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BIOCHEMICAL PROFILING OF THE COAGULATION–FIBRINOLYSIS SYSTEM AND A THREE-TIER STRATIFIED TESTING WORKFLOW IN NON-SMALL CELL LUNG CANCER

BIOHEMIJSKO PROFILISANJE SISTEMA KOAGULACIJE–FIBRINOLIZE I TROSTEPENI STRATIFIKOVANI DIJAGNOSTIČKI PROTOKOL KOD NEMIKROCELULARNOG KARCINOMA PLUĆA

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Summary

Background: In routine clinical practice, the general workflow for coagulation–fibrinolysis testing has not been specifically designed for non-small cell lung cancer (NSCLC), and its ability to identify and assess thrombotic risk in this population remains limited. The lack of NSCLC-specific stratified testing design, disease-specific positive thresholds, and standardized testing timing and cycles limits these conventional workflows. This study aimed to characterize the full pathway biochemical profile of the coagulation–fibrinolysis system in patients with NSCLC and to establish and validate an optimized, standardized testing workflow tailored to this population.
Methods: A total of 148 subjects who met the inclusion and exclusion criteria between January 2024 and June 2025 were enrolled, including 64 healthy controls, 45 patients with benign lung disease, and 39 patients with pathologically confirmed NSCLC. Key exclusion criteria included receipt of anticoagulant or antiplatelet therapy within 3 months before enrolment, unqualified blood specimens, and incomplete clinical data. After fasting, venous blood samples had been collected. Pretreated, routine coagulation–fibrinolysis indices, coagulation factor activities, fibrinolytic regulatory markers, and vascular endothelial injury markers were quantitatively measured using a Sysmex CS-5100 fully automated coagulation analyser, a Sysmex XN-1000 fully automated haematology analyser, and a Roche Cobas e 602 fully automated chemiluminescence immunoassay analyser. Standardized

Kratak sadržaj

Uvod: U rutinskoj kliničkoj praksi, opšti tok rada za ispitivanje sistema koagulacije–fibrinolize nije posebno dizajniran za nemikrocelularni karcinom pluća (NSCLC), a njegova sposobnost da identifikuje i proceni trombotički rizik u ovoj populaciji je i dalje ograničena. Nedostatak stratifikovanog načina testiranja specifičnog za NSCLC, bolesti-specifičnih pozitivnih graničnih vrednosti, kao i standardizovanog vremena i ciklusa testiranja, ograničavaju primenu ovih konvencionalnih pristupa. Cilj ove studije je bio da se odredi biohemijski profil celokupnog puta sistema koagulacije–fibrinolize kod pacijenata sa NSCLC i da se uspostavi i validira optimizovan i standardizovan tok testiranja prilagođen ovoj populaciji.
Metode: U studiju je uključeno ukupno 148 ispitanika koji su ispunjavali kriterijume uključivanja i isključivanja u periodu od januara 2024. do juna 2025. godine, među kojima je bilo 64 zdravih kontrolnih pacijenata, 45 pacijenata sa benignim plućnim bolestima i 39 pacijenata sa patohistološki potvrđenim NSCLC. Ključni kriterijumi isključenja su obuhvatali primenu antikoagulantne ili antitrombocitne terapije u periodu od 3 meseca pre uključenja, neadekvatne uzorke krvi i nepotpune kliničke podatke. Nakon gladovanja, uzimani su uzorci venske krvi. Kvantitativno su određivani prethodno tretirani, rutinski pokazatelji koagulacije–fibrinolize, aktivnosti koagulacionih faktora, regulatorni markeri fibrinolize i markeri oštećenja vaskularnog endotela, korišćenjem potpuno automatizovanog koagulacionog analizatora Sysmex CS-5100, hematološk-

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quality control was implemented throughout the analytical process, and the performance of the testing systems was verified. At the same time, an NSCLC-specific three-tier stratified precision testing workflow was established and compared in a paired manner with the conventional non-differentiated routine clinical testing workflow.
Results: Compared with both the Control group and the benign lung disease group, the NSCLC group showed significant differences in routine coagulation–fibrinolysis indices, coagulation factor activities, fibrinolytic regulatory markers, and endothelial injury markers ($P<0.05$), with significantly elevated procoagulant indices and imbalances in fibrinolytic regulatory markers. For the same specimens, the results obtained before and after workflow optimization remained highly consistent, with intraclass correlation coefficients (ICCs) for all indices ≥0.90. After optimization, the median within-run coefficient of variation (CV) decreased from 2.9 to 1.7%, and the median between-run CV decreased from 5.4 to 3.4%. The relative deviation from international reference materials was also reduced ($P<0.001$). In clinical application, the median specimen turnaround time fell from 101.0 min to 69.0 min, while the median average testing cost per patient decreased from 332.0 yuan to 238.0 yuan ($P<0.05$).
Conclusion: The NSCLC-specific three-tier stratified coagulation–fibrinolysis testing workflow established in this study improved analytical performance and workflow efficiency while remaining clinically practical, providing a standardized laboratory approach for coagulation assessment and early thrombotic risk management in patients with NSCLC, particularly for early warning of thrombotic events.

