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Development of a thrombin-antithrombin complex detection kit and study in venous thromboembolism complicated by cervical cancer

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Abstract

Objective Our study successfully developed an assay kit for thrombin-antithrombin complex (TAT) and demonstrated the predictive value of plasma TAT concentration in the development of venous thromboembolism (VTE) in patients with cervical cancer.

Method A retrospective analysis was conducted on 177 patients with cervical cancer who received treatment at the Affiliated Hospital of Jiangnan University in Wuxi City from July 1, 2023 to October 1, 2023. This study provides a comprehensive analysis of cervical cancer patients and their VTE risk factors. The patients were divided into two groups: 27 cases with VTE (Thrombosis group) and 150 cases without VTE (Non-thrombotic group). Additionally, the patients were classified into four stages based on tumor stage: 42 cases of stage I, 45 cases of stage II, 62 cases of stage III, and 28 cases of stage IV. The control group consisted of 80 healthy patients undergoing medical check-ups. Thrombin-antithrombin complex (TAT), fibrinolytic enzyme-α2-fibrinolytic inhibitor complex (PIC), thrombomodulin (TM), and tissue-type plasminogen activator inhibitor 1 complex (t-PAIC) were detected using quantitative chemiluminescence immunoassay. The study assessed the variations in thrombotic marker levels among cervical cancer patients of different stages through a receiver operating characteristic (ROC) curve.

Result The TAT reagent demonstrated a detection limit of 0.048 ng/mL, with a linear R value of 0.9997, indicating high accuracy and precision. The reagent's accelerated stability was also excellent, with deviations of less than 10%. Furthermore, the correlation coefficient of this method with Hyson Mecon was $R^2 = 0.9683$. Notably, in patients with cervical cancer, TAT and PIC levels were found to be elevated compared to those of the healthy population. Cervical cancer patients who developed thrombosis had significantly elevated levels of TAT and fibrinogen degradation products (FDP) compared to those who did not. Furthermore, patients with stage III-IV cervical cancer exhibited higher levels of the six markers than those with stage I-II during staging. Notably, the combination of four or six markers significantly improved the sensitivity and specificity of the diagnosis, as demonstrated by the ROC curves.

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Conclusion Our developed TAT test kit has excellent performance and low cost, making it a clinically valuable tool for widespread use. Elevated TAT levels have significant predictive value for thrombosis occurrence in cervical cancer patients. The combination of t-PAIC, TM, TAT, PIC, D-dimer(D-D), and FDP markers is superior to using a single marker for diagnosing VTE in patients with malignant tumors. Screening cervical cancer patients for the six markers is essential to aid in active prophylaxis, determine optimal treatment timing, and implement nursing interventions to improve prognosis, reduce venous thrombosis incidence and mortality, and prolong survival time.

Keywords Cervical cancer, Venous thromboembolism, Thrombosis markers, Thrombin-antithrombin III complex , Chemiluminescence kit development

Introduction

Cancer is one of the common causes of death in both developing and developed countries, especially in China [1]. According to GLOBOCAN, there were approximately 19,292,789 new cancer cases and 9,958,133 cancer-related deaths worldwide in 2020. In 2022, it is estimated that there will be around 110,000 new cervical cancer cases and 60,000 associated fatalities in China [2, 3]. Since Trousseau first elucidated the association between Venous Thrombosis (VTE) and cancer, a multitude of studies have substantiated the correlation between malignancies and the coagulation system. Cancer cells exhibit procoagulant properties that induce platelet aggregation, facilitate metastatic spread, and enhance tumor progression [4]. Cancer patients face a 4–7 times increased risk of developing venous thromboembolism compared to the general population [5]. VTE is a prevalent complication among individuals with malignant tumors, which can be categorized into Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). It ranks as the third leading cause of mortality worldwide [6]. The incidence of thrombosis in gynecological cancers is about 40.8%, and the literature shows that the incidence of cervical cancer-related VTE is 3% ~ 30% [7, 8]. A study on the incidence of VTE in different types of gynecological cancers showed that cervical cancer patients had the highest incidence of VTE (41.5%), this was followed by vulvar and vaginal cancer (41.4%), endometrial cancer (39.9%) and ovarian cancer (39.3%). This result was different from previous research results showing that the incidence of VTE in ovarian cancer patients was higher than that in cervical cancer patients, but these studies all confirmed that there was no statistical difference in the incidence of VTE in different types of gynecological cancer [8, 9]. In any case, cervical cancer patients are at a higher risk of VTE due to extensive vascular network at the surgical site, fasting before surgery, prolonged bed rest after surgery, elevated internal abdominal pressure and other reasons [9, 10]. The occurrence of VTE aggravates the mortality and recurrence rate of cervical cancer patients, increases the economic burden of patients, and patients are prone to psychological problems such as fear and anxiety [11, 12]. It affects the prognosis and quality of life of patients. How to screen and evaluate VTE early is crucial for timely anticoagulation therapy to improve patient prognosis and reduce the risk of death. A comprehensive approach involving clinical assessment, plasma D-dimer (DD) measurement, and imaging studies has been proposed and validated for the diagnosis of venous VTE. Since its introduction in the late twentieth century, DD testing has emerged as a crucial diagnostic tool for patients with suspected VTE [10]. Although DD testing exhibits high sensitivity, its specificity is comparatively lower, and positive results cannot consistently differentiate thromboembolic events from various other conditions that may elevate DD levels, such as malignancies, pregnancy, infections, and others [13].

