

Decreased expression levels of Annexin V⁺/CD41⁺ platelet-derived microparticles in peripheral circulation blood and their auxiliary diagnostic value for patients with lung cancer complicated by pulmonary thromboembolism: An exploratory analysis

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Research Article

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Abstract

Background

Lung cancer is associated with an increased risk of pulmonary thromboembolism (PTE), but the auxiliary biomarkers for identifying lung cancer-associated PTE remain limited. Platelet-derived microparticles are involved in platelet activation, coagulation, inflammation, and cancer-associated thrombosis; however, their circulating expression patterns in patients with lung cancer complicated by PTE have not been fully clarified. This study aimed to investigate the expression pattern of circulating Annexin V⁺/CD41⁺ platelet-derived microparticle-like events in patients with lung cancer-associated PTE and to evaluate their exploratory diagnostic value when combined with conventional coagulation parameters.

Methods

Flow cytometry was employed to analyze the expression levels of Annexin V⁺/CD41⁺ platelet-derived microparticles in peripheral circulation blood from 26 patients with lung cancer complicated by PTE, 23 patients with lung cancer without PTE, and 23 healthy individuals. Results of clinical laboratory parameters, including activated partial thromboplastin time (APTT), fibrinogen, D-dimers, fibrin/fibrinogen degradation products (FDP), and thyroid function, were collected, and their association with PTE was examined. An exploratory joint predictive model was developed using coagulation parameters combined with Annexin V⁺/CD41⁺ platelet-derived microparticles.

Results

The expression levels of Annexin V⁺/CD41⁺ platelet-derived microparticles in peripheral circulation blood were significantly lower in the PTE group than in the non-PTE and healthy control groups. Univariate analysis revealed statistically significant associations of Annexin V⁺/CD41⁺ platelet-derived microparticles, APTT, fibrinogen, FDP, D-dimers, FT3, and T3 levels with the presence of PTE in patients with lung cancer. Multivariate logistic regression analysis revealed that Annexin V⁺/CD41⁺ platelet-derived microparticles, APTT, and D-dimers are independent predictors of PTE in patients with lung cancer. The column plot model developed based on these three biomarkers demonstrated good discriminative power in this exploratory cohort (area under the curve = 0.92).

Conclusion

Reduced expression levels of Annexin V⁺/CD41⁺ platelet-derived microparticles in the peripheral circulation of patients with PTE and lung cancer suggest they may reflect dynamic changes during thrombus formation associated with lung cancer. The combination of Annexin V⁺/CD41⁺ platelet-derived microparticles with APTT and D-dimers could provide a novel minimally invasive diagnostic approach for patients with PTE and lung cancer, although further validation is required in larger prospective cohorts.

Introduction

Lung cancer (LC) ranks among the most prevalent and lethal malignant tumors globally and imposes a significant societal and healthcare burden [1]. Among the causes of mortality of patients with cancer, venous thromboembolism (VTE) is a major complication and significant risk factor for death, along with tumor progression itself [2]. Pulmonary embolism (PE) typically occurs when a thrombus from the venous system travels through the bloodstream and obstructs the pulmonary artery or its branches. Pulmonary thromboembolism (PTE), the primary form of PE, has an annual incidence ranging from 11.7 to 131.9 per 100,000 individuals and a mortality rate as high as 30% for untreated acute cases, reflecting its substantial disease burden. Moreover, its clinical manifestations and conventional laboratory tests lack specificity [3, 4], making early diagnosis challenging. Traditional pulmonary angiography has been regarded as the gold standard for diagnosing PTE; however, its clinical application remains limited due to its invasive nature, high cost, and associated risks such as contrast agent allergies. Currently, computed tomography pulmonary angiography (CTPA) is a pivotal imaging modality for the clinical diagnosis of PE; however, it is limited by radiation exposure, contrast agent allergies, and incompatibility with certain patient populations [5]. Therefore, identifying simple, non-invasive, and reproducible blood biomarkers for PTE risk stratification and auxiliary diagnosis in patients with LC is of substantial clinical importance. In recent years, platelet-derived microparticles (PMPs), which promote extracellular vesicle release during platelet activation, have garnered attention for their role in tumor-associated hypercoagulability states [6]. However, the behavioral patterns of PMP-related biomarkers in the peripheral circulation and their diagnostic value in patients with LC and PTE require further clarification.

In recent years, the role of platelets and their derivatives as blood biomarkers of tumor progression has garnered increasing attention. Platelets are not only central to the hemostatic process, but also play pivotal roles in tumor progression, metastasis, and thrombosis through dynamic interactions with tumor cells, serving as a potential source for liquid biopsy in cancer [7, 8]. PMPs are extracellular vesicles derived from activated or apoptotic platelets that are typically released via membrane budding. They carry platelet-associated membrane proteins, lipids, nucleic acids, and various bioactive molecules and participate in coagulation activation, inflammatory regulation, intercellular communication, and disease progression [9, 10]. Elevated circulating PMP levels in patients with LC may correlate with tumor growth, invasion, angiogenesis, and metastasis and are associated with non-small cell LC progression [11, 12]. In non-tumor populations, elevated PMP levels have been associated with PTE and may have diagnostic value [13]. Furthermore, prospective cohort studies indicate that high PMP levels are associated with increased future risks of VTE, suggesting that PMPs not only serve as markers of platelet activation but may also participate in the pathological mechanisms underlying VTE development [14]. In colorectal cancer, activated platelets can induce neutrophil extracellular trap formation and enhance procoagulant activity, implying that platelet-neutrophil interactions may contribute to tumor-associated VTE processes [15]. However, No targeted clinical studies are currently present to investigate whether PMP-related biomarkers in the peripheral circulation can aid in diagnosing PTE in patients with LC.