Keywords: biomarkers, coagulation, fibrinolysis, lung neoplasms, non-small-cell, neoplasm metastasis, thrombosis

og analizatora Sysmex XN-1000 i hemiluminiscentnog imunoanalizatora Roche Cobas e 602. Standardizovana kontrola kvaliteta sprovođena je tokom celokupnog analitičkog procesa, a performanse sistema testiranja su verifikovane. Istovremeno je uspostavljen trostepeni stratifikovani precizni dijagnostički protokol specifičan za NSCLC i upoređen uparenim pristupom sa konvencionalnim, nediferenciranim rutinskim kliničkim protokolom testiranja.
Rezultati: U poređenju sa kontrolnom grupom i grupom sa benignim plućnim bolestima, grupa sa NSCLC je pokazala statistički značajne razlike u rutinskim parametrima koagulacije–fibrinolize, aktivnostima koagulacionih faktora, regulatornim markerima fibrinolize i markerima oštećenja endotela ($P<0,05$), uz značajno povišene prokoagulantne parametre i disbalans regulatornih markera fibrinolize. Za iste uzorke, rezultati dobijeni pre i nakon optimizacije toka rada ostali su visoko konzistentni, sa intraklasnim koeficijentima korelacije (ICC) ≥ 0,90 za sve parametre. Nakon optimizacije, medijana koeficijenta varijacije unutar serije (CV) smanjena je sa 2,9 na 1,7%, dok je medijana CV između serija smanjena sa 5,4 na 3,4%. Relativno odstupanje u odnosu na međunarodne referentne materijale je takođe smanjeno ($P<0,001$). U kliničkoj primeni, medijana vremena obrade uzorka smanjena je sa 101,0 min na 69,0 min, dok je medijana prosečnog troška testiranja po pacijentu smanjena sa 332,0 juana na 238,0 juana ($P<0,05$).
Zaključak: Trostepeni stratifikovani protokol testiranja koagulacije–fibrinolize specifičan za NSCLC, uspostavljen u ovoj studiji, unapredio je analitičke performanse i efikasnost toka rada, uz zadržavanje kliničke primenljivosti. Ovaj pristup obezbeđuje standardizovan laboratorijski okvir za procenu koagulacije i rano upravljanje trombotičkim rizikom kod pacijenata sa NSCLC, naročito u pogledu ranog upozorenja na trombotičke događaje.

Ključne reči: biomarkeri, koagulacija, fibrinoliza, tumori pluća, nemikrocelularni, metastaze tumora, tromboza

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. In 2025, approximately 2.2 million new lung cancer cases and 1.8 million related deaths were reported globally. Among Pathological types of lung cancer, non-small cell lung cancer (NSCLC) accounts for 80–85% (1). Thromboembolic events are the second leading cause of death in patients with NSCLC, ranking only behind progression of the primary tumour. The risk of thrombosis in these patients is 4–7 times higher than that in healthy individuals, with an overall incidence of 10–30% (2). In NSCLC patients who develop thrombotic events, the 1-year all-cause survival rate is reduced by more than 40% compared with those without thrombotic complications (3). Disruption of coagulation–fibrinolysis homeostasis is a central pathological basis for thrombotic events in malignant tumours. Meanwhile, tumour cells can promote invasion, distant metastasis, and immune escape through sustained activation of the coagulation pathway. Dynamic changes in related biochemical markers are closely involved in the development and progression of NSCLC and also serve as a core laboratory basis for clinical evaluation of disease status (4).

Previous studies have shown that routine coagulation–fibrinolysis biochemical indices, including fibrinogen, D-dimer, prothrombin time, and activated partial thromboplastin time, are significantly associated with tumour stage, pathological subtype, treatment response, and long-term prognosis in patients with NSCLC. Some key indices have already been incorporated into classic assessment models for cancer-associated thrombotic risk (5, 6). At present, quantitative testing of coagulation–fibrinolysis-related indices has been widely implemented in clinical laboratories and provides basic biochemical support for the evaluation of patients with NSCLC (7, 8). Even so, current testing strategies still largely rely on non-differentiated general workflows and lack standardized, disease-specific designs directed at the pathological features of NSCLC. In addition, no unified NSCLC-specific standards are available for marker combinations, testing timing, or thresholds for result interpretation in routine clinical workflows. As a result, the clinical utility of these test re-

sults remains insufficient, and precise stratification and early warning of thrombotic risk are difficult to achieve (9, 10).

In the present study, we examined biomarkers across the full coagulation–fibrinolysis pathway to define the characteristic biochemical profile of patients with NSCLC and to clarify its relationship with tumour clinicopathological features. On this basis, we established an NSCLC-specific three-tier stratified precision-testing workflow. We compared the optimized workflow with the conventional general workflow with respect to laboratory analytical performance and clinical application value. Taken together, these findings help clarify the pathological and biochemical basis of coagulation–fibrinolysis abnormalities in NSCLC and support a standardized testing strategy that is better aligned with this patient population. They also suggest that biochemical indices may have greater value in risk stratification and day-to-day clinical management, providing a more objective laboratory basis for the early prevention of cancer-associated thrombotic complications.

Materials and Methods

Study design and study subjects

This was a single-centre retrospective cohort study conducted from January 2024 to June 2025, with data extracted from the electronic medical record and laboratory information system of Chifeng Municipal Hospital. A total of 148 subjects who met the inclusion and exclusion criteria were enrolled, including 64 in the control group, 45 in the benign lung disease group, and 39 in the pathologically confirmed NSCLC group. No statistically significant differences were observed among the three groups in age, sex distribution, or other baseline characteristics ($P>0.05$), indicating good baseline comparability (*Table I*).

Table I Baseline characteristics of the study groups.

Statistical Item	Control group (n=64)	Benign group (n=45)	NSCLC Group (n=39)	t or χ^2	P
Age (years)	58.4±10.8	62.4±9.9	60.7±8.3	2.226	0.112
Gender, Male/Female	38/26 (59.4/40.6)	30/15 (66.7/33.3)	24/15 (61.5/38.5)	0.606	0.739
BMI (kg/m^2)	23.9±3.0	23.5±3.2	23.1±2.6	0.879	0.418
Smoking history	22 (34.4)	18 (40.0)	19 (48.7)	2.08	0.354
Previous history of thrombosis	0 (0.0)	1 (2.2)	2 (5.1)	3.221	0.2
Hypertension	18 (28.1)	15 (33.3)	14 (35.9)	0.75	0.687
Type 2 diabetes mellitus	9 (14.1)	10 (22.2)	7 (17.9)	1.22	0.543
Coronary heart disease	5 (7.8)	4 (8.9)	7 (17.9)	2.83	0.243
Pathological subtype	-	-	-	-	-
Adenocarcinoma	-	-	28 (71.8)	-	-
Squamous cell carcinoma	-	-	9 (23.1)	-	-
Other NSCLC subtypes	-	-	2 (5.1)	-	-
TNM staging	-	-	-	-	-
Stage I-II	-	-	11 (28.2)	-	-
Stage III	-	-	15 (38.5)	-	-
Stage IV	-	-	13 (33.3)	-	-
Tumour differentiation grade	-	-	-	-	-
Well/moderately differentiated	-	-	17 (43.6)	-	-
Poorly differentiated	-	-	22 (56.4)	-	-
Distant metastasis	-	-	13 (33.3)	-	-