As research advances in thrombosis detection, various thrombosis markers—including Thrombin-antithrombin III Complex (TAT), plasmin-α2-plasmin inhibitor complex (PIC), Thrombomodulin (TM), and Tissue Plasminogen Activator-inhibitor Complex (t-PAIC)—are employed to assess alterations in the coagulation system among clinical patients. A retrospective study conducted in Japan suggests that TAT is a valuable predictor for the onset of DVT in colorectal cancer patients post-surgery [14]. TAT represents a 1:1 complex of thrombin and antithrombin, serving as an indicator of thrombin generation within blood vessels. The activation of the coagulation system leads to thrombin production, which subsequently binds with antithrombin to form an inactive complex. The presence of TAT in circulation signifies coagulation system activation, indicating an elevated risk for thrombosis [15]. TM has been demonstrated to play a role in regulating both the coagulation and fibrinolysis systems within the human body; its plasma levels correlate with diseases associated with vascular endothelial injury [16, 17]. PIC reflects fibrinolysis system activation and exhibits greater sensitivity compared to DD and fibrin degradation products (FDP) [18]. t-PAIC indicates impairment within the fibrinolysis system and serves as an independent prognostic factor for cardiovascular mortality [19]. Notably, quantitative assessment of TAT levels in plasma holds significant diagnostic value for

bloodstream infections, deep vein thrombosis, and disseminated intravascular coagulation [20, 21].

Currently, the predominant methods for detecting TAT are Enzyme-Linked Immunosorbent Assay (ELISA) and chemiluminescence immunoassay. The chemiluminescence immunoassay is characterized by its high sensitivity and specificity, excellent reproducibility, and extensive linear range, making it the leading platform for the development of in vitro diagnostic reagents. Notably, the TAT chemiluminescence immunoassay reagent kit produced by Sysmex Corporation of Japan holds a significant share in both domestic and international markets.

This study aims to detect the TAT content in plasma utilizing a double antibody sandwich assay, establishing a method that is highly accurate, stable, repeatable, and possesses a wide linear range with a short detection time to meet clinical application requirements. Building on this foundation, we will investigate the variations in plasma concentrations of novel thrombosis markers (TAT, TM, PIC, and t-PAIC) among patients at different stages of cervical cancer. Furthermore, we will assess the diagnostic efficacy of these markers in cervical cancer patients complicated by deep vein thrombosis, thereby providing valuable insights for the detection and prevention of deep vein thrombosis in this patient population.

Materials and methods

Study population

A total of 177 patients diagnosed with cervical cancer and treated at the Affiliated Hospital of Jiangnan University in Wuxi between July 1, 2023, and October 1, 2023, were included in this study. The clinical staging of these patients was conducted according to the 2018 FIGO staging system for cervical cancer [22]. Patients who were first screened for thrombosis before surgery and diagnosed with DVT by color Doppler ultrasound or angiography and pulmonary embolism (PE) by spiral CT pulmonary angiography and MRI pulmonary angiography were grouped into the thrombosis group. Additionally, a control group consisting of 80 healthy individuals who underwent physical examinations at the hospital during the same timeframe was selected. The study protocol received approval from the Human Ethics Review Committee of the Affiliated Hospital of Jiangnan University in Wuxi, and written informed consent was obtained from either the patients or their close relatives.

The inclusion criteria are as follows: (1) All malignant tumors were confirmed by pathology and imaging; (2) complete clinical data were available; (3) no pharmacological prophylaxis for DVT was used; (4) informed consent was obtained.

The exclusion criteria are as follows: (1) recent use of anticoagulants; (2) hereditary or acquired thrombophilia; (3) a clear inflammatory complication or increased inflammatory markers after admission; (4) a history of thrombosis; (5) cardiac disease; (6) diabetes or hypertension; (7) liver disease.

Blood-sampling

A total of 2.7 mL of venous whole blood was collected from patients who fulfilled the inclusion criteria and subsequently transferred into BD vacuum blood collection tubes containing sodium citrate as an anticoagulant. The tubes were inverted and mixed thoroughly to ensure homogeneity. The blood samples were then centrifuged at 3500 rpm for 15 min with a radius of 16.4 cm to facilitate plasma separation. The resulting plasma samples were aliquoted into Eppendorf tubes and stored at −80°C for future analysis, which was completed within 24 h.