The hypercoagulable state is a pathological process that precedes thrombus formation, resulting from an imbalance between the coagulation, anticoagulation, and fibrinolytic systems. Its core characteristics

include enhanced platelet activation, increased thrombin production, elevated fibrin deposition, and secondary activation or relative deficiency of the fibrinolytic response, ultimately shifting blood flow from a physiological hemostatic equilibrium toward pathological thrombus formation. Patients with LC exhibit a higher risk of VTE, with tumor stage, histological type, and treatment factors potentially influencing VTE occurrence. Additionally, platelet activation and tumor-cell-platelet interactions may contribute to tumor progression [16, 17]. Platelet-derived microparticles carry various membrane proteins and bioactive molecules that participate in processes such as hemostasis, thrombosis, angiogenesis, inflammatory regulation, and tumor progression [18]. Given the absence of unified laboratory diagnostic criteria for tumor-associated hypercoagulability, a comprehensive evaluation of platelet activation and coagulation/fibrinolytic markers aids in identifying high-risk patients with thrombosis and provides a basis for subsequent anticoagulation management and clinical monitoring.

In this study, we focused on Annexin V⁺/CD41⁺ platelet-derived microparticles in the peripheral circulation of patients with LC complicated by PTE, aiming to preliminarily investigate their clinical value in the auxiliary diagnosis of PTE-associated LC. Additionally, we retrospectively analyzed the relationships of coagulation function, blood biochemical parameters, and tumor markers with PTE and developed a combined auxiliary diagnostic model based on Annexin V⁺/CD41⁺ platelet-derived microparticles and coagulation indicators to provide an exploratory basis for PTE risk assessment in patients with LC.

Materials and Methods

Ethical Approval

This study was approved by the Medical Ethics Committee of Guangxi Medical University Affiliated Cancer Hospital (Approval Number: LW2025030) and was conducted in strict compliance with the provisions of the Declaration of Helsinki. All the participants provided written informed consent.

Study Participants

This study included patients who were hospitalized at the Affiliated Tumor Hospital of Guangxi Medical University from January 2025 to June 2026 and provided peripheral blood samples along with complete clinical data, comprising 26 patients with LC complicated by PTE, 23 patients with LC without PTE, and 23 healthy individuals undergoing physical examinations. LC was diagnosed based on a comprehensive evaluation of China's Lung Cancer Diagnosis and Treatment Guidelines, pathological findings, and clinical data [19]. The diagnosis of PTE was established according to relevant diagnostic consensus criteria combined with clinical manifestations, laboratory test results, and imaging evidence such as CTPA [5,20].

Reagents and Instruments

The Annexin V-APC reagent was purchased from Lianke Company; the anti-human CD41 antibody (PerCP/Cyanine 5.5 anti-human CD41) was obtained from Biolegend Company; the 5X Binding Buffer was supplied by Lianke Company; sodium citrate-coated blood collection tubes were sourced from Beckon-Dickinson, USA; and absolute counting fluorescent microspheres (Blank Calibration threshold

microspheres, 1.0–1.4 μm) were acquired from Beckman Coulter, USA. The BD Accuri C6 flow cytometer was equipped with 488 nm and 640 nm lasers for detecting CD41-PerCP-Cy5.5 and Annexin V-APC fluorescence signals, respectively.

Collection of Clinical Data

We collected baseline demographic data (age, gender) for each patient with LC along with six coagulation parameters including prothrombin time (PT), fibrinogen (FIB), thrombin time, activated partial thromboplastin time (APTT), D-dimers (DD), and fibrin/fibrinogen degradation products (FDP); eight hematological parameters including red blood cell count, white blood cell count, platelet count, hemoglobin, neutrophil count, lymphocyte count, monocyte count, and eosinophil count; 14 biochemical parameters including triiodothyronine (T3), Tetraiodothyronine (T4), Free Triiodothyronine (FT3), Free Tetraiodothyronine (FT4), Thyroid-Stimulating Hormone (TSH), Squamous Cell Carcinoma Antigen, Cytokeratin 19 Fragment Antigen 21-1, Carcinoembryonic Antigen, Carbohydrate Antigen 125, Carbohydrate Antigen 153, Triglycerides, Total cholesterol, High-density lipoprotein cholesterol, and low-density lipoprotein cholesterol, and pathological staging.

Whole Blood Sample Processing

All blood samples were obtained as 3.7 mL of fasting venous blood collected early in the morning and anticoagulated with sodium citrate. The samples were gently inverted to mix thoroughly, centrifuged at $180 \times g$ at room temperature for 10 min, and the supernatant plasma was collected. Subsequently, the plasma was centrifuged again at $2500 \times g$ at room temperature for 10 minutes; the supernatant was discarded, and the precipitate was collected as the plasma particle-enriched fraction, which was stored at -80°C for analysis.

Flow Cytometry Detection of PMPs

The plasma particle-enriched fraction stored at -80°C was retrieved, dissolved at room temperature, resuspended in 1 mL of phosphate-buffered saline (PBS), and vortexed at 2800 rpm for 1 min. After centrifugation at $3200 \times g$ for 15 minutes at 4°C , 1 mL of the supernatant was collected; subsequent centrifugation at $20000 \times g$ for 5 minutes at 4°C resulted in discarding of the supernatant. The particle precipitate was placed on ice, resuspended in 100 μL of $1\times$ Binding Buffer, followed by addition of 5 μL of Annexin V-APC and 5 μL of CD41-PerCP-Cy5.5 antibody, and incubated under light protection at 4°C for 15 minutes. A flow cytometer tube was filled with 500 μL of PBS and the aforementioned sample solution, followed by analysis using a flow cytometer. Data were analyzed using FlowJo software. Double-distilled water filtered through a 0.1 μm membrane served as the background control, whereas dual fluorescent standard microspheres (1.0 μmol and 1.4 μmol) were used to establish event analysis gates for small particles based on forward and side scatter spectra. Given the sensitivity limitations of conventional flow cytometry in detecting submicron-sized extracellular vesicles (EVs), this study defined events within the detectable particle range that were double-positive for Annexin V and CD41 as Annexin V⁺/CD41⁺ platelet-derived particles, as recommended for EV research and flow cytometric reporting [21,22] (see Figure 1).