Inclusion and exclusion criteria

The inclusion criteria were as follows: the control group consisted of individuals undergoing physical examination who had normal coagulation and cardiopulmonary function and no history of acute or chronic organic disease, malignant tumour, or thrombosis; the Benign group included patients with non-malignant pulmonary conditions, such as benign pulmonary nodules, community-acquired pneumonia, and chronic obstructive pulmonary disease, confirmed by imaging or pathology, and without a history of malignant tumours or severe organ dysfunction. Patients with acute exacerbation of COPD or severe pneumonia were excluded; the NSCLC group included patients with pathologically confirmed NSCLC who had been followed for at least 6 months after diagnosis, with core follow-up endpoints including venous thromboembolism (VTE) events and all-cause death. VTE in this group was confirmed by lower-extremity compression ultrasonography or CT pulmonary angiography.

The exclusion criteria were as follows: receipt of anticoagulant or antiplatelet therapy within 3 months before enrolment; concomitant hematologic disease, severe hepatic or renal insufficiency, a history of thrombosis within 6 months before enrolment, or malignant tumours at other sites; unqualified specimens with severe haemolysis, lipemia, or clotting; and incomplete clinical or follow-up data.

Specimen collection and laboratory pretreatment

For all study subjects, fasting venous blood samples were collected from the cubital vein after more than 8 h of fasting within 24 h after admission, whereas samples from the control group were collected on the day of physical examination. Blood was drawn into 3.2% sodium citrate vacuum anticoagulant tubes (blood-to-anticoagulant ratio, 9:1; BD, USA) for coagulation–fibrinolysis testing and into EDTA-K2 vacuum anticoagulant tubes (BD, USA) for platelet count determination.

After collection, the samples were gently inverted 8 times to mix, then centrifuged at 3000×g for 15 min at room temperature (18–25 °C) to obtain platelet-poor plasma. Vigorous agitation and sample haemolysis were avoided throughout the process. Routine assays were completed within 2 h after sample collection. Plasma specimens intended for characteristic marker detection were aliquoted at 500 μL per tube and immediately stored in an ultra-low-temperature freezer at -80 °C (Thermo Fisher Scientific, USA), with a maximum storage time of 12 months. Before batch testing, the samples were thawed once in a 37 °C water bath, and the number of freeze–thaw cycles did not exceed 2 to avoid degradation of target biomarkers and ensure the stability of test results.

Routine coagulation–fibrinolysis biochemical testing

All indices in this section were measured on a Sysmex CS-5100 fully automated coagulation analyser (Sysmex Corporation, Japan; core assay parameters: 1–200% linear range for coagulation factor activity, 0.1–20.0 mg/L FEU linear range for D-dimer) using matched original reagents, calibrators, and quality control materials. The testing environment was maintained at a constant temperature of 20–24 °C. To exclude system error, the testing conditions before and after optimization were kept completely identical. PT and INR were determined by the one-stage clotting method. The reagent used had an international sensitivity index (ISI) of 1.08, and INR was calculated according to the internationally accepted formula. APTT was measured using an ellagic acid-activated activated partial thromboplastin time assay. FIB was determined by the Clauss method, and quantification was completed using a standard curve ranging from 0.5 to 8.0 g/L prepared with matched calibrators. D-dimer and FDP were measured by latex-enhanced immunoturbidimetry, with linear ranges of 0.1–20.0 mg/L FEU and 0.5–80.0 mg/L, respectively.

Detection of coagulation factor activity and platelet count

Coagulation factor activity was measured by the one-stage clotting method on the same Sysmex CS-5100 fully automated coagulation analyser using matched original human factor-deficient plasma (Sysmex Corporation, Japan; catalogue No. 20013 for FVII, 20014 for FVIII, 20016 for FX), calibrators, and reagents, and the results were expressed as percentages (%) in international standard units. Activity of intrinsic coagulation factor VIII was determined using the APTT-based system, whereas activities of extrinsic coagulation factors VII and X were measured using the PT-based system. In both settings, standard curves were generated with matched calibrators over a concentration gradient of 0–200%. The linear detection range was 1–200%, and samples outside this range were retested after dilution.

Platelet count was measured using the sheath-flow direct current impedance method combined with nucleic acid fluorescence staining on a Sysmex XN-1000 fully automated haematology analyser (Sysmex Corporation, Japan; core assay parameter: $10–5000\times10^9$/L linear range for platelet count). Matched original reagents and calibrators were used, the CBC+DIFF mode was selected, and the linear range was $10–5000\times10^9$/L.

Detection of fibrinolytic regulatory markers and vascular endothelial injury markers

A double-antibody sandwich chemiluminescence immunoassay was used for detection on a Roche Cobas e 602 fully automated chemiluminescence immunoassay analyser (Roche Diagnostics, Switzerland; core assay parameter: ≤0.1 ng/mL analytical sensitivity for target protein markers), together with matched original calibrators, quality control materials, and reagents. The testing environment was maintained at 22 °C, and all reactions were performed at 37 °C. The general assay procedure was as follows: the plasma sample was incubated at 37 °C for 9 min with streptavidin-coated magnetic microparticles and a biotinylated target-specific monoclonal antibody; after washing, a ruthenium-labelled second antibody was added and incubated for another 9 min; after a second wash, the electrochemiluminescence signal was measured, and quantification was completed using a calibration curve generated by 6-point calibration. The core assay parameters for target markers were as follows: PAI-1 (linear range: 1.0–150.0 ng/mL; reference interval: 5.0–45.0 ng/mL), t-PA (linear range: 0.5–50.0 ng/mL; reference interval: 1.0–12.0 ng/mL), vWF (linear range: 10–300%; reference interval: 50–160%), TM (linear range: 0.5–100.0 TU/mL; reference interval: 2.0–8.0 TU/mL).