Main reagents and materials

The TAT antigen was purchased from Yawei Century Technology Co., Ltd. (Shenzhen, China), and the TAT antibody was provided by Jiangsu Baiming Biotechnology Co., Ltd. (Wuxi, China). The calibration standards, substrates, etc. were provided by Jiangsu Baiming Biotechnology Co., Ltd. (Wuxi, China). The chemiluminescence immunoassay instrument was purchased from AnTu Experimental Instrument Co., Ltd. (Zhengzhou, China). The pH meter was purchased from Mettler-Toledo (Switzerland).

Preparation of biotinized antibodies

Dissolve 1 mg of coated antibody and replace the buffer in the desalting column with a phosphate buffer, collecting approximately 1 mL. Subsequently, add 1 mg of activated biotin to the 1 mL phosphate buffer, then introduce 100 μL of this solution into the antibody mixture and thoroughly mix at room temperature for one hour. Transfer the Biotin-conjugated antibody to the desalting column for purification. Finally, measure the absorbance of the Biotin-antibody conjugate at 280 nm and calculate a protein recovery rate of 94%.

Preparation of horseradish peroxidase (HRP) conjugated antibody

Weigh out horseradish peroxidase (HRP) and dissolve it in deionized water. Subsequently, add a 0.1 mol/L sodium periodate ($NaIO_4$) solution and stir the mixture at room temperature in the dark for 20 min. Following this, transfer the reaction mixture to a refrigerator set at 4°C and dialyze overnight using a sodium acetate solution (1 mmol/L, pH = 4.4) to eliminate impurities. Next, incorporate 4 μL of glycerol and incubate the solution at room temperature in the dark for an additional 30 min. Introduce TAT-labeled antibody into the conjugate and

perform dialysis overnight at 4°C in a carbonate buffer (0.2 mol/L, pH = 9.5) to further remove contaminants. Add 0.1 mL of sodium borohydride ($NaBH_4$) to the conjugate and mix thoroughly before incubating it at 4°C in darkness for two hours. Stir equal volumes of saturated ammonium sulfate into the mixture and continue incubation at 4°C for one hour more. Centrifuge at 5000 rpm for fifteen minutes, discarding the supernatant afterward. Resuspend the precipitate in phosphate-buffered saline (PBS), then add equal volumes of glycerol before storing it at −20°C.

Experimental process

The TAT, PIC, TM, and t-PAIC contents in the sample were detected by a chemiluminescence double antibody sandwich method (Fig. 1). After adding 30 μL of the sample to the microplate precoated with streptavidin and biotin-conjugated bovine serum albumin, 80 μL of biotin-conjugated antibody was added, and the plate was shaken for 2 min and incubated at 37℃ for 10 min. The plate was then washed three times. The antibody-HRP enzyme-labeled antibody was added, and the plate was incubated at 37℃ for 10 min again. The TAT in the sample will form an antibody-antigen–antibody HRP enzyme complex with the enzyme-labeled antibody. The unbound reactants on the solid phase were washed away, and the chemiluminescence substrate was added. The chemiluminescence substrate emits light under the catalytic action of the HRP enzyme, and the Relative Light Units(RLU) is read using a chemiluminescence immunoassay instrument. Finally, the concentration of TAT, PIC, TM, and t-PAIC in the sample is calculated using the calibration curve.

Data analysis

Data were analyzed using SPSS 25.0. Continuous variables with normal distribution were expressed as mean ± standard deviation, while those with non-normal distribution were expressed as median [quartile (P25, P75)]. Binary logistic regression was used to analyze the relationship between t-PAIC, TAT, PIC, TM, DD, FDP, and VTE. The diagnostic efficiency was evaluated using the receiver operating characteristic (ROC) curve, and the maximum Youden index was used as the critical value. $P < 0.05$ indicates statistically significant differences.

Result

Optimization of TAT antibody working concentration

A chessboard method experiment was conducted using biotin-labeled antibody and HRP-labeled antibody, and different antibody concentrations were selected for detection. The RLU values of 6 standard substances were determined, as shown in Supplementary Table 1. When the concentration of TAT-Biotin was 1 μg/mL and the concentration of TAT-HRP was 1.5 μg/mL, the signal values of the calibration standards were appropriate and the ratios were close. Under the same conditions, a lower antibody concentration was chosen to reduce costs, and this combination was selected as the working concentration of the antibodies.