Comparison Analysis of PMPs and Clinical Outcomes

A comparative analysis was conducted on the proportions of Annexin V⁺/CD41⁺ PMPs and their clinical parameters between 26 patients with LC with concurrent PTE and 23 patients with LC without PTE. Only parameters showing statistically significant differences were included in subsequent analyses.

Logistic Regression Analysis and Construction/Validation of Prognostic Column Charts

Through univariate and multivariate logistic regression analysis, the relationship between Annexin V⁺/CD41⁺ PMPs and clinical indicators with LC complicated by PTE was evaluated. Parameters with statistically significant P-values were selected, and diagnostic boxplots for LC complicated by PTE were constructed using the "rms" and "ggplot2" packages in R software.

Statistical Analysis

Statistical analysis of the collected data was performed using R software (version 4.3.3) and SPSS 26.0. Continuous variables conforming to a normal distribution were expressed as mean $\pm$ standard deviation, while those not conforming were expressed as medians (interquartile range). Nonparametric comparisons among multiple groups were conducted using the Kruskal-Wallis test, homogeneity of variance was assessed using the Levene test, and nonparametric comparisons between two groups were performed using the Mann-Whitney U test. Univariate logistic regression analysis was used to identify candidate variables associated with PTE in patients with LC, with statistically significant variables included in the multivariate logistic regression model. Column charts were constructed based on the multivariate analysis results, and receiver operating characteristic and calibration curves were used to evaluate the model discrimination power and calibration accuracy. Statistical significance was set at $p < 0.05$.

Results

Expression Levels of PMPs in Peripheral Blood of Patients With LC and Healthy Individuals

Flow cytometry results demonstrated that the proportion of Annexin V⁺/CD41⁺ PMPs was significantly lower in the patients with LC with PTE group than in the patients with LC without PTE group and the healthy control group. The median values were 91.5% for patients with LC without PTE, 88.8% for the healthy control group, and 72.3% for patients with LC in the PTE group. The Annexin V⁺/CD41⁺ PMP expression level in the patients with LC with PTE group was markedly lower than those in both the patients with LC without PTE group and the healthy control group (Figure 2, $P < 0.05$).

Association Between PMPs, Clinical Indicators, and PTE

To identify candidate biomarkers associated with PE in patients with LC, this study compared the PE-positive and PE-negative LC groups for Annexin V⁺/CD41⁺ PMPs, coagulation function, hematological biochemical parameters, tumor markers, and clinical staging. The results demonstrated statistically significant differences in the levels of expression of PMPs, APTT, FIB, FDP, DD, FT3, and T3 between the two groups (Table 1, $P < 0.05$).

Construction and Validation of Diagnostic Line Graphs

Univariate analysis of 49 clinically diagnosed patients with LC demonstrated significant associations between PMPs, APTT, FIB, FDP, DD, FT3, and T3 levels and LC complicated by PTE (Table 2, $P < 0.05$). Multivariate logistic regression analysis using forward stepwise variable selection revealed that PMP levels (OR, 0.91; 95% CI, 0.85–0.97), APTT (OR, 0.82; 95% CI, 0.70–0.95), and DD (OR, 1.32; 95% CI, 1.01–1.71) were independently associated with LC complicated by PTE (Table 2, $P < 0.05$). Combining the three biomarkers, PMPs, APTT, and DD, we established a logistic regression model for the auxiliary identification of PTE in LC (Figure 3A). The model equation was: $\text{logit}(z) = 13.31 - 0.10 \times \text{PMPs} - 0.20 \times \text{APTT} + 0.27 \times \text{DD}$, with significant overall model fit (Figure 3B, area under the curve [AUC] = 0.92, $P < 0.05$). As shown in Figure 3C, the calibration curve of this model closely approximated the ideal curve (slope = 1). These results indicate that the combined model exhibits good discriminative power and calibration trends for PTE in LC within this exploratory cohort; however, its stability and generalizability require further validation in independent cohorts.

Discussion

This study involved an exploratory analysis of the characteristic changes and auxiliary diagnostic value of Annexin V⁺/CD41⁺ PMP expression levels in peripheral circulation blood among patients with LC complicated by PTE. Flow cytometry is a commonly used method for detecting circulating microparticle phenotypes and is particularly suitable for analyzing microparticles that are positive for specific cell source markers. Our findings revealed that Annexin V⁺/CD41⁺ PMPs were significantly reduced in the peripheral circulation blood of the LC+PTE group; Annexin V⁺/CD41⁺ PMPs, APTT, FIB, FDP, DD, FT3, and T3 levels were independently associated with LC+PTE; a combined model constructed from Annexin V⁺/CD41⁺ PMPs, APTT, and DD demonstrated good discriminative power for this exploratory cohort (AUC = 0.92, $P < 0.05$). Based on these results, we propose that Annexin V⁺/CD41⁺ PMPs in the peripheral circulation may serve as a novel hematological biomarker for the auxiliary diagnosis of LC complicated by PTE. The differential indicators identified in this study warrant further discussion; however, their specific biological significance requires further investigation in light of the pathophysiological mechanisms underlying tumor-associated hypercoagulability and thrombosis.

