu Pharmaceutical Co., Ltd., Shandong, China; National Drug Approval No. H37021358) on the same schedule. Patients in the combination therapy group received the same chemotherapy backbone as above, with the addition of camrelizumab (AiRuika; Suzhou Suncadia Biopharmaceutical Co., Ltd., Jiangsu, China; National Drug Approval No. S20190027), a PD-1 inhibitor, administered at a fixed dose of 200 mg via intravenous infusion every three weeks for 6 weeks. Dose adjustments or treatment delays were permit-

ted based on individual tolerance and adverse events, in accordance with treatment guidelines [22].

Data extraction and outcome measures

Data were extracted retrospectively from electronic medical records, laboratory information systems, and radiology archives by independent researchers. Extracted data included demographic characteristics, clinical and pathological features, treatment details, serial laboratory results (tumor markers, coagulation/fibrinolytic parameters), imaging reports, and recorded adverse events.

Primary outcomes: (1) Incidence of VTE within 6 months after treatment initiation; (2) Changes in key coagulation/fibrinolytic biomarkers from baseline to post-treatment. Secondary Outcomes: (1) Short-term clinical efficacy; (2) Changes in tumor marker levels; (3) Incidence of other adverse events; (4) Survival outcomes.

Short-term clinical efficacy: Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors version 1.1 guidelines. Contrast-enhanced computed tomography was performed at baseline and after 6 weeks of treatment. Responses were classified as follows [23]: Complete Response (CR): Disappearance of all target lesions for at least 4 weeks; Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, with no new lesions or progression of non-target lesions, lasting for at least 4 weeks; Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease (PD); PD: At least a 20% increase in the sum of diameters of target lesions, or the appearance of one or more new lesions. The objective response rate (ORR) was defined as the proportion of patients achieving CR or PR. The disease control rate (DCR) was defined as the proportion of patients achieving CR, PR, or SD.

Blood tests: Peripheral venous blood (10 mL) was collected from each patient after an overnight fast at two time points: before and 24 hours after completing the 6-week treatment. For coagulation tests, 5 mL of blood was drawn into vacuum tubes containing 3.2% (0.109 M) sodium citrate as anticoagulant. Another 5 mL was collected in ethylenediaminetetraacetic acid tubes for complete blood count analysis.

(1) Tumor biomarker levels, including carcinoembryonic antigen (CEA; catalog 05200067, Roche Diagnostics, Switzerland), carbohydrate antigen 125 (CA125; catalog 07005717, Roche), and cytokeratin 19 fragment (CYFRA21-1; catalog 06656011, Roche), were quantitatively measured using an electrochemiluminescence immunoassay on a Roche Cobas e801 automated immunoassay analyzer.

(2) Coagulation parameters were assessed on automated coagulation and hematology analyzers. Prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, and D-dimer were measured on a Sysmex CS-5100 fully automated coagulation analyzer (Sysmex, Japan) using Siemens reagents (clotting method for PT, APTT, and fibrinogen; immunoturbidimetric assay for D-dimer). FDPs were also determined on the Sysmex CS-5100 system using a Siemens immunoturbidimetric assay reagent. PICs were measured by a chemiluminescence immunoassay on a Shine i2900 automated chemiluminescence analyzer (Guangzhou Wondfo Biotech Co., Ltd., Guangzhou, China) using the manufacturer's reagents. Platelet counts were obtained from complete blood count analysis performed on a Sysmex XN-20 automated hematology analyzer (Sysmex, Japan) using the manufacturer's reagents.

Adverse events: Adverse events occurring within six months after treatment initiation were identified and graded based on medical records. VTE events were diagnosed using Doppler ultrasonography for deep vein thrombosis (**Figure 2**) and computed tomography pulmonary angiography for pulmonary embolism (**Figure 3**). Non-VTE adverse events - including leukopenia, thrombocytopenia, and hepatic or renal impairment - were documented in accordance with the Common Terminology Criteria for Adverse Events v5.0 [24].

Follow up

Patients were followed up every three months systematically, either through outpatient visits or telephone interviews. The follow-up period lasted for two years, ending in December 2024. The first six months after treatment initiation served as the primary observation period for