Quality control and performance verification

Before analysis, specimen rejection criteria were clearly defined, and standardized procedures for collection, centrifugation, and storage were strictly followed. During analysis, internal quality control materials at two concentration levels, high and low, were tested simultaneously in each batch [Bio-Rad Laboratories (Shanghai) Co., Ltd.], with 2 levels of controls tested at the start and end of each batch. The Westgard multirule quality control procedure was applied, including the 1_{3s}, 2_{2s}, R_{4s}, 4_{1s}, and 10_{x} rules. Patient specimens were tested only after quality control results were confirmed within the acceptable range. Quality control materials were also inserted regularly during testing to monitor system stability. After analysis, qualified laboratory physicians reviewed all results, and abnormal findings were rechecked for confirmation. During the study period, the pass rate in the external quality assessment was 100% across all test items, ensuring the traceability and comparability of the results. Before the study began, performance verification was completed for all analytical systems, with within-run precision CV<5%, between-run precision CV<8%, relative deviation from target values <10% for external quality assessment samples, linear regression coefficient R^2>0.99, and carryover rate <0.5%, with no cross-interference.

Workflow design and comparison scheme

Before optimization, the clinical workflow followed a routine, non-differentiated general testing model. All NSCLC patients, regardless of disease stage or thrombotic risk, received the same testing panel comprising four routine coagulation tests plus D-dimer. There were no standardized indications for testing characteristic markers, which were added only when coagulation abnormalities, thrombotic events, or explicit clinical requests arose. No standardized testing interval was defined. Results were interpreted using general reference intervals for healthy individuals, and only numerical reports were issued, without risk warning or clinical decision prompts.

After optimization, a three-tier stratified precision-testing workflow was established based on the coagulation–fibrinolysis biochemical profile of NSCLC, and all NSCLC patients underwent evaluation according to this workflow. Tier 1 served as the initial screening level and covered all newly diagnosed, hospitalized, and follow-up patients. Four routine coagulation items, plus D-dimer, were tested; standardized testing time points were defined, and NSCLC-specific positive screening thresholds were set. Patients with negative results underwent routine periodic re-examination, whereas those with positive results entered tier 2 testing. Tier 2 was designed for risk stratification and targeted patients with positive initial screening results, perioperative patients, and those receiving treatment for middle- to advanced-stage disease. FDP, platelet count, and PAI-1 were additionally tested within 48 h, and patients were classified into low-, intermediate-, and high-risk groups according to the results; those in the intermediate- and high-risk groups proceeded to tier 3 testing. Tier 3 served as the precision assessment level and was intended for high-risk patients, patients with confirmed thrombotic events, and those with marked fluctuations in laboratory indices. Full-pathway characteristic marker testing was additionally completed within 72 h to define the type of coagulation–fibrinolysis imbalance, with standardized result interpretation and clinical decision recommendations provided accordingly. The specific numerical values of the trigger thresholds for stratified testing are provided in the Results section as cut-off values. Details are shown in *Table II*.

Statistical analysis

Data were analyzed using SPSS 26.0. Quantitative data were tested for normality using the Shapiro–Wilk test. Data conforming to a normal distribution were expressed as mean ± standard deviation, comparisons among multiple groups were performed using one-way analysis of variance, and

Table II Comparison of the conventional and optimized coagulation–fibrinolysis testing workflows in patients with non-small cell lung cancer.

Comparison Dimension	Before Optimization	After Optimization
Core target population	Undifferentiated coverage for all NSCLC patients without population stratification	Stratified matching based on disease stage and thrombotic risk, covering the whole diagnosis and treatment cycle of NSCLC patients
Test indicator system	Unified panel: routine 4 coagulation tests + D-Dimer; no clear indication for characteristic markers, only passive supplementary testing	Three-tier stratified system:
		Tier 1 Primary Screening: routine 4 coagulation tests + D-Dimer
		Tier 2 Risk Stratification: primary screening indicators + FDP + PLT + PAI-1
		Tier 3 Precise Assessment: tier 2 indicators + FVIII activity + vWF + TM + PLG + t-PA
Test timing and cycle	No standardized cycle, only random testing at admission/follow-up, no dynamic monitoring rules	Standardized timing and cycle:
		Primary screening: once at initial diagnosis/admission, and once per cycle before anti-tumour treatment
		Stratified testing: completed within 48 hours after positive primary screening
		Dynamic monitoring: 1-4 week re-examination cycle matched by risk level
Result interpretation rules	Adopted general reference interval for healthy population, no NSCLC-specific cut-off value, no stratified interpretation criteria	Adopted NSCLC population-specific cut-off values, set stratified interpretation thresholds according to disease stage and risk level, and clarified hierarchical response rules for positive results
Laboratory testing pathway	Unified batch testing for all specimens without priority distinction and exclusive quality control requirements	Pathway matched by stratification level: routine batch testing for primary screening specimens, emergency priority channel for high-risk specimens, and exclusive internal quality control for NSCLC specimens throughout the process
Clinical connection process	Only issued test numerical reports without result interpretation prompts and a risk warning mechanism	Hierarchical report mode: positive primary screening automatically triggers risk prompts, high-risk results are simultaneously pushed with clinical early warning, and supported by standardized result interpretation and decision-making recommendations

pairwise comparisons were conducted using the LSD-t test. Non-normally distributed data were expressed as median (interquartile range, IQR), comparisons among multiple groups were performed using the Kreskas–Wallis H test, and Bonferroni correction was applied for pairwise comparisons. Categorical data were expressed as the number of cases (percentage), and intergroup comparisons were performed using the Pearson χ^2 test or Fisher's exact test. Trend analysis of indicator detection rates was performed using the Cochran–Armitage trend test, and consistency between pre- and post-optimization results was evaluated using the intraclass correlation coefficient (ICC). All statistical tests were two-sided, with $\alpha=0.05$ as the significance level, and $P<0.05$ was considered statistically significant.