Janowska-Wieczorek et al. found that activated platelet-derived microvesicles (PDMVs) can transfer platelet-associated integrin CD41 to the surface of LC cells and promote LC cell migration, angiogenesis, and metastasis [23]. In non-tumor or acute PTE populations, elevated expression levels of PDMVs or procoagulant particles in the peripheral circulation have also been reported to be associated with PTE development [13,24]. The present study focused on the specific clinical context of patients with lung cancer with PTE and observed significantly reduced levels of expression of Annexin V⁺/CD41⁺ PMPs in the peripheral circulation of the LC+PTE group, which is markedly distinct from results of previous studies that consistently reported elevated levels of procoagulant particles during thrombosis. This discrepancy suggests that changes in Annexin V⁺/CD41⁺ PMPs in patients with LC and PTE may not simply mirror the pattern of PMP elevation seen in non-tumor PTE cases but could be influenced by multiple factors, including tumor-associated procoagulant networks, stages of thrombosis, therapeutic interventions, and

measurement time points. From a pathophysiological perspective, cancer-related thrombosis is a complex process involving tumor cells, platelets, endothelial cells, inflammatory cells, and the coagulation/fibrinolytic system. Tissue factor (TF) expression, release of procoagulant EVs, formation of neutrophil extracellular traps, modulation by inflammatory mediators, and procoagulant activation of platelets can contribute to thrombin generation and fibrin formation [25,26]. Coagulation-promoting platelet activation can provide membrane surface space for the coagulation response through phosphatidylserine exposure, thereby facilitating venous thrombosis [27]; experimental venous thrombosis models have also demonstrated that leukocyte-derived particles and PMPs are correlated with thrombus weight and TF activity [28]. Consequently, PMPs are generally regarded as procoagulants in hypercoagulable states and in thrombotic processes. The observed reduction of Annexin V⁺/CD41⁺ PMPs in this study does not negate their procoagulant properties but rather suggests that these detectable particles undergo redistribution or transient changes following LC-associated PTE formation. The decreased levels of Annexin V⁺/CD41⁺ PMPs in the peripheral circulation of the LC+PTE group can be interpreted as a clinical phenomenon of "consumptive depletion." Post-thrombotic conditions are not static but involve dynamic processes, including immune cell infiltration, fibrinolysis, collagen degradation, thrombus organization, and recanalization. Neutrophils, monocytes/macrophages, and endothelial cells participate in deep vein thrombus resolution and tissue remodeling [29]. A bidirectional amplification relationship exists between inflammation and the coagulation system, where systemic inflammation can promote coagulation activation via multiple pathways [30]. Based on these multidimensional findings, the reduced levels of Annexin V⁺/CD41⁺ PMPs in the peripheral circulation of patients with LC and PTE may be associated with local capture after thrombus formation, circulatory redistribution, inflammation-related clearance, or therapeutic factors. During the aforementioned physiological and pathological processes, Annexin V⁺/CD41⁺ PMPs may undergo a "rise–consumptive decline–rebalancing" pattern alongside thrombus formation. Additionally, patients with LC may receive anticoagulant therapy, chemotherapy, targeted therapy, or immunotherapy before or after PTE onset, all of which can influence platelet activation status, coagulation/fibrinolysis parameters, and circulating microparticle levels. Furthermore, the duration between PTE onset and blood sampling may affect peripheral circulatory PMP levels, and the state of circulating microparticles may differ following acute thrombosis, imaging confirmation, anticoagulation therapy, or during the stable phase. Due to insufficient stratification in this study regarding anticoagulant drug types, antitumor treatment regimens, duration of medication, and the interval from PTE onset to blood sampling, therapeutic factors and sampling time points may represent potential confounding variables. Therefore, further research is required to elucidate the phenomenon and mechanisms underlying the "consumptive decline." Furthermore, this study employed flow cytometry to define the Annexin V⁺/CD41⁺ event, which reflects the platelet-derived procoagulant particulate phenotype; however, it lacked nanoparticle tracking analysis, transmission electron microscopy, and validation using CD61/CD42b, CD62P, and Triton X-100 lysis assays. This study focuses solely on the flow-cytometry-defined Annexin V⁺/CD41⁺ PMPs and provides corresponding explanations.

The results of this study showed that patients with LC and PTE had shortened APTT and decreased FIB levels, accompanied by elevated FDP and DD concentrations. Shortened APTT serves as an indirect indicator of relative activation of the coagulation system. Malignant tumors can promote coagulation

system activation through mechanisms such as TF expression, release of inflammatory cytokines, and production of procoagulant EVs, leading to enhanced thrombin generation in some patients [25,26,31]. Concurrently, inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) can induce endothelial and monocyte activation, enhance TF expression, strengthen platelet activation, and impair anticoagulant/fibrinolytic regulation, collectively contributing to an inflammatory hypercoagulable state. Shortened APTT may reflect overall coagulation system activation and elevated levels of acute-phase coagulation factors such as factor VIII and fibrinogen; however, it is not a specific marker of TF pathway activation. FIB, an acute-phase response protein, often increases under inflammatory or neoplastic conditions, and its decrease may indicate substrate depletion following persistent activation of the coagulation system. However, during prolonged or intense activation of the coagulation system, fibrinogen is continuously converted into fibrin and participates in thrombus formation. Secondary fibrinolytic activation promotes fibrin/fibrinogen degradation, resulting in decreased FIB levels. Therefore, a decrease in FIB should not be interpreted as a state of hypocoagulability in patients with LC complicated by PE; it requires comprehensive evaluation alongside DD, FDP, platelet count, PT/APTT, and clinical thrombotic burden. This study observed a concurrent reduction in APTT along with decreased FIB, suggesting simultaneous activation of the coagulation system and depletion of coagulation substrates. A shortened APTT may reflect enhanced coagulation activity, increased thrombin production, or elevated activity of coagulation factors such as factor VIII, whereas reduced FIB level may indicate consumptive changes following persistent coagulation activation. Thus, this combination of shortened APTT and decreased FIB level is more appropriately described as an abnormal coagulation phenotype characterized by coexisting coagulation activation and substrate depletion, rather than serving as a sole evidence for hypocoagulability or disseminated intravascular coagulation (DIC). If accompanied by significantly elevated DD or FDP, decreased platelet count, prolonged PT, or elevated DIC scores, the possibility of chronic or occult DIC should be considered [32,33,34]. The hypercoagulable state associated with LC may involve persistent expression or presence of thromboplastin (TF) on tumor cells and tumor-derived EVs. TF can activate the extrinsic coagulation pathway via the TF/FVIIa complex, promote thrombin generation, and amplify the coagulation cascade, thereby contributing to cancer-related thrombosis [26]. Furthermore, platelet-derived EVs can expose phosphatidylserine and carry platelet activation-associated molecules, providing a procoagulant surface for thrombin generation and further exacerbating the tumor-associated hypercoagulable state [35].