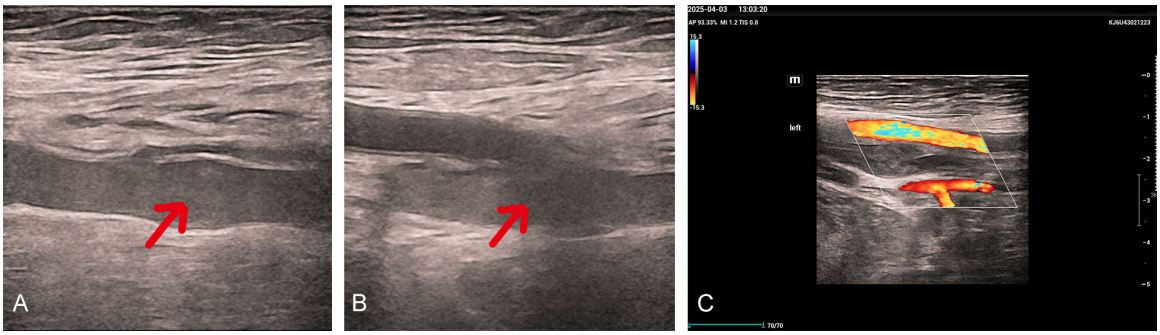

Figure 2. Ultrasound showing acute deep vein thrombosis. A. Gray-scale ultrasound of the left common femoral vein reveals an enlarged vein (red arrow); B. Gray-scale compression ultrasound shows the vein is non-compressible (red arrow), with low echogenicity within the lumen; C. Corresponding color flow and spectral Doppler imaging demonstrate absence of flow within the vein.

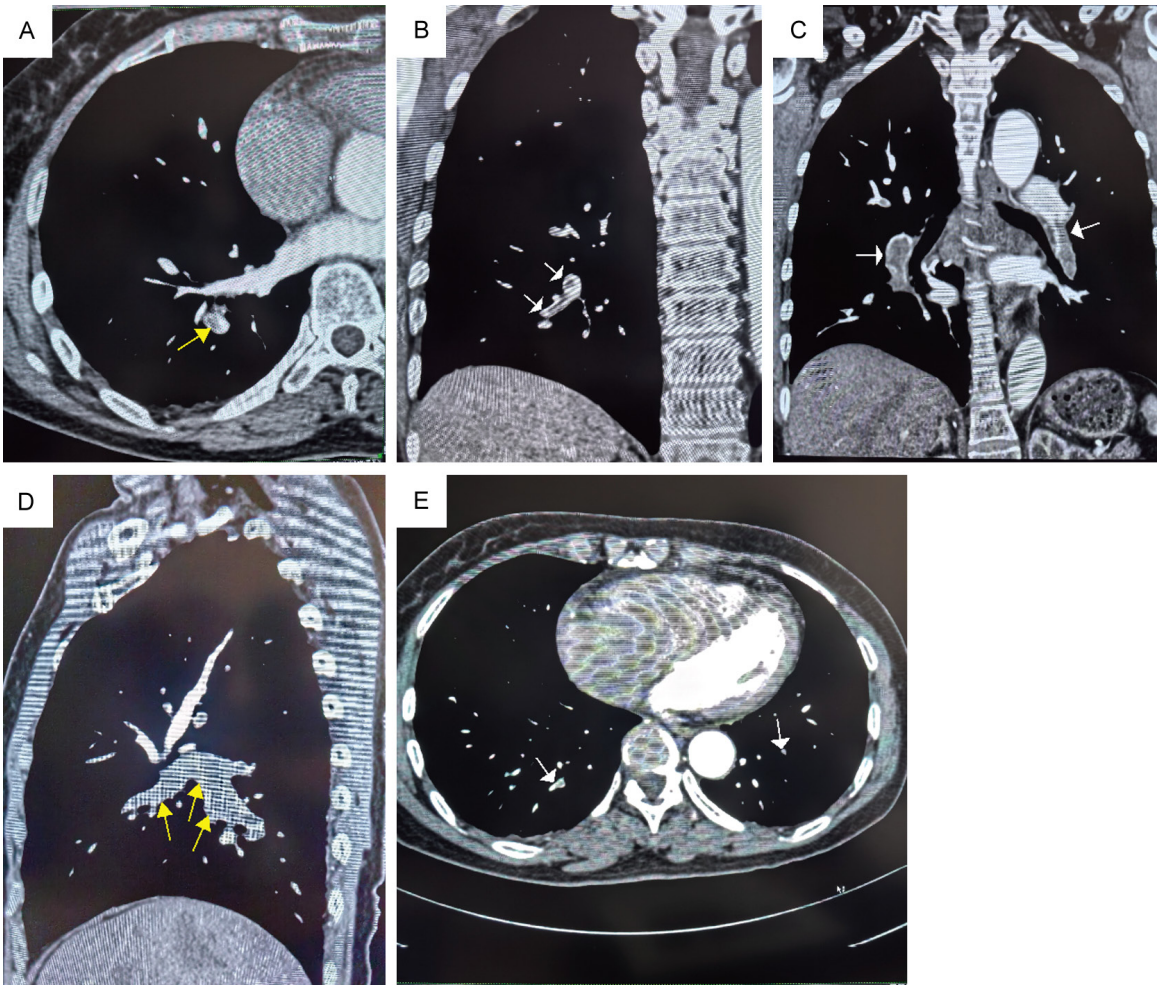

Figure 3. Direct findings of acute pulmonary embolism on CTPA. A. Axial CTPA orthogonal to the dilated posterior segmental pulmonary artery of the right lower lobe shows a central filling defect surrounded by a rim of contrast, demonstrating the "polo-mint" sign of acute pulmonary embolism (yellow arrow); B. Coronal CTPA along the long axis of the segmental pulmonary artery of the right lower lobe shows a central filling defect with parallel contrast lines around it, consistent with the "railway track" sign of acute pulmonary embolism (white arrow); C. Coronal CTPA reveals extensive large pulmonary emboli, presenting as occlusive and mural filling defects with edges forming sharp angles with the vessel wall (white arrow); D. A large low-density embolus covering the bifurcation of the right pulmonary artery, exhibiting a "saddle embolus" morphology (yellow arrow); E. Axial CTPA of the lung base shows dilation