Results

Differences in routine coagulation–fibrinolysis biochemical indices among groups before optimization

Before optimization, the differences among groups in the three basic coagulation indices, PT, INR, and APTT, were all statistically significant ($P<0.05$). For FIB, D-dimer, and FDP, which reflect fibrin formation and degradation, the between-

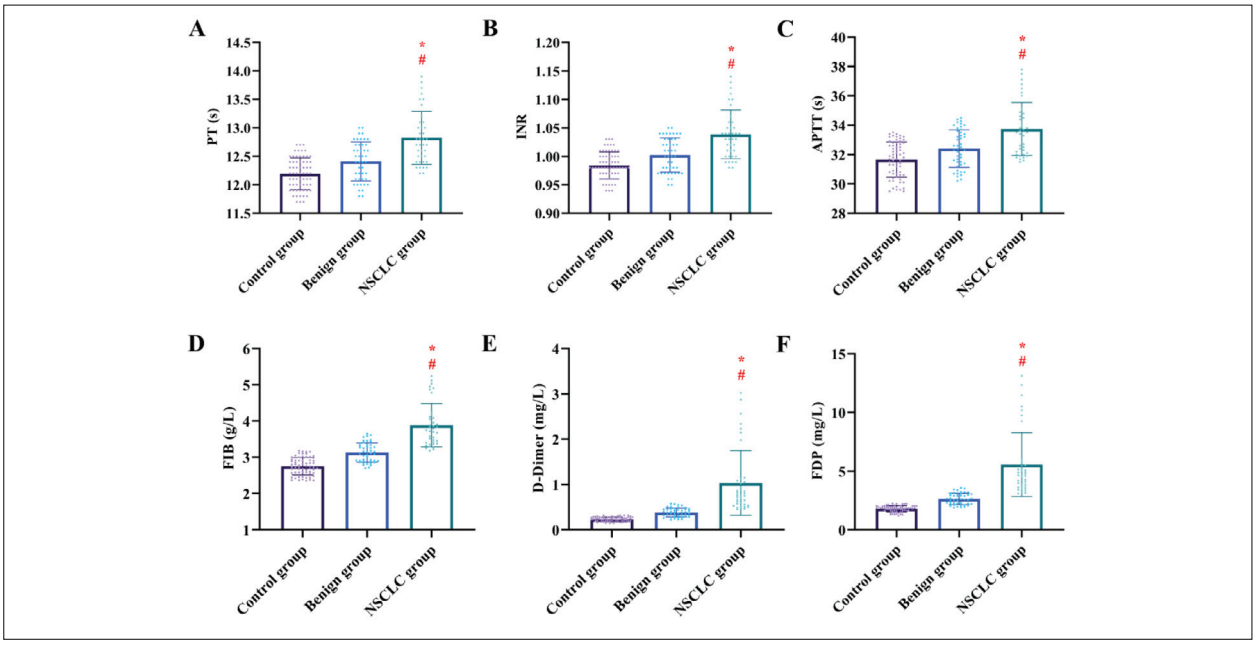

Figure 1 Routine coagulation–fibrinolysis indices before workflow optimization in the study groups.

(A) Prothrombin time (PT). (B) International normalized ratio (INR). (C) Activated partial thromboplastin time (APTT). (D) Fibrinogen (FIB). (E) D-dimer. (F) Fibrin/fibrinogen degradation products (FDP). Routine coagulation–fibrinolysis indices were compared among the control, benign, and NSCLC groups before workflow optimization. *P<0.05 vs the control group; #P<0.05 vs the benign group.

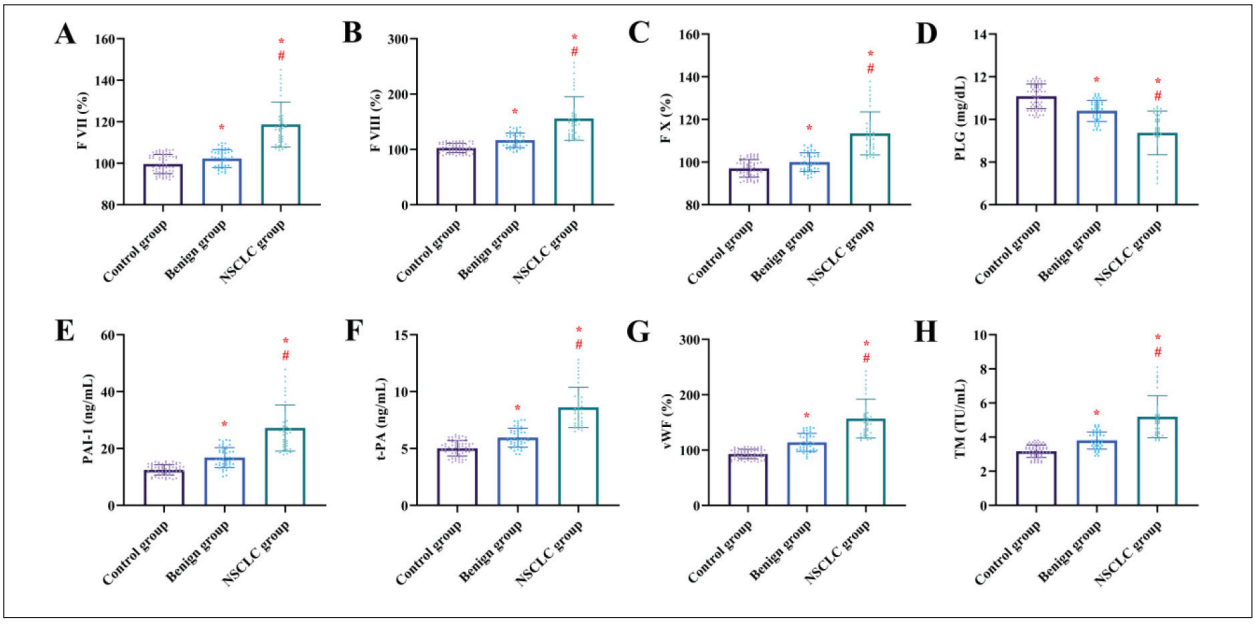

Figure 2 Characteristic coagulation–fibrinolysis biomarkers before workflow optimization in the study groups.