Optimum incubation time

The duration of incubation significantly influences the relative light units (RLU); therefore, varying incubation times were selected for standard samples 1–5, as well as low-value and high-value plasma samples. The results are illustrated in Fig. 2 and Supplementary Table 2. Notably,

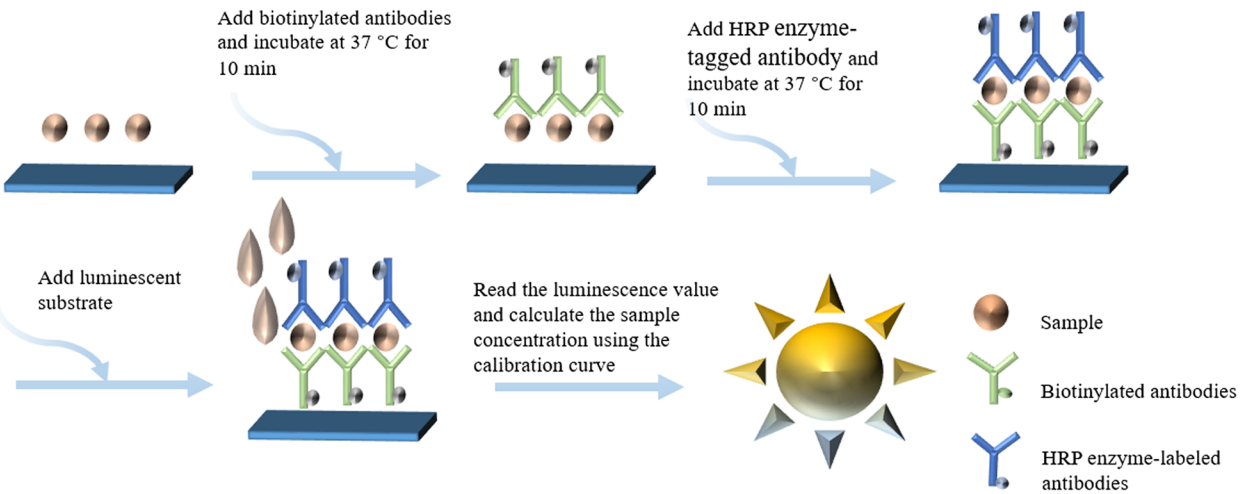

Fig. 1 Chemiluminescence double antibody sandwich method

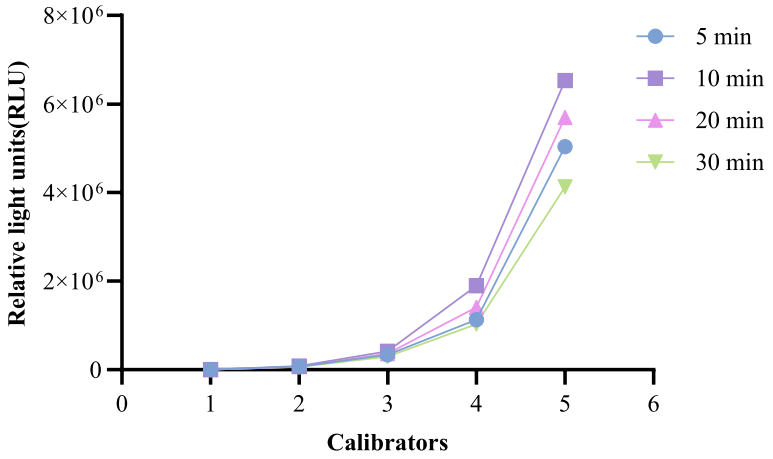

Fig. 2 Influence of the 37℃ incubation time

Table 1 Verification results of the limit of detection

Indicators	Test1	Test2	Test3
M	5459.8	5460.8	5461.8
SD	338.58	289.72	312.35
M + 2SD	6164.16	6265.44	5978.70
LOD (ng/mL)	0.045	0.048	0.039

Abbreviation: M Mean, *SD* Standard deviation, *LOD* Limit of detection

at an incubation period of 10 min, the signal values for all calibration standards were generally elevated. Opting for a shorter incubation time can substantially decrease the overall testing duration and enhance detection throughput; consequently, the reaction conditions of the kit were established at 37°C for a duration of 10 min.

Method performance evaluation

The performance of the method is assessed in accordance with the EP guidelines established by the Clinical and Laboratory Standards Institute (CLSI).

Limit of detection

The mean and standard deviation of RLU of 20 standard dilutions were calculated, and M + 2SD was brought into the standard curve to obtain the concentration values of 0.045 ng/mL, 0.048 ng/mL, 0.039 ng/mL, respectively, so the lowest detection limit of the method was 0.048 ng/mL, which is shown in Table 1.

Linearity

The high-value samples close to the upper limit of the linear range were diluted into a number of concentrations in a certain proportion, of which the low-value concentration samples had to be close to the lower limit of the linear range. The results of linear fitting by least squares method with the calibration product concentration as the horizontal coordinate and the mean value of RLU as the vertical coordinate (Fig. 3) are as follows, which shows that there is a good linear relationship between the theoretical concentration of the samples and the actual concentration, and the linear regression equation after the fitting is $y = 0.9914x\text{-}0.6358$, $R = 0.9995$.