of the posterior basal segmental pulmonary artery of the right lower lobe with an occlusive filling defect, consistent with acute pulmonary embolism (white arrow). There is also a small pulmonary embolus in the posterior basal segmental pulmonary artery of the left lower lobe (white arrow). CTPA, computed tomography pulmonary angiography.

Table 1. Demographic and clinical characteristics of the two groups

Parameters	Chemotherapy-Alone Group (n=116)	Combination Therapy Group (n=102)	t/χ^2	p
Gender [n (%)]			0.710	0.399
Male	68 (58.62)	54 (52.94)		
Female	48 (41.38)	48 (47.06)		
Age (years)	63.42±8.73	64.18±9.26	0.624	0.533
BMI (kg/m^2)	22.36±3.14	22.89±3.42	1.199	0.232
Education level [n (%)]			0.611	0.434
High school and below	72 (62.07)	58 (56.86)		
University and above	44 (37.93)	44 (43.14)		
Smoking status [n (%)]			0.182	0.670
Current or former	76 (65.52)	64 (62.75)		
Never	40 (34.48)	38 (37.25)		

BMI: body mass index.

recording adverse events related to the treatment. Overall survival (OS) and progression-free survival (PFS) were monitored throughout the entire follow-up period. All relevant data were obtained through retrospective review of electronic medical records, imaging reports, and follow-up documentation.

Statistical analysis

All statistical analyses were performed using SPSS software (version 29.0; IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed continuous data are presented as mean ± standard deviation and compared between the two groups using the independent-samples t-test. For within-group comparisons of normally distributed parameters, the paired-samples t-test was used. Non-normally distributed continuous data are expressed as median with interquartile range and compared using the Mann-Whitney U test. For categorical variables, which are presented as frequencies and percentages [n (%)], the Chi-square (χ^2) test or Fisher's exact test was utilized as appropriate. A multivariable logistic regression analysis was performed to identify independent risk factors for VTE. Key continuous variables (D-dimer, FDP) were converted into categorical variables based on clinically established cut-off points (D-dimer: 0.7 mg/L [25]; FDP: 5.0 μg/mL [26]) prior to inclusion in the regression. Variables with a *p*-value <0.10 in univariate analysis were initially entered into the multivariable model. To avoid overfitting and maintain parsimony, backward stepwise selection (likelihood-ratio test) was applied, retaining variables with P<0.05 in the final model. Model adequacy was evaluated using the Hosmer-Lemeshow goodness-of-fit test. Multicollinearity among independent variables was assessed by calculating variance inflation factors; all variance inflation factor values were below 2, indicating no substantial multicollinearity. Survival outcomes (OS and PFS) were visualized using Kaplan-Meier curves, and differences between groups were compared with the log-rank test. All tests were two-tailed, and statistical significance was set at P<0.05.

Results

Baseline characteristics of patients

Analysis of baseline demographic and clinical characteristics demonstrated no significant differences between the combination therapy group (n=102) and the chemotherapy-alone group (n=116) in terms of gender, age, body mass index, smoking status, ECOG performance status, histology, clinical stage, metastasis, or PD-L1 expression level (all P>0.05; **Tables 1**, **2**). This indicates that the two groups

Table 2. Clinical and pathological characteristics of the two groups [n (%)]

Parameters	Chemotherapy-Alone Group (n=116)	Combination Therapy Group (n=102)	χ^2	p
ECOG performance-status score			0.278	0.870
0	42 (36.21)	36 (35.29)		
1	58 (50.00)	54 (52.94)		
2	16 (13.79)	12 (11.76)		
Histology			0.624	0.732
Squamous cell carcinoma	43 (37.07)	34 (33.33)		
Adenocarcinoma	67 (57.76)	64 (62.75)		
Large cell carcinoma	6 (5.17)	4 (3.92)		
Clinical stage			0.162	0.687
III	44 (37.93)	36 (35.29)		
IV	72 (62.07)	66 (64.71)		
Metastasis			0.297	0.586
Multiple	78 (67.24)	65 (63.73)		
Single	38 (32.76)	37 (36.27)		
Brain metastases	20 (17.24)	18 (17.65)	0.006	0.937
PD-L1 TPS			0.412	0.814
<1%	36 (31.03)	28 (27.45)		
1-49%	48 (41.38)	46 (45.10)		
≥50%	32 (27.59)	28 (27.45)		

ECOG: Eastern Cooperative Oncology Group; PD-L1: programmed death-ligand 1; TPS: tumor proportion score.