(A) Factor VII activity (FVII). (B) Factor VIII activity (FVIII). (C) Factor X activity (FX). (D) Plasminogen (PLG). (E) Plasminogen activator inhibitor-1 (PAI-1). (F) Tissue plasminogen activator (t-PA). (G) von Willebrand factor (vWF). (H) Thrombomodulin (TM). Characteristic coagulation–fibrinolysis biomarkers were compared among the control, benign, and NSCLC groups before workflow optimization. *P<0.05 vs the control group; #P<0.05 vs the benign group.

group differences were even more pronounced (P<0.05). Pairwise comparisons after Bonferroni correction showed that the NSCLC group had higher levels of all the above indices than the control group and the benign group (P<0.05), whereas no statistically significant difference was observed between the control group and the benign group (P>0.05) (*Figure 1*).

Expression characteristics of characteristic coagulation–fibrinolysis biochemical markers before optimization

Analysis of characteristic markers across the full coagulation–fibrinolysis pathway showed that, in the NSCLC group, the activities of extrinsic and intrinsic coagulation factors (FVII, FVIII, and FX), PAI-1, and the levels of vWF and TM were higher than those in the other two groups, whereas PLG levels were lower than those in the control group and the benign group ($P<0.05$) (*Figure 2*).

Routine coagulation–fibrinolysis biochemical test results and consistency verification after optimization

After optimization, the routine coagulation indices obtained under the three-tier stratified workflow showed the same pattern of between-group variation as that observed before optimization, and the differences in PT, INR, and APTT among groups remained statistically significant ($P<0.05$). Paired consistency analysis of the same specimens showed good agreement between the results obtained before and after optimization. The intraclass correlation coefficients (ICCs) of all routine coagulation indices were ≥0.90, and the lower bounds of the 95% confidence intervals were not lower than 0.91, indicating that workflow optimization did not compromise the stability or accuracy of routine index detection (*Figure 3*).

Detection performance of characteristic coagulation–fibrinolysis biochemical markers after optimization

For the characteristic biochemical markers, the stratified workflow after optimization produced between-group differences fully consistent with those observed before optimization, and all indices showed statistically significant differences among groups ($P<0.05$). The ICCs for the same specimens before and after optimization were all ≥0.92, indicating good consistency (*Figure 4*).

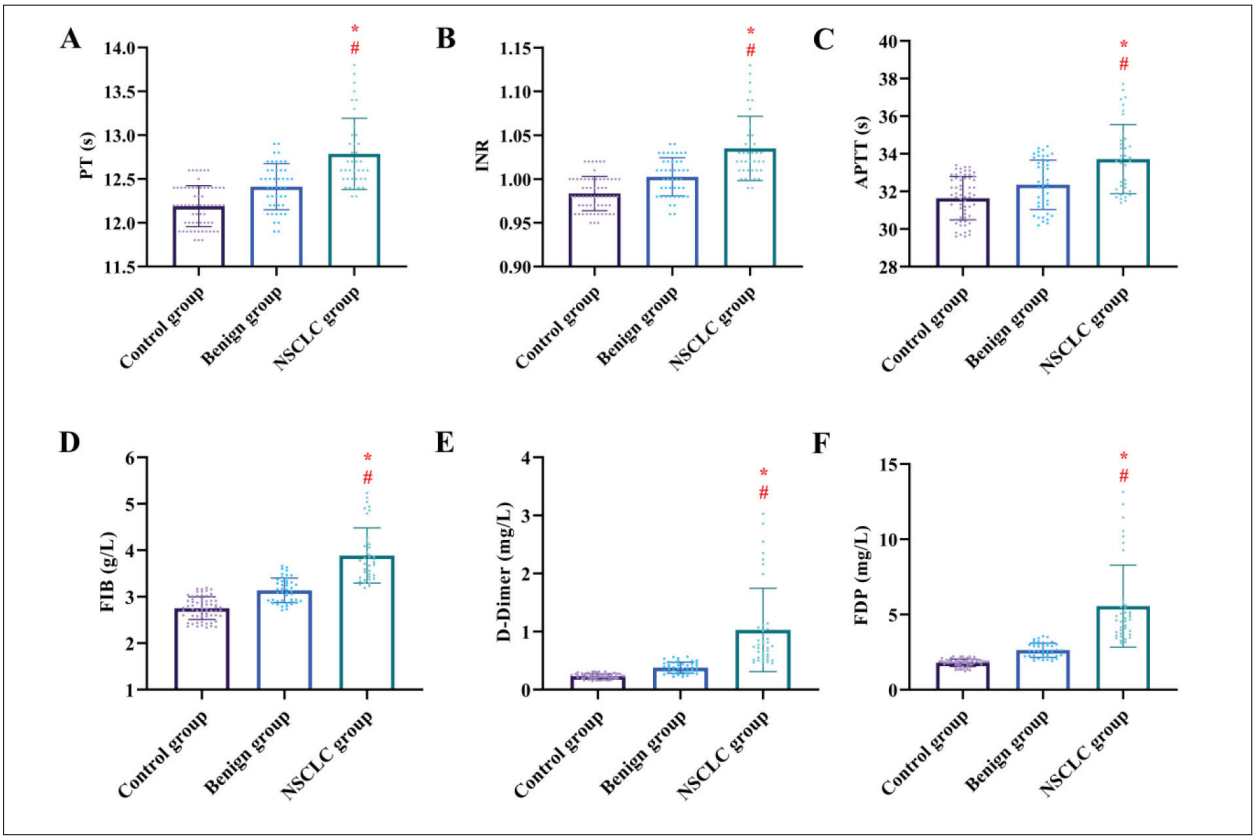

Figure 3 Routine coagulation–fibrinolysis indices after workflow optimization in the study groups.