Furthermore, both FDP and DD levels are significantly elevated in patients with LC with PE compared with those without thrombosis. This phenomenon reflects the persistent activation of the coagulation system and secondary enhancement of the fibrinolytic system, representing one of the hallmark laboratory features of tumor-associated hypercoagulability. First, the direct mechanism underlying the elevated FDP and DD levels involves a simultaneous increase in fibrin synthesis and degradation following excessive activation of the coagulation system. In LC and other malignancies, tumor cells continuously express and secrete TF, cancer-derived EVs, and proinflammatory cytokines, thereby triggering the extrinsic coagulation pathway and accelerating thrombin production [35]. Thrombin promotes the conversion of fibrinogen to fibrin, leading to microthrombus formation within blood vessels. The fibrinolytic system is activated, with plasmin degrading fibrin and releasing substantial amounts of FDP and DD [26]. Thus, elevated FDP and

DD levels not only indicate thrombus formation but also serve as a comprehensive marker of concurrent hypercoagulability and fibrinolytic system activation. Second, tumor-specific procoagulant mechanisms exacerbate this process. LC cells highly express TF, carcinoembryonic antigen, and adhesion molecules, which can activate platelets and endothelial cells via tumor-derived EVs, further promoting fibrin deposition [10,35]. Additionally, chronic inflammation associated with malignancies (e.g., IL-6, TNF- α) upregulates fibrinogen synthesis and enhances the coagulation cascade [35]. Therefore, some patients with LC may have persistent elevation of DD and FDP levels, which are regarded as biochemical markers of "subclinical thrombosis," even before the occurrence of clinically visible thrombi [36]. Furthermore, elevated FDP and DD levels may reflect compensatory activation of the fibrinolytic system. When continuous fibrin deposition occurs within blood vessels, the body initiates compensatory thrombolysis by activating the plasminogen-plasmin system, leading to significant increases in FDP and DD [26]. This "hypercoagulable with hyperfibrinolytic" state represents a hallmark pathophysiological feature of tumor-associated thrombosis. DD levels have been positively correlated with the risk of VTE, thrombotic burden, and recurrence probability, and can serve as prognostic and anticoagulant efficacy monitoring indicators [37,10]. Additionally, elevated DD and FDP levels have been associated with tumor burden and tissue necrosis. Tumor necrosis and apoptosis release large amounts of phospholipids, DNA, and procoagulant substances, further activating the coagulation system; thus, the rise in FDP and DD reflects both thrombus formation and potential contributions from coagulation-fibrinolytic reactions in necrotic tumor areas [10,35]. Elevated FDP and DD levels further suggest synchronous activation of the coagulation and fibrinolytic processes in patients with LC and PTE. Thrombin promotes the conversion of fibrinogen into fibrin, which is subsequently degraded by plasmin to produce FDP, whereas cross-linked fibrin breaks down to release DD. Therefore, elevated FDP and DD levels indicate activation of the coagulation-fibrinolytic system, although their specificity remains limited and requires interpretation in conjunction with imaging studies, such as CTPA, and clinical context. TF-positive EVs (TF+EVs) derived from tumor cells represent one of the key mechanisms underlying cancer-associated coagulation activation and are associated with thrombosis in various disease contexts [31]. In patients with LC complicated by PE, elevated levels of FDP and DD, shortened APTT, and decreased FIB concentration coexist, potentially reflecting an "activation-consumption" process involving TF-mediated coagulation initiation, platelet-derived particle procoagulant effects, and secondary fibrinolysis. Subsequent extended studies incorporating dynamic DD monitoring, thrombin generation assays, and TF+EV detection may provide a more comprehensive assessment of the coagulation–fibrinolysis balance and thrombotic risk in patients with LC.

Regarding thyroid function-related parameters, both FT3 and T3 levels were lower in the patients in the LC and PTE than in those with LC without PTE, while the differences in FT4 and TSH levels were not significant. These findings suggest that patients with LC and PTE may have a non-thyroidal disease syndrome or a low T3 state. Non-thyroidal disease syndromes are associated with severe systemic diseases and inflammatory states potentially linked to reduced peripheral conversion of T4 to T3, altered regulation of the hypothalamic-pituitary-thyroid axis, and tissue metabolic redistribution [38–40]. Thyroid hormones influence myocardial contraction, vascular tone, and peripheral circulatory homeostasis; thus, decreased FT3/T3 ratios may reflect abnormalities in systemic metabolism and cardiovascular regulation under tumor and thrombotic stress [41]. However, these results do not establish a direct correlation

between low T3 levels and LC complicated by PTE. They also do not indicate that low T3 directly promotes thrombosis through endothelial dysfunction. Low T3 levels may represent an endocrine phenotype of the inflammation-hypercoagulability state, sharing common pathological mechanisms with endothelial activation, platelet activation, microparticle release, and increased venous thromboembolic risk. Therefore, reduced FT3 and T3 levels are suitable exploratory indicators of systemic stress and metabolic redistribution, although their independent predictive values require further validation in larger studies.

The joint model developed in this study demonstrates that Annexin V⁺/CD41⁺ PMPs, APTT, and DD are independently associated with PTE in patients with LC. The combined diagnostic model achieved an AUC of 0.92, indicating strong discriminative power within this exploratory cohort. Previous studies have utilized DD combined with PMPs for PTE assessment with favorable diagnostic performance [13]. Compared with DD alone, the inclusion of Annexin V⁺/CD41⁺ PMPs and APTT may provide a more comprehensive reflection of PTE risk in patients with LC across three dimensions: platelet activation/microparticle phenotype, coagulation system activation, and elevated fibrinolytic products. However, the limited sample size and variable selection processes may have led to overestimation of the AUC, and the model has not undergone independent external validation. Therefore, this model is currently best suited as an exploratory tool for auxiliary identification and risk stratification of PTE in patients with LC and cannot replace imaging modalities such as CTPA.