Table 3. Comparison of short-term treatment response between the two groups [n (%)]

Parameters	Chemotherapy-Alone Group (n=116)	Combination Therapy Group (n=102)	χ^2	p
CR	4 (3.45)	8 (7.84)		
PR	32 (27.59)	44 (43.14)		
SD	48 (41.38)	34 (33.33)		
PD	32 (27.59)	16 (15.69)		
ORR (CR+PR)	36 (31.03)	52 (50.98)	8.97	0.003
DCR (CR+PR+SD)	84 (72.41)	86 (84.31)	4.476	0.034

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; ORR: objective response rate; DCR: disease control rate.

were well-matched and comparable at baseline.

Short-term clinical efficacy

Short-term treatment response was evaluated. The results were better in the combination therapy group. The ORR was 50.98% for patients who received immunotherapy plus chemotherapy, which was significantly higher than the ORR of 31.03% in the chemotherapy-alone group (P=0.003). The DCR was also higher in the combination group (84.31%) compared to the chemotherapy-alone group (72.41%) (P=0.034). These data are shown in **Table 3**.

Tumor marker levels

Before treatment, all tumor marker levels were comparable between the two groups (all P>0.05). After treatment, tumor marker levels showed a greater reduction in the combination therapy group compared to the chemotherapy-alone group. The post-treatment CEA level was 62.17±30.28 ng/mL in the combination group versus 76.89±31.42 ng/mL in the chemotherapy-alone group (t=3.512, P<0.001). Similarly,

Table 4. Within- and between-group comparisons of tumor marker levels before and after treatment

Parameters	Time	Chemotherapy-Alone Group (n=116)	Combination Therapy Group (n=102)	t	p
CEA (ng/mL)	Baseline	98.36±42.18	101.24±45.73	0.483	0.629
	After	76.89±31.42	62.17±30.28	3.512	<0.001
t		4.396	7.194		
p		<0.001	<0.001		
CA125 (ng/mL)	Baseline	74.33±32.17	77.18±34.62	0.630	0.530
	After	58.76±18.45	46.42±15.83	5.263	<0.001
t		4.522	8.161		
p		<0.001	<0.001		
CYFRA21-1 (ng/L)	Baseline	7.82±2.46	7.94±2.57	0.356	0.722
	After	4.13±1.87	3.28±1.64	3.572	<0.001
t		12.861	15.437		
p		<0.001	<0.001		

CEA: carcinoembryonic antigen; CA125: carbohydrate antigen 125; CYFRA21-1: cytokeratin 19 fragment.

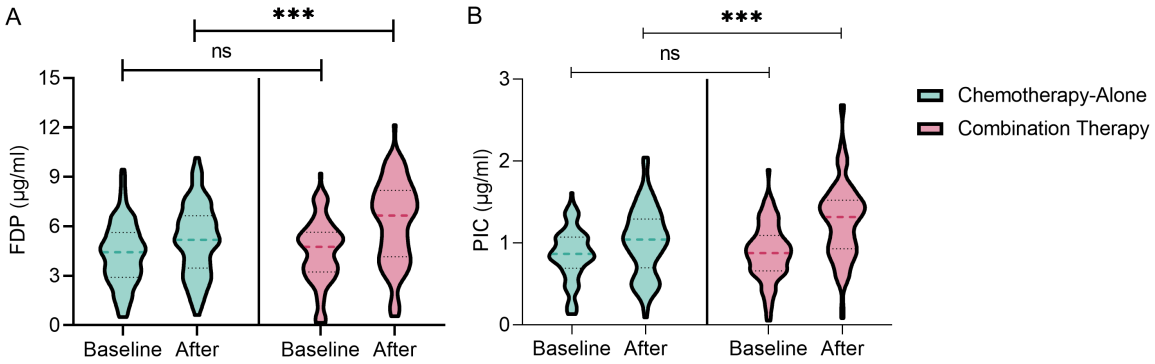

Figure 4. Changes in fibrinolytic markers before and after treatment. A. FDP (μg/mL); B. PIC (μg/mL). FDP: fibrinogen degradation products; PIC: plasmin-antiplasmin complexes. Ns: no significant difference; ***P<0.001.

post-treatment CA125 levels were 46.42±15.83 ng/mL versus 58.76±18.45 ng/mL (t=5.263, P<0.001), and CYFRA21-1 levels were 3.28±1.64 ng/L versus 4.13±1.87 ng/L (t=3.572, P<0.001) (**Table 4**).