(A) Prothrombin time (PT). (B) International normalized ratio (INR). (C) Activated partial thromboplastin time (APTT). (D) Fibrinogen (FIB). (E) D-dimer. (F) Fibrin/fibrinogen degradation products (FDP). Routine coagulation–fibrinolysis indices were compared among the control, benign, and NSCLC groups after workflow optimization. The same pattern of between-group differences was maintained after optimization. *$P<0.05$ vs the control group; #$P<0.05$ vs the benign group.

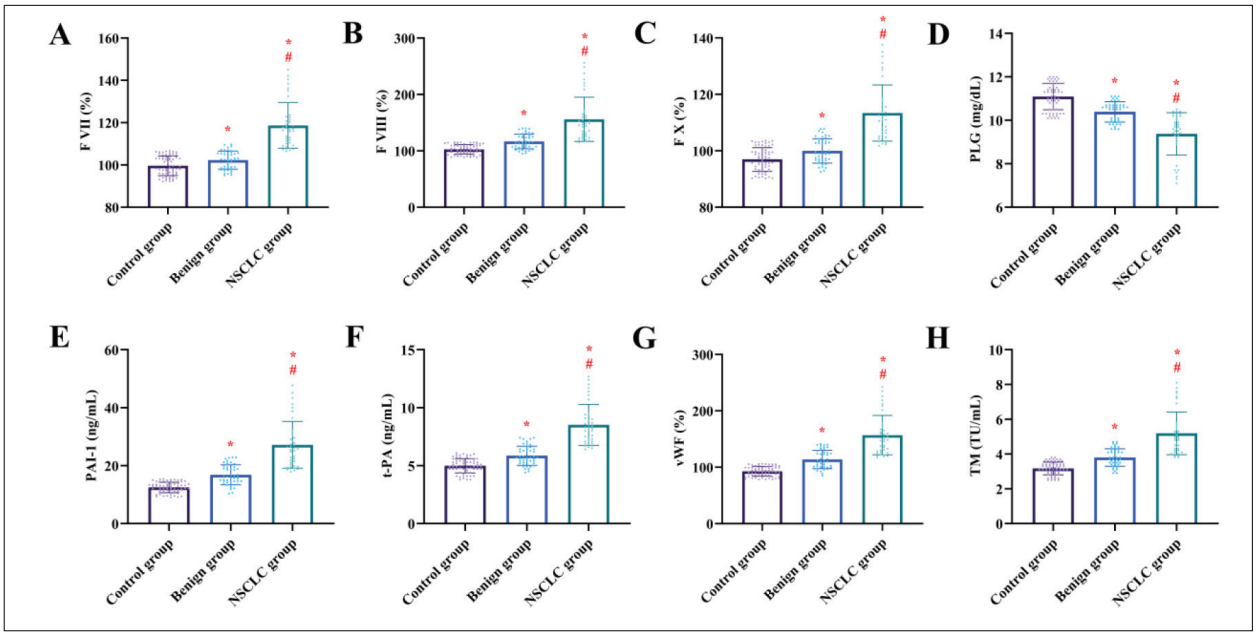

Figure 4 Characteristic coagulation–fibrinolysis biomarkers after workflow optimization in the study groups.

(A) Factor VII activity (FVII). (B) Factor VIII activity (FVIII). (C) Factor X activity (FX). (D) Plasminogen (PLG). (E) Plasminogen activator inhibitor-1 (PAI-1). (F) Tissue plasminogen activator (t-PA). (G) von Willebrand factor (vWF). (H) Thrombomodulin (TM). Characteristic coagulation–fibrinolysis biomarkers were compared among the control, benign, and NSCLC groups after workflow optimization. The between-group differences remained consistent with those observed before optimization. *P<0.05 vs the control group; #P<0.05 vs the benign group.

Table III Analytical performance of the testing workflow before and after optimization.

Performance Evaluation Index	Traditional General Process Before Optimization	Three-Tier Stratified Process After Optimization	Z	P
Intra-batch coefficient of variation (CV, %)	2.9 (2.7, 3.2)	1.7 (1.5, 1.9)	4.782	<0.001
Inter-batch coefficient of variation (CV, %)	5.4 (4.9, 5.7)	3.4 (3.2, 3.7)	4.516	<0.001
Relative deviation from international reference materials (%)	6.0 (5.3, 6.6)	3.3 (2.9, 3.7)	8.743	<0.001
Specimen retest rate (%)	4 (2.7)	2 (1.4)	Fisher's exact test	0.684
Unqualified specimen rate (%)	6 (15.4)	0 (0.0)	Fisher's exact test	0.025

Note: For specimen retest rate and unqualified specimen rate, values outside the parentheses are the number of cases, and values inside the parentheses are the corresponding percentage (%).

Laboratory performance verification results of the testing workflows before and after optimization

The full-process laboratory performance of the two workflows before and after optimization was evaluated, and the results showed that the optimized workflow improved both analytical performance and operational efficiency. In terms of precision, the within-run and between-run coefficients of variation (CVs) after optimization were lower than those before optimization (P<0.001). In terms of accuracy, the relative deviation between test results and international reference materials was also reduced after optimization (P<0.001). In addition, the specimen retesting rate did not change after optimization (P=0.684), whereas the rates of unqualified specimens and consumable waste both decreased (P=0.025) (*Table III*).

Table IV Clinical utility of the testing workflow before and after optimization in patients with non-small cell lung cancer.