Accuracy

The assay was repeated three times for the high-value QC and low-value QC, respectively, and the average value of the measured concentration results was recorded as (M), and the relative deviation (Bi) was calculated according to Bi(%) = (M-T)/T*100%, respectively, where Bi is the relative deviation, M is the average value of the measured concentration, and T is the theoretical concentration, and the results were shown in Supplementary Table 3, and the relative deviations were all within 5%.

Precision

Prepare three batches of reagents and conduct the detection of high-value and low-value control items ten times for each batch. Calculate the mean value (M) and standard deviation (SD) of the results, then determine the coefficient of variation (CV) using the formula CV(%) = (SD/M) * 100%. Here, CV represents the coefficient of variation, M denotes the mean concentration value, and SD indicates the standard deviation. The results are presented in Supplementary Table 4, with a precision threshold of 3% or lower.

Acceleration stability

Five batches of reagents were incubated at 37°C, with samples collected on days 1, 4, and 7 for analysis. The

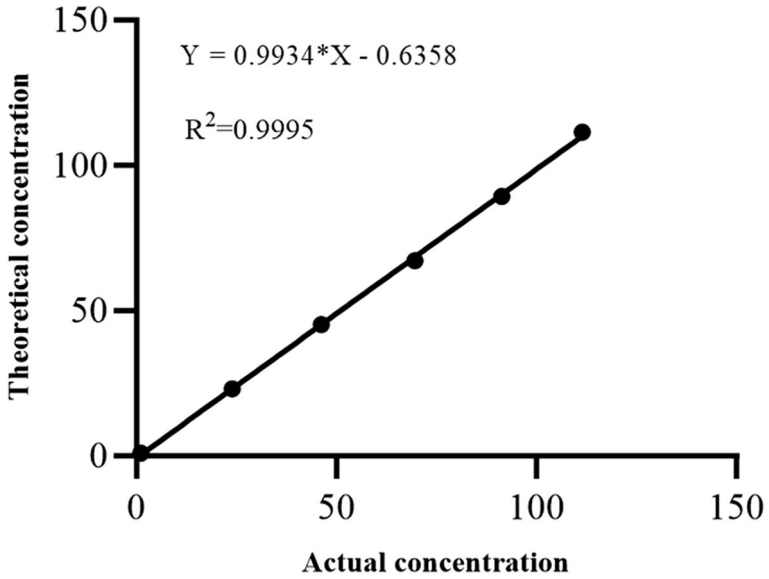

Fig. 3 Linear testing of TAT reagents

performance metrics of the reagents stored at 4°C were compared to those subjected to incubation. The results presented in Table 2 demonstrate that the deviation of the reagents maintained at 37°C over varying durations from those kept at 4°C is less than 10%, indicating that the method exhibits commendable stability.

Clinical evaluation

The HISCL-800 kit from Sysmex (Japan) was used to test 130 clinical plasma samples. The analysis resulted in a correlation coefficient of $R^2 = 0.9683$, indicating excellent agreement between the two assays (Fig. 4).

General features

A total of 177 patients diagnosed with cervical cancer were included in the study, comprising 42 individuals in Stage I, 45 in Stage II, 62 in Stage III, and 28 in Stage IV. The mean age of the thrombosis group was recorded at 52.37 ± 11.35 years, whereas the non-thrombosis group had a mean age of 55.27 ± 10.24 years. Additional demographic information is presented in Supplementary Table 5.

Characteristics and risk factors of thrombosis in patients with cervical cancer

Supplementary Table 6 presents an overview of the incidence of thrombosis in patients with cervical cancer. Among the cohort of 177 cervical cancer patients, 27 were diagnosed with VTE. Within this group, four patients were identified as having PE, one patient exhibited both DVT and PE, while no fatalities were reported; all instances of PE were non-fatal. Specifically, ten out of the twenty-seven patients experienced VTE in the left leg, eleven in the right leg, and six had bilateral involvement. Two cases involved proximal DVT while sixteen presented with distal DVT. Notably, all VTE occurrences were asymptomatic. To further elucidate the risk factors associated with thrombosis among cervical cancer

Table 2 Accelerated stability test results

	S1		S2		S3		S4		S5	
	test1	tes2	test1	tes2	test1	tes2	test1	tes2	test1	tes2
4°1Day	5823	5844	80097	82289	394211	403,916	1804187	1823406	6418172	6429542
37°1Day	5733	5667	78976	78758	388867	388,245	1784274	1733298	6112683	6071570
37°4Day	5625	5634	79749	78411	384194	385,240	1775541	1792015	6256376	6103575
37°7Day	5559	5588	77185	77160	375501	376844	1692983	1752580	6021054	6060396
Day 1 decline	−2%	−3%	−1%	−4%	−1%	−4%	−1%	−5%	−5%	−6%
Day 2 decline	−3%	−4%	0%	−5%	−3%	−5%	−2%	−2%	−3%	−5%
Day 3 decline	−5%	−4%	−4%	−6%	−5%	−7%	−6%	−4%	−6%	−6%