Fibrinolytic system status

We evaluated the fibrinolytic system by measuring two key markers: FDP and PIC. Both were measured before and after treatment in the chemotherapy-alone and combination therapy groups. Before treatment, FDP levels were similar between the two groups: 4.32±1.87 μg/mL in the chemotherapy-alone group versus 4.46±1.92 μg/mL in the combination therapy group (t=0.523, P=0.601). Pretreatment PIC levels also showed no significant difference: 0.86±0.32 μg/mL versus 0.89±0.34 μg/mL (t=0.593, P=0.554). After treatment, both markers increased significantly within each group, with a more pronounced rise in the combination therapy group. Post-treatment FDP was 5.18±2.13 μg/mL in the chemotherapy-alone group compared to 6.27±2.48 μg/mL in the combination therapy group (t=3.497, P<0.001). Similarly, post-treatment PIC was 1.02±0.41 μg/mL in the chemotherapy-alone group versus 1.28±0.46 μg/mL in the combination therapy group (t=4.300, P<0.001). These results show that fibrinolytic activity increased more markedly after combination therapy than after chemotherapy alone (**Figure 4**).

Coagulation function

Coagulation function was assessed by measuring platelet count, D-dimer, fibrinogen, PT, and APTT before and after treatment in both

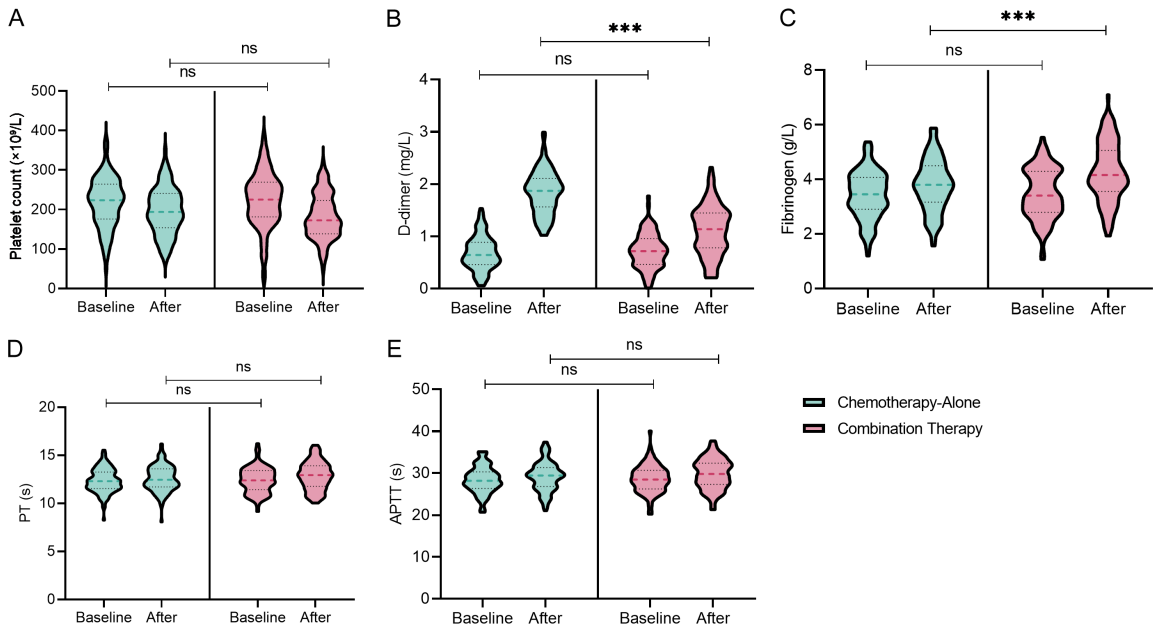

Figure 5. Changes in coagulation parameters before and after treatment. A. Platelet count (×10^9/L); B. D-dimer (mg/L); C. Fibrinogen (g/L); D. PT (s); E. APTT (s). PT: prothrombin time; APTT: activated partial thromboplastin time; Ns: no significant difference; ***P<0.001.

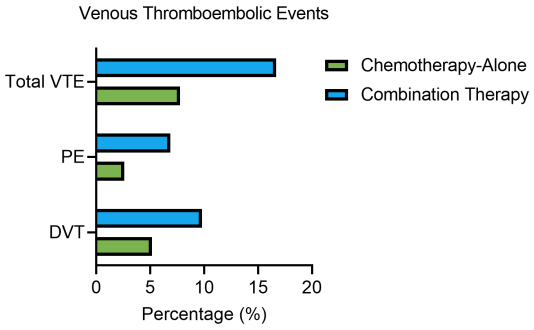

Figure 6. Incidence of venous thromboembolic events [n (%)]. DVT: deep vein thrombosis; PE: pulmonary embolism; VTE: venous thromboembolism.