This study has certain limitations. First, it was a single-center, small-sample exploratory analysis, and the sample size limited the stability of the multivariate model, potentially increasing the risk of overfitting. Second, PMP detection was based on the definition of Annexin V⁺/CD41⁺ PMPs via flow cytometry. While this method reflects platelet-derived procoagulant particle-like events, further validation using nanoparticle tracking analysis, transmission electron microscopy, CD61/CD42b, CD62P, or Triton X-100 lysis was not performed because of constraints in funding and research equipment. Thus, the study failed to provide a comprehensive characterization of PMPs. Third, dynamic sampling was not conducted, making it impossible to assess the temporal changes in PMPs before PTE onset, during the acute phase, post-treatment, or during the stable phase, which represents a future research direction. Fourth, anticoagulant therapy, antitumor treatment, tumor stage, pathological type, and blood collection timing may influence PMP-like events and coagulation parameter levels; however, this study lacked adequate stratification. Finally, the association between decreased FT3/T3 levels and PTE remains clinically correlated and cannot be interpreted as a causal factor. Future studies should employ multicenter prospective cohorts, dynamic monitoring, more comprehensive EV characterization methods, and external validation cohorts to confirm these findings.

Conclusion

In summary, Annexin V⁺/CD41⁺ PMPs, APTT, FIB, DD, FDP, FT3, and T3 levels demonstrated statistically significant associations with the occurrence of PTE in patients with LC. The reduced levels of Annexin V⁺/CD41⁺ PMPs in the peripheral circulation of patients with LC and PTE may reflect dynamic changes following thrombosis secondary to LC, although the underlying mechanism requires further validation. The model developed from Annexin V⁺/CD41⁺ PMPs combined with APTT and DD demonstrated robust

auxiliary diagnostic potential in this exploratory cohort and can serve as a reference tool for PTE risk assessment and auxiliary diagnosis in patients with LC; however, it cannot replace imaging modalities such as CTPA, and its clinical utility warrants further validation through large-scale, multicenter, prospective studies.

Abbreviations

APTT

activated partial thromboplastin time

AUC

area under the curve

CTPA

computed tomography pulmonary angiography

DD

D-dimers

DIC

disseminated intravascular coagulation

EVs

extracellular vesicles

FDP

fibrin/fibrinogen degradation products

FIB

fibrinogen

FT3

free Triiodothyronine

FT4

free Tetraiodothyronine

IL-6

interleukin-6

LC

lung cancer

PDMVs

platelet-derived microvesicles

PE

pulmonary embolism

PMPs

platelet-derived microparticles

PT

prothrombin time

PTE

pulmonary thromboembolism

T3
Triiodothyronine
T4
Tetraiodothyronine
TF
tissue factor
TNF- α
tumor necrosis factor-alpha
TSH
Thyroid-Stimulating Hormone
VTE
venous thromboembolism

Declarations

Ethics approval and consent to participate:

This study was approved by the Medical Ethics Committee of Guangxi Medical University Affiliated Cancer Hospital (Approval Number: LW2025030) and was conducted in strict compliance with the provisions of the Declaration of Helsinki. All the participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available because they contain potentially identifiable clinical information and are subject to institutional ethical restrictions. De-identified data may be made available from the corresponding author upon reasonable request and with permission from the Medical Ethics Committee of Guangxi Medical University Affiliated Cancer Hospital.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KB: Design of the study, bioinformatics analysis, experimental validation, and manuscript writing; PG, RL, CY: Clinical specimen collection, data curation, and writing-review-editing. JK: Guidance on study design, bioinformatics analysis, experimental validation, and final manuscript editing, review, and revision; All authors read and approved the final manuscript.

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Tables

Table 1 to 2 are available in the Supplementary Files section.