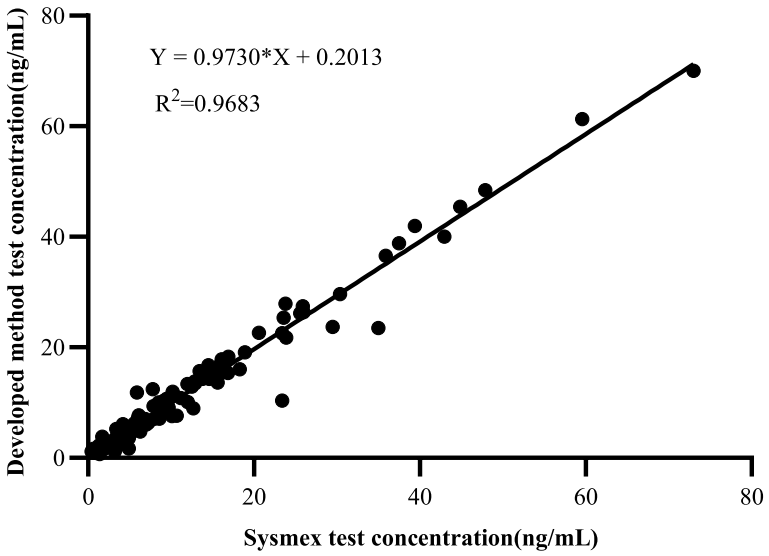

Fig. 4 Comparison of blood sample correlations

patients, this study analyzed case histories from recruited individuals to assess relevant variables. The findings showed that patients with tumors larger than 50 mm who

Table 3 Clinical characteristics of the VTE and non-VTE groups

Preoperative risk factors	Thrombosis group *n* = 9	Non-thrombosis group *n* = 150	t/χ^2	*P*-value
Age[a], yeas, mean ± SD (range)	52.37 ± 11.35 (33–77)	55.27 ± 10.24 (29–83)	−0.448	0.655
BMI[b](kg/m^2) (range)	24.656 ± 5.09 (13.1–31.8)	24.299 ± 4.96 (13.1–34.1)	−0.375	0.707
Smoke or not[b], n(%)	2(22.22%)	15(10%)	1.328	0.247
FIGO stage[b], n(%)			1.872	0.596
I	1(11.11%)	38(25.33%)		
II	2(22.22%)	43(28.67%)		
III	3(33.33%)	41(27.33%)		
IV	3(33.33%)	28(18.67%)		
Adjuvant chemotherapy[b], n(%)	5(55.56%)	25(16.67%)	7.025	**0.019**
Pathological pattern[b], n(%)			1.910	0.403
Squamous cell	6(66.67%)	121(80.67%)		
Adenocarcinoma	2(22.22%)	22(14.67%)		
Adenosquamous	1(11.11%)	6(4%)		
Other	0(0%)	1(0.67%)		
Cervical cancer tumor size[b], n(%)			6.920	**0.016**
≥ 50 mm	6(66.67%)	39(26%)		
< 50 mm	3(33.33%)	111(74%)		

Significant *P*-values are indicated in bold print

Abbreviation: BMI Body mass index, *FIGO* Federation International of Gynecology and Obstetrics, *VTE* Venous thromboembolism, n Number of patients

[a] t-test

[b] Fisher exaet test

received adjuvant chemotherapy had an increased risk of thrombosis. For additional details refer to Table 3.

Comparison of t-PAIC, TAT, TM, and PIC between healthy control and cervical cancer groups

As shown in Table 4, we found that TAT and PIC were significantly higher than those of the control group, and the difference was statistically significant ($P<0.001$ or $P<0.05$).

Comparison of t-PAIC, TAT, PIC, TM, D-D, and FDP between thrombus and no thrombus groups of cervical cancer patients

As shown in Table 5, the TAT and FDP of the thrombosis group were significantly higher than those of the non-thrombosis group, with statistical significance ($P<0.001$), while the other few items did not have statistical significance.

Comparison of t-PAIC, TAT, PIC, TM, D-D, and FDP in patients with stage I-II versus stage III-IV cervical cancer

As shown in Table 6, there was no significant difference ($P>0.05$) in t-PAIC, TM, and PIC concentrations between the two groups; however, there was a significant difference ($P<0.05$) in TAT concentration, with the TAT concentration of the advanced group being significantly higher than that of the early group.