the chemotherapy-alone and combination therapy groups. At baseline, the two groups did not differ significantly in any parameter: platelet count (218.36±62.47×10^9/L vs. 224.18±68.32×10^9/L; t=0.657, P=0.512), D-dimer (0.68±0.32 mg/L vs. 0.72±0.34 mg/L; t=0.896, P=0.371), fibrinogen (3.42±0.87 g/L vs. 3.48±0.92 g/L; t=0.474, P=0.636), PT (12.36±1.24 s vs. 12.42±1.31 s; t=0.344, P=0.731), and APTT (28.42±3.16 s vs. 28.67±3.24 s; t=0.564, P=0.573). After treatment, D-dimer levels were significantly higher in the chemotherapy-alone group compared to the combination therapy group (1.12±0.48 mg/L vs. 1.84±0.41 mg/L; t=12.126, P<0.001). Fibrinogen levels also increased significantly in the combination therapy group (4.26±1.08 g/L vs. 3.78±0.94 g/L; t=3.544, P<0.001). Platelet counts decreased in both groups after treatment, but the inter-group difference was not statistically significant (182.73±56.28×10^9/L vs. 196.42±58.34×10^9/L; t=1.757, P=0.080). PT and APTT values showed no significant changes between the two groups after treatment (**Figure 5**).

Adverse events

VTE occurred significantly more frequently in the combination therapy group (16.67%) compared to the chemotherapy-alone group (7.76%; P=0.043; **Figure 6**). Regarding other adverse events, hematological toxicities were more common with combination therapy. Specifically, the incidence of leukopenia (55.88% vs. 37.07%, P=0.005) and thrombocytopenia (40.20% vs. 25.00%, P=0.016) was markedly higher in the combination group. No significant differences were observed between the two groups in terms of gastrointestinal reactions, alopecia, or hepatic/renal impairment (**Table 5**).

Table 5. Incidence of treatment-related adverse events [n (%)]

Parameters	Chemotherapy-Alone Group (n=116)	Combination Therapy Group (n=102)	t	p
Gastrointestinal reactions	38 (32.76)	42 (41.18)	1.656	0.198
Alopecia	51 (43.97)	49 (48.04)	0.363	0.547
Leukopenia	43 (37.07)	57 (55.88)	7.737	0.005
Thrombocytopenia	29 (25.00)	41 (40.20)	5.749	0.016
Hepatic/renal impairment	17 (14.66)	23 (22.55)	2.257	0.133

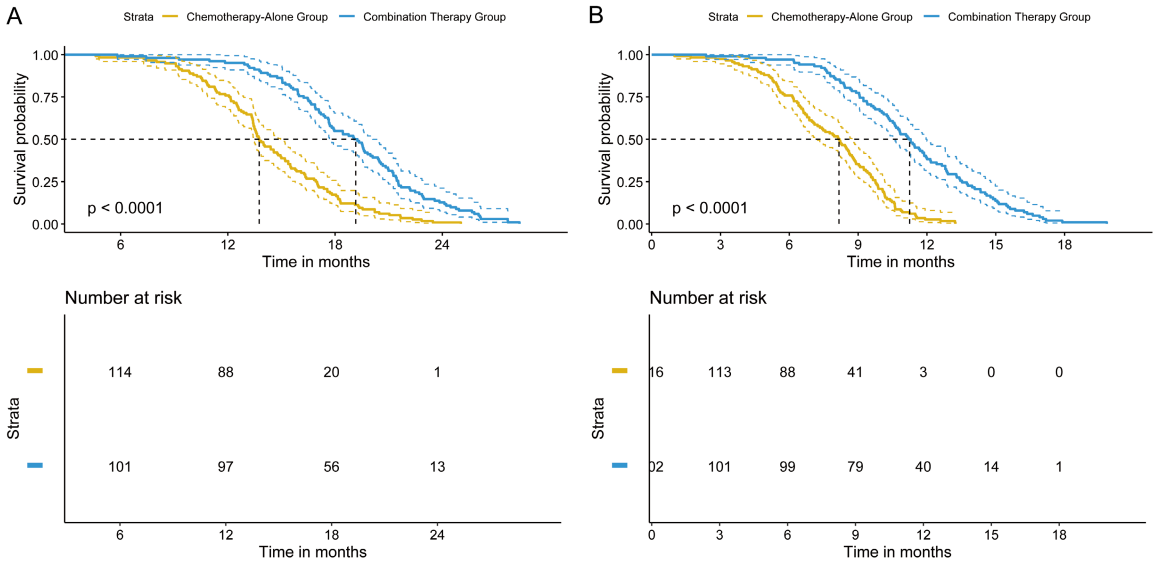

Figure 7. Comparison of OS and PFS between the two groups. A. OS; B. PFS. OS: overall survival; PFS: progression-free survival.

Survival outcomes

Survival outcomes were analyzed. Patients in the combination therapy group had better OS and PFS than those in the chemotherapy-alone group. The mean OS was longer in the combination therapy group (18.94±4.26 months) compared to the chemotherapy-alone group (14.36±3.82 months), a difference that was statistically significant (P<0.001). Similarly, the mean PFS was longer in the combination therapy group (11.37±3.18 months) than in the chemotherapy-alone group (7.82±2.46 months), which was also significant (P<0.001). These results, shown in **Figure 7A** and **7B**, indicate that combination therapy improves both OS and PFS compared to chemotherapy alone.