Figures

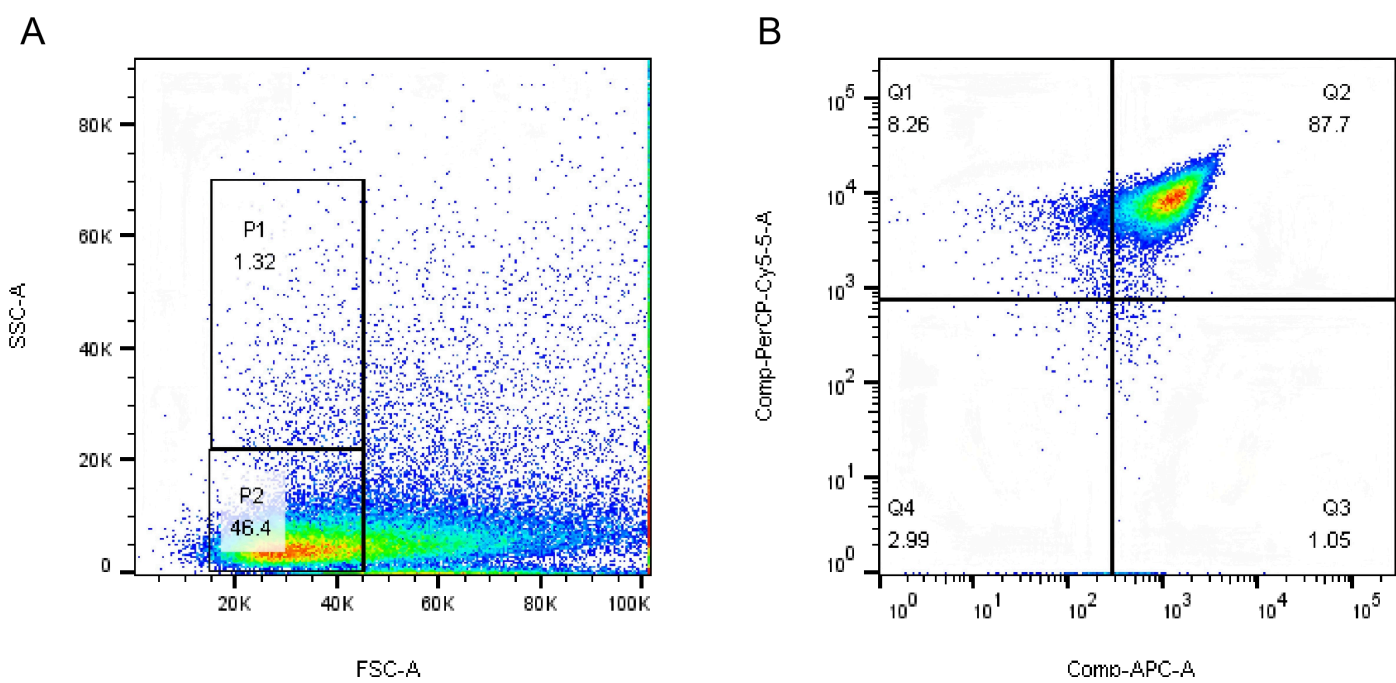


Figure 1

Flow cytometric detection of Annexin V⁺/CD41⁺ platelet-derived microparticles

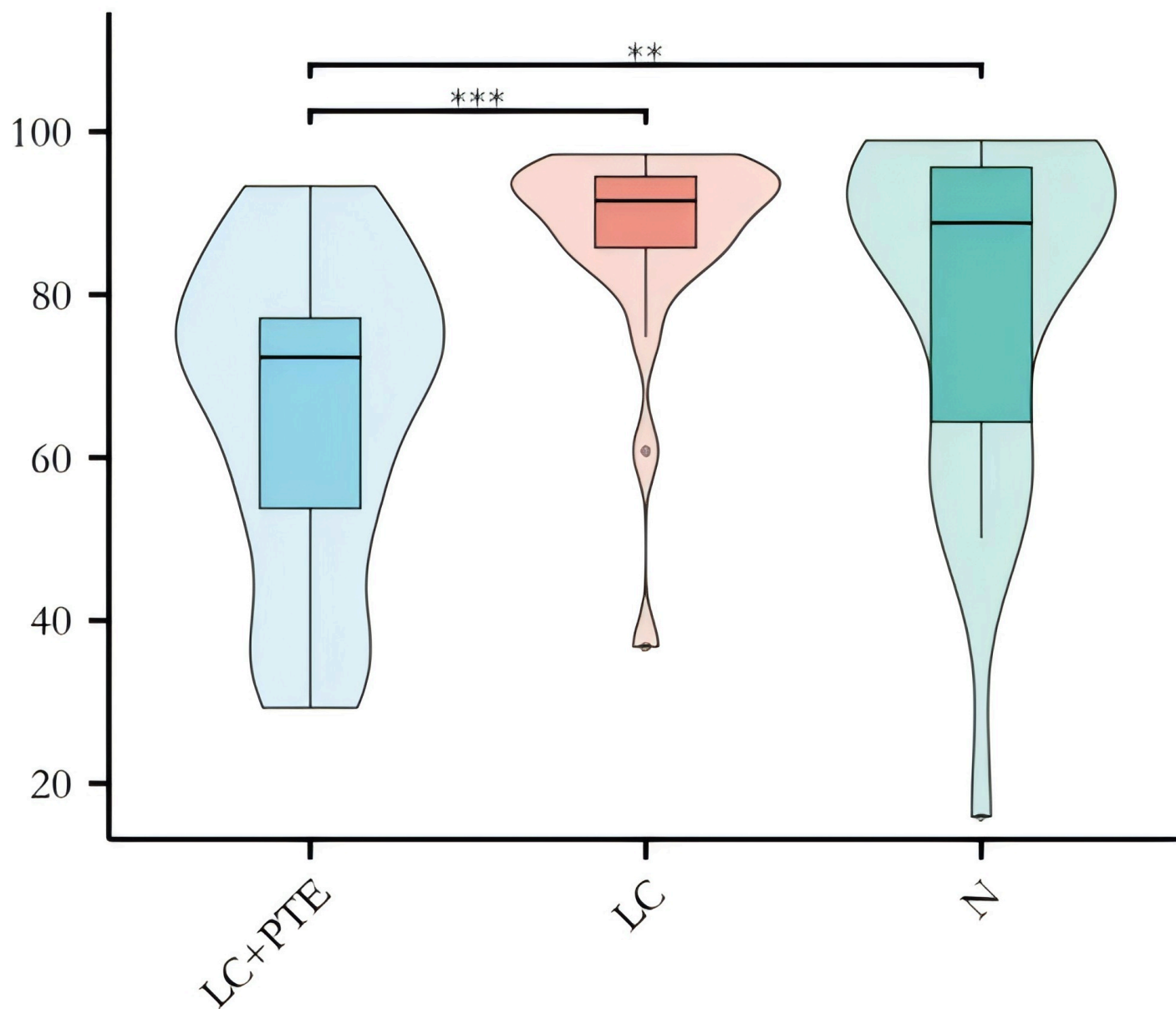


Figure 2

Proportions of V⁺/CD41⁺ platelet-derived microparticles in different groups.

LC+PTE: lung cancer with pulmonary arterial thromboembolism;

LC: lung cancer without pulmonary arterial thromboembolism;

HC: healthy control group;

, P<0.01; *, P<0.001.