Evaluation of the value of each marker in the diagnosis of VTE in patients with cervical cancer using receiver operating characteristic (ROC) curve analysis

When diagnosing VTE, the AUCs of t-PAIC, TM, TAT, PIC, D-Dimer, and FDP were 0.670, 0.689, 0.791, 0.710, 0.700, and 0.718, respectively. The critical values of t-PAIC, TM, TAT, PIC, D-Dimer, and FDP were 10.580 ng/mL, 11.624 TU/mL, 15.627 ng/mL, 0.822 ng/mL, 0.515 mg/L, and 5.000 mg/L, respectively. TAT has

Table 4 Comparison of thrombotic markers between healthy individuals and cervical cancer patients

Thrombosis markers	Control Group ($n=80$)	Cervical cancer group ($n=177$)	Z Value	*P* Value
t-PAIC (ng/mL)	4.49 (3.04 ~ 7.21)	5.82 (4.07 ~ 7.94)	−2.508	0.012
TAT (ng/mL)	3.27 (2.65 ~ 3.91)	10.43 (6.61 ~ 13.87)	−11.251	< 0.001
TM (TU/mL)	6.27 (4.49 ~ 8.44)	5.57 (4.02 ~ 10.92)	−0.177	0.860
PIC (ng/mL)	0.32 (0.20 ~ 0.50)	0.70 (0.44 ~ 0.95)	−7.839	< 0.001

Abbreviation: VTE Stomach cancer, *t-PAIC* Tissue plasminogen activator inhibitor complex, *TAT* Thrombin-antithrombin complex, *TM* Thrombomodulin, *PIC* α2-plamininhibitor-plasmin complex

* $P<0.05$ considered statistically significant

Table 5 Comparison of thrombosis markers between two groups of patients

Thrombosis markers	Thrombosis group ($n=27$)	Non-thrombosis group ($n=150$)	Z Value	*P* Value
t-PAIC (ng/mL)	11.29 (4.10 ~ 13.72)	5.63 (4.04 ~ 7.27)	−2.811	0.005
TAT (ng/mL)	19.07 (13.61 ~ 31.84)	9.43 (6.36 ~ 12.64)	−4.806	< 0.001
TM (TU/mL)	14.33 (4.14 ~ 15.44)	5.22 (3.96 ~ 7.93)	−3.117	0.002
PIC (ng/mL)	0.94 (0.82 ~ 1.26)	0.68 (0.43 ~ 0.92)	−3.466	0.001
FDP (mg/L)	6.30 (2.80 ~ 7.10)	2.65 (1.80 ~ 4.30)	−3.604	< 0.001
D-D (mg/L)	0.70 (0.53 ~ 1.36)	0.40 (0.26 ~ 0.48)	−3.303	0.001

Table 6 Comparison of thrombosis markers in patients with early and advanced cervical cancer (x ± s)

Thrombosis markers	Stage I-II ($n=87$)	Stage III-IV ($n=90$)	Z Value	*P* Value
t-PAIC (ng/mL)	5.38 (3.93 ~ 7.05)	6.50 (4.10 ~ 10.36)	−2.033	0.042
TAT (ng/mL)	8.77 (5.94 ~ 11.71)	11.32 (8.06 ~ 17.18)	−3.531	< 0.001
TM (TU/mL)	4.81 (3.79 ~ 7.41)	7.30 (4.20 ~ 14.59)	−3.565	0.001
PIC (ng/mL)	0.65 (0.42 ~ 0.92)	0.80 (0.47 ~ 0.99)	−2.099	0.036
FDP (mg/L)	2.30 (1.70 ~ 4.50)	3.30 (2.20 ~ 5.25)	−2.429	0.015
D-D (mg/L)	0.38 (0.23 ~ 0.49)	0.45 (0.31 ~ 0.74)	−2.043	0.041

good diagnostic value for VTE, with a sensitivity of 74.1% and a specificity of 91.3%. The AUC of t-PAIC was 0.670, and its diagnostic efficiency was the lowest (Supplementary Table 7, Fig. 5-A). When the six markers were combined, the AUCs of "t-PAIC + TM + TAT + PIC" and "t-PAIC + TM + TAT + PIC + D-D + FDP" were significantly higher than the AUC of "D-D + FDP". The AUC, sensitivity, and specificity of "D-D + FDP" were 0.688, 51.9%, and 88.7%, respectively, while the AUC, sensitivity, and specificity of "t-PAIC + TM + TAT + PIC" were 0.894, 81.5%, and 91.3%. The AUC, sensitivity, and specificity of "t-PAIC + TM + TAT + PIC + D-D + FDP" were 0.912, 85.2%, and 88.7% (Supplementary Table 8, Fig. 5-B).