Efficacy Evaluation Index	Traditional General Process Before Optimization (n=39)	Three-Tier Stratified Process After Optimization (n=39)	Z/χ^2	P
Test turnaround time (TAT), min	101.0 (86.5, 115.5)	69.0 (59.0, 76.5)	4.826	<0.001
Average test cost per patient, CNY	332.0 (274.0, 378.0)	238.0 (219.5, 266.5)	4.258	<0.001
Proportion of unnecessary tests (%)	13 (33.3)	5 (12.8)	4.622	0.032
Clinical decision compliance rate (%)	31 (79.5)	37 (94.9)	4.129	0.042
Missed diagnosis rate of thrombotic events within 6 months (%)	6 (15.4)	0 (0.0)	Fisher's exact test	0.025

Comparison of clinical application efficiency between the workflows before and after optimization

Based on paired analysis of 39 patients with NSCLC, the optimized workflow reduced the median specimen turnaround time (TAT) from 101.0 min to 69.0 min. It lowered the median average testing cost per patient from 332.0 yuan to 238.0 yuan (P<0.001). The proportion of unnecessary testing also decreased after optimization (P=0.032), whereas the clinical decision conformity rate increased (P=0.042). Among 6 confirmed VTE events during the 6-month follow-up, the missed-diagnosis rate was 15.4% under the pre-optimization workflow, but fell to 0% after optimization (P=0.025) (*Table IV*).

Discussion

By characterizing the full coagulation–fibrinolysis pathway in patients with NSCLC, this study established a three-tier stratified testing workflow and conducted paired validation against the conventional general workflow on the same analytical platform. Our paired comparison showed that the optimized strategy improved both analytical performance and workflow efficiency, suggesting that it may be a practical way to refine laboratory testing of coagulation-related indices in NSCLC.

From the perspective of medical biochemistry, the abnormalities involving multiple links of the coagulation–fibrinolysis pathway in patients with NSCLC essentially reflect cascade-like disruption of coagulation homeostasis driven by tumour cells (3, 11). In our cohort, characteristic changes were observed across the extrinsic coagulation initiation pathway, the intrinsic amplification pathway, fibrinolytic regulation, and endothelial injury markers. These alterations were directly related to tumour cell biology. Tissue factor, which is highly expressed by tumour cells, can directly activate the extrinsic coagulation pathway and drive increases in FVII and FX activity (12). Meanwhile, inflammatory mediators continuously released within the tumour microenvironment may induce endothelial cells to shift toward a procoagulant phenotype. This change promotes the release of endothelial injury markers, such as vWF and TM, and, at the same time, suppresses fibrinolytic activity by upregulating PAI-1 expression. The result is a biochemical profile marked by hypercoagulability, fibrinolytic imbalance, and endothelial injury occurring in parallel (13, 14). Because the present analysis covered the pathway as a whole rather than focusing solely on routine indices, it offers a fuller biochemical picture of coagulation abnormalities associated with NSCLC (15).

Previous studies on cancer-associated coagulation markers have repeatedly highlighted the same contradiction: although many characteristic markers have been shown in mechanistic studies to be closely linked to tumour-related coagulation abnormalities, their practical value in clinical laboratories has often been lower than expected (16, 17). Our results offer a laboratory-based explanation for this discrepancy. The issue does not seem to lie in the biochemical specificity of the markers themselves, but rather in the design logic of the conventional general workflow. In the traditional model, Patients are managed using a non-differentiated »one-size-fits-all« strategy and receive only four routine coagulation tests plus D-dimer, while characteristic markers are added only after obvious abnormalities have already appeared in routine indices (18). Under such a framework, low-abundance biochemical abnormalities in early-stage NSCLC are easily missed (19).

In contrast, the key idea behind the three-tier stratified workflow established here was not simply to refine the analytical method for a single marker, but to align the biochemical profile of NSCLC with the entire laboratory testing process. The initial screening tier relies on routine markers already widely used

in clinical practice, thereby preserving accessibility and basic screening capacity. The second and third tiers then introduce characteristic markers stepwise based on the initial screening results, avoiding unnecessary testing in low-risk patients while preserving full-pathway biochemical information for those at higher risk (20). This design affected more than just marker selection: turnaround time was shortened, unnecessary testing was reduced, and analytical precision and accuracy improved. One likely reason is that stratified testing reduces unnecessary batch analysis, shortens the time specimens remain at room temperature, lowers the risk of degradation in unstable biochemical markers, and allows more targeted internal quality control, thereby further reducing systematic error. Unlike earlier studies that focused solely on methodological refinements of individual indices (21, 22), this end-to-end workflow optimization is closer to the actual operating conditions of clinical laboratories. It should be easier to implement on routine testing platforms.

Nonetheless, this was a single-centre retrospective study with only 39 NSCLC cases, most of whom had middle- to late-stage adenocarcinoma. Whether the optimized workflow is equally applicable to early-stage NSCLC, squamous cell carcinoma, and rarer pathological subtypes still needs to be verified in larger cohorts. In addition, validation was performed on only one analytical platform. Because reagent traceability and reference intervals may differ across testing systems, the adaptability of this workflow to multiple platforms remains to be confirmed in multicentre studies. Finally, the present study evaluated testing performance only within 6 months. During longer follow-up, the optimal stratified testing interval remains to be determined. In addition, the study did not assess the impact of different anticancer treatments (e.g., chemotherapy, targeted therapy, immunotherapy) on coagulation markers and workflow performance; this could serve as a direction for future research.

Conclusion

This study clarified the characteristic full-pathway biochemical profile of the coagulation–fibrinolysis system in patients with NSCLC. On this basis, the three-tier stratified testing workflow established here improved laboratory operational efficiency and the ability to identify abnormal biochemical signals without compromising analytical accuracy. Taken together, these results offer a standardized approach to optimizing coagulation–fibrinolysis testing for patients with NSCLC in the clinical laboratory and add laboratory-based evidence to current work on the biochemical basis of tumour-associated coagulation abnormalities.

Consent to publish

All authors gave final approval of the version to be published.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

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Conflict of interest statement

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

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