Univariate and multivariable logistic regression analysis of risk factors for VTE

A univariate logistic regression analysis was conducted to assess potential risk factors for VTE occurrence. As shown in **Table 6**, receipt of combination therapy (odds ratio [OR]=2.801, P=0.016), elevated post-treatment D-dimer level (≥0.7 mg/L vs. <0.7 mg/L; OR=1.449, P<0.001), elevated post-treatment FDP level (≥5.0 μg/mL vs. <5.0 μg/mL; OR=2.850, P=0.008), and clinical stage IV disease (vs. stage III; OR=2.199, P=0.025) were significantly associated with an increased risk of VTE.

Multivariable analysis identified four independent risk factors for VTE (**Table 7**): receiving combination therapy (adjusted OR=2.367, P=0.012), elevated post-treatment D-dimer level (≥0.7 mg/L; adjusted OR=1.338, P<0.001), elevated post-treatment FDP level (≥5.0 μg/mL; adjusted OR=2.528, P=0.008), and clinical stage IV disease (adjusted OR=1.980, P=0.019).

Discussion

This retrospective cohort study provides a comprehensive comparison of the effects of ICI

Table 6. Univariate logistic regression analysis for VTE risk

Variable	β	OR	95% CI	p
Treatment group (combination vs. chemotherapy-alone)	1.030	2.801	1.214-6.467	0.016
Post-treatment D-dimer (≥0.7 mg/L vs. <0.7 mg/L)	0.371	1.449	1.173-1.791	<0.001
Post-treatment FDP (≥5.0 μg/mL vs. <5.0 μg/mL)	1.047	2.850	1.314-6.183	0.008
Clinical stage (IV vs. III)	0.788	2.199	1.103-4.386	0.025

All categorical variables were coded as 1 for the characteristic (listed first) vs. 0 for the reference category (listed second). VTE: venous thromboembolism; FDP: fibrin degradation products; OR: odds ratio; CI: confidence interval.

Table 7. Multivariate logistic regression for VTE risk

Variables	β	Adjusted OR	95% CI	p
Treatment group (combination vs. chemotherapy-alone)	0.861	2.367	1.204-4.651	0.012
Post-treatment D-dimer (≥0.7 mg/L vs. <0.7 mg/L)	0.291	1.338	1.138-1.573	<0.001
Post-treatment FDP (≥5.0 μg/mL vs. <5.0 μg/mL)	0.927	2.528	1.272-5.024	0.008
Clinical Stage (IV vs. III)	0.683	1.980	1.122-3.494	0.019

All categorical variables were coded as 1 for the characteristic (listed first) vs. 0 for the reference category (listed second). VTE: venous thromboembolism; FDP: fibrin degradation products; OR: odds ratio; CI: confidence interval.

combination therapy with chemotherapy versus chemotherapy alone on coagulation biomarkers, thrombotic risk, and clinical outcomes in patients with NSCLC. Our findings indicate that while ICI combined with PTx confers superior antitumor efficacy and survival benefits, it is also associated with a distinct pattern of alterations in the hemostatic system and an increased risk of VTE.

Regarding short-term treatment outcomes, the combination therapy group showed a higher ORR and DCR than the chemotherapy-alone group. These findings are consistent with and support the results of major clinical trials. For example, Qiu et al. [27] reported that the combination of an immune checkpoint inhibitor with chemotherapy brings about greater tumor reduction and improved disease control in patients with advanced NSCLC, further corroborating the higher ORR and DCR observed in our combination therapy group. The enhanced efficacy may be attributed to the synergistic effect of chemotherapy and immunotherapy: chemotherapy can induce immunogenic cell death and increase tumor antigen exposure, which may in turn augment the activity of ICI. This combined mechanism may create a more potent antitumor microenvironment, though it may also affect other physiological systems, such as coagulation [28, 29].

In agreement with the stronger tumor response observed, combined treatment also induced a pronounced reduction in important tumor markers, including CEA, CA125, and CYFRA21-1. The more robust decline in these markers aligns with the greater antitumor efficacy of the combination regimen. Prior studies suggest that immunotherapy may alter the tumor microenvironment - for example, by modulating apoptosis and antigen presentation-thereby accelerating the decrease in circulating biomarkers [30]. Moreover, the reduction in these markers reflects effective suppression of tumor growth and dissemination, processes that may also influence systemic pathways such as coagulation and fibrinolysis. The work by Chiu et al. [31] highlights the interaction between immune activation and tumor biology, a perspective that is also supported by our findings.