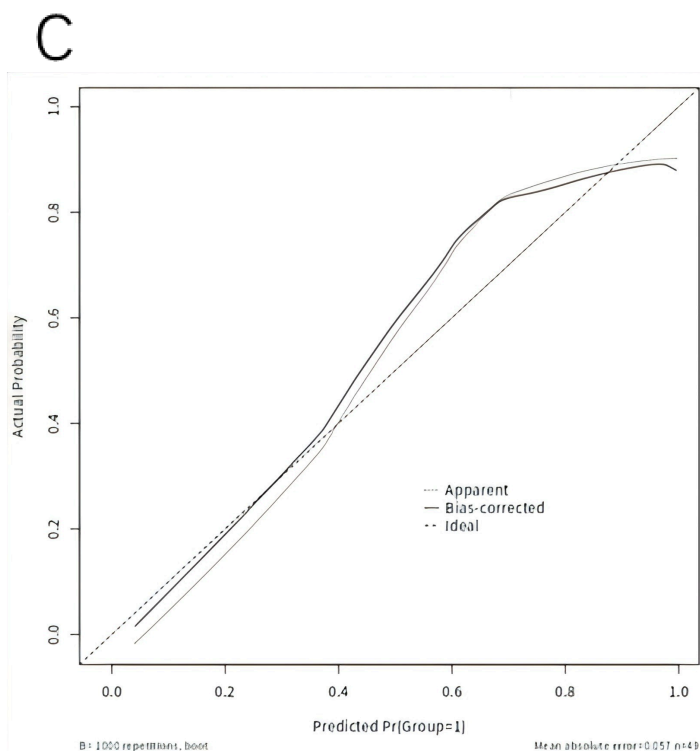
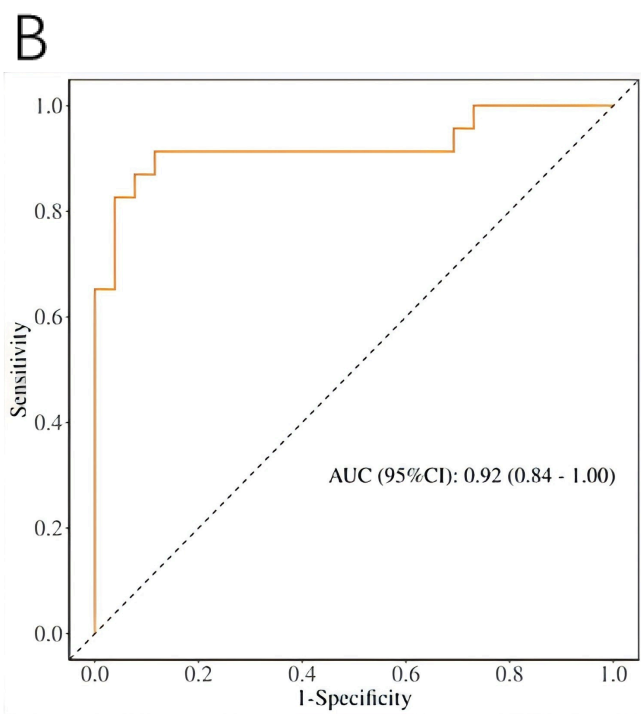
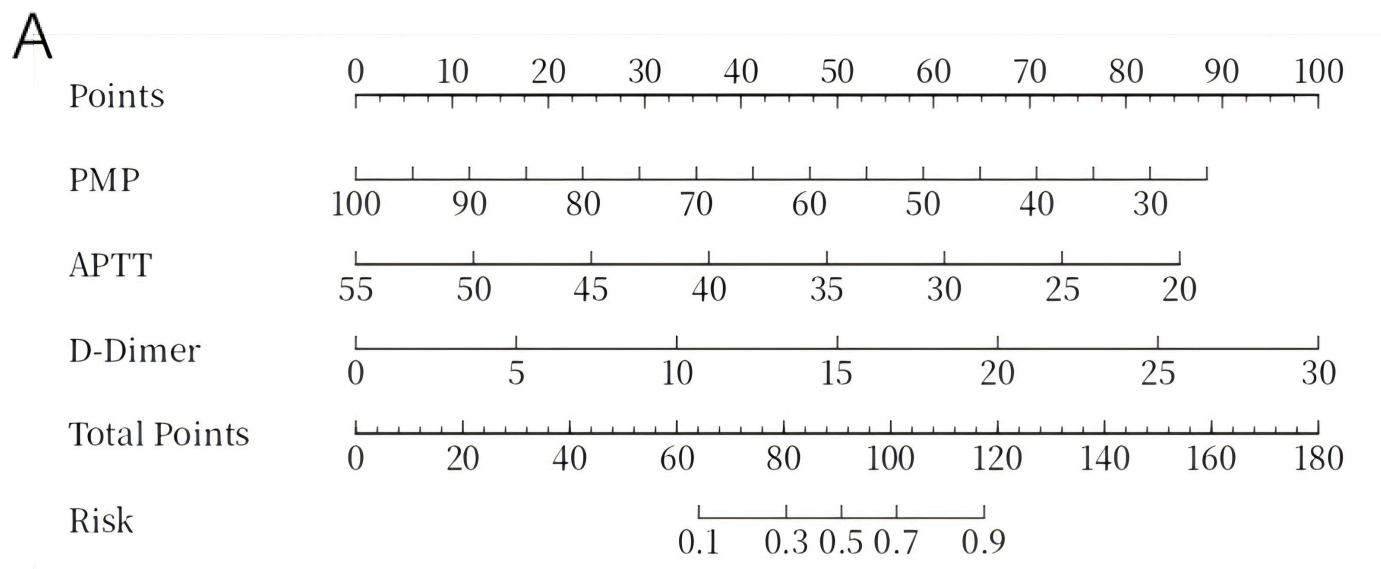


Figure 3

Auxiliary diagnostic model for lung cancer-associated PTE. A, nomogram based on Annexin V⁺/CD41⁺ platelet-derived microparticles, APTT, and D-dimer; B, receiver operating characteristic curve; C, calibration curve.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.BaselineclinicalandlaboratorycharacteristicsofpatientswithlungcancerwithandwithoutPTE.docx](#)
- [Table2.Resultsofforwardstepwiselogisticregressionanalysis.docx](#)