Analysis of the fibrinolytic system showed that the increase in FDP and PIC were greater in the combination therapy group compared to the chemotherapy-alone group. This indicates enhanced fibrinolysis when chemotherapy is combined with an ICI. While chemotherapy itself can disrupt hemostasis [32], we propose that ICIs further amplify this effect: the heightened fibrinolysis observed may result from multiple mechanisms. Activated T-cells and other immune cells, expanded under ICI treatment, can release inflammatory cytokines and activate endothelial cells, thereby increasing tissue plasminogen activator and promoting fibrinolysis [33, 34]. Furthermore, immune-related adverse events, such as subclinical vasculitis,

could contribute to vascular perturbation and increased fibrin turnover, as hypothesized by Liu et al. [21] in their review of systemic inflammatory syndromes associated with ICIs. Therefore, the pronounced fibrinolytic activity in the combination group may reflect a more robust systemic immune activation and inflammatory response triggered by the ICI.

Concomitantly, the evaluation of coagulation parameters showed that combination therapy resulted in a greater post-treatment elevation of D-dimer and fibrinogen levels compared to chemotherapy alone. D-dimer, a marker of fibrin formation and degradation, is a well-established indicator of hypercoagulable states and is linked with an increased risk of VTE and poorer prognosis in cancer patients, as reported by Koch et al. [35] and Cosmi et al. [36]. Fibrinogen, an acute-phase reactant, also rose significantly, further reflecting a shift toward a prothrombotic state. These findings indicate that combining immunotherapy with chemotherapy creates a stronger prothrombotic environment than chemotherapy alone. This enhanced effect probably stems from several interacting mechanisms. Chemotherapy causes direct damage to the endothelium and promotes the release of procoagulant microparticles. The addition of ICIs may amplify this process through mechanisms related to the immune system [37]. Activated T-cells can express tissue factor, the main initiator of the coagulation cascade. Moreover, effective immune activation causes a systemic inflammatory state characterized by high levels of cytokines, which in turn can activate endothelial cells, platelets, and the coagulation system at the same time, leading to increased thrombin generation and fibrin deposition [37, 38]. The concept of immunothrombosis - which describes the interplay between innate immune responses and coagulation pathways - provides a reasonable framework to understand our results. As noted by Lyon et al. [39], cancer-associated thrombosis is a multifactorial process. Our data show that using ICIs adds an important immunological dimension to this risk profile.

Indeed, the higher occurrence of VTE observed in the combination therapy group directly corresponds to the laboratory findings in these patients. Such monitoring holds significant value for patient risk stratification. The multivariable analysis conducted in this study identified several independent risk factors for VTE, including receipt of combination therapy, elevated post-treatment D-dimer and FDP levels, and advanced disease stage. These results enhance the biological plausibility of our work by linking our laboratory evidence of heightened coagulation activation, increased fibrinolysis, and clinical VTE events. Emerging broader toxicity profiles of ICI-containing regimens, as highlighted in reviews such as that by McKenzie et al. [40], further contextualize our findings. Our data contribute to this evolving understanding, underscoring that the increased thrombotic risk associated with combination therapy warrants vigilant monitoring and consideration of early preventive measures, particularly in patients with advanced disease and high baseline risk [39].

Other adverse events, such as hematological toxicities, occurred more frequently in the combination therapy group compared to chemotherapy alone. Specifically, leukopenia and thrombocytopenia were significantly more common with the combined regimen. These findings align with prior reports, such as the network meta-analysis by Peng et al. [14] in gastrointestinal cancers, which similarly documented increased hematologic toxicity with chemo-immunotherapy combinations. The likely explanation involves the synergistic myelosuppressive effects of chemotherapy and immunotherapy on hematopoietic precursor cells [13, 16].

Despite the observed hemostasis changes and increased toxicity, survival outcomes showed a clear advantage for combination therapy over chemotherapy alone, with significantly longer overall survival and progression-free survival. These findings reconfirm the survival benefit reported in major clinical trials and support the real-world effectiveness of this treatment approach. The coexistence of improved survival and elevated VTE risk suggests that the oncological benefit likely outweighs the increased thrombotic risk. But this also shows the need to mitigate VTE risk, thereby preserving quality of life and safeguarding the potential survival gains [41, 42].

There are some limitations in the study. Its retrospective design makes it susceptible to potential selection and confounding factors,

which may influence the observed outcome. Additionally, the 2-year followup period is relatively short. Future work should validate these findings in larger, multicenter prospective cohorts. Long-term studies with regular measurements are needed to better understand the temporal dynamics of coagulation activation and thrombotic risk. Further investigation into different ICIs and treatment regimens and their specific effects on thrombosis is also warranted. Ultimately, these efforts could support the development of risk-prediction models based on clinical and laboratory parameters, helping to identify patients who might benefit from primary thromboprophylaxis when receiving immunotherapy combined with chemotherapy.

Conclusion

In conclusion, the combination of ICIs and chemotherapy provides superior tumor control and improved survival outcomes for patients with NSCLC compared to chemotherapy alone. However, this regimen is associated with significantly greater activation of coagulation and fibrinolysis, leading to an increased incidence of VTE. These findings highlight the complex interplay between cancer, the immune system, and hemostasis. An integrated management strategy is therefore needed, one that effectively addresses both the oncological benefits and the treatmentrelated thromboembolic risks.

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Disclosure of conflict of interest

None.

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