Discussion

This study included a total of 177 patients diagnosed with cervical cancer, among whom 27 exhibited thrombosis. Analysis of influencing factors showed that patients with tumors larger than 50 mm and receiving chemotherapy were at a higher risk of thrombosis. These findings indicate that healthcare professionals should implement targeted health education and thrombosis prevention strategies for these patients. Thrombosis is a multifaceted process influenced by various factors associated with the coagulation system, fibrinolysis system, and endothelial function, among others [23]. Compared to healthy individuals, cancer patients exhibit an elevated risk of thrombosis development. This study demonstrates that cervical cancer patients present significantly higher levels of TAT, TM, and PIC in their plasma compared to healthy controls. Previous studies have demonstrated that TM, PIC, TAT, and t-PAIC can respond to thrombosis arising from the coagulation system, fibrinolysis system, and endothelial system while also serving as prognostic indicators for patients [24]. Tumor cells continuously stimulate the synthesis of substantial amounts of thrombin via tissue factor; TAT is a sensitive complex formed by thrombin and its inhibitor, representing an early marker of coagulation activation. An increase in a patient's TAT level by 1 ng/mL correlates with a 1.66-fold elevation in the risk of hypercoagulability [25]. Numerous studies have demonstrated that plasma TAT levels can significantly increase prior to the formation of a blood clot, indicating its potential clinical utility for the early diagnosis of thrombotic lesions, guiding anticoagulation treatment timing, and facilitating re-thrombosis detection [26]. The findings from this study reveal that plasma TAT levels in cervical cancer patients with thrombosis are elevated

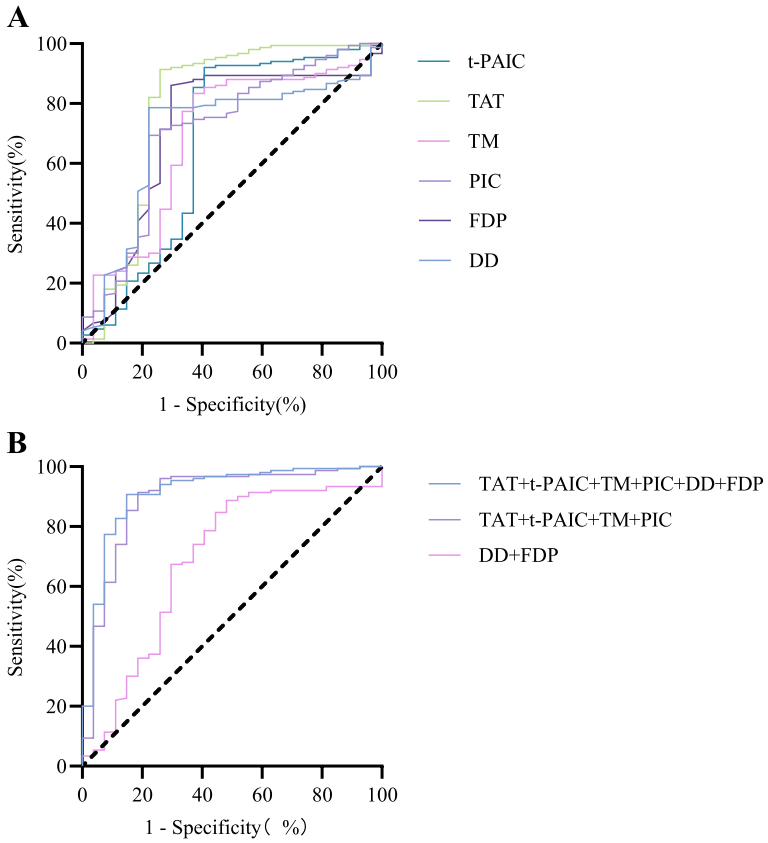

Fig.5 **A** Single-item ROC detection. **B** Combined ROC detection

compared to those without thrombosis, suggesting a role for TAT in the thrombotic processes associated with cervical cancer. Thrombomodulin (TM), as the receptor for thrombin on endothelial cells, inhibits thrombin activity while enhancing protein C activation, thereby serving as a sensitive biomarker for assessing vascular endothelial injury severity [27]. This study indicates that plasma TM levels in patients with stage III-IV cervical cancer are significantly higher than those in stage I-II patients, implying an increased susceptibility to vascular endothelial injury among late-stage cervical cancer patients which correlates with elevated TM levels. Some researchers have indicated that combining blood clot markers with D-dimer testing may yield a more precise evaluation of coagulation function in cancer patients and reflect their hypercoagulable state at an earlier stage [24]. The analysis of ROC curves for plasma t-PAIC, TM, TAT, and PIC in diagnosing venous thrombosis among cervical cancer patients reveals AUC values of 0.670, 0.689, 0.791, and 0.710 respectively. Notably, the combined diagnostic AUC for these four biomarkers is 0.894. The sensitivity and specificity associated with this combination surpass those observed with individual markers, indicating that while single-item indicators possess clinical relevance in diagnosing venous thrombosis in cervical cancer patients, the integration of all four significantly enhances diagnostic performance metrics such as AUC, sensitivity, and specificity. These biomarkers serve as initial or intermediate products within the venous thrombosis pathway and provide a multifaceted perspective on hemostasis by reflecting aspects of coagulation processes, fibrinolysis mechanisms, and endothelial function; thus they can be utilized as reference indices for early prediction of venous thromboembolism in this patient population. Furthermore, their application aids in timely identification of high-risk cervical cancer patients predisposed to thrombotic events and facilitates targeted interventions aimed at improving overall prognosis.

This study also developed a TAT assay kit that employs a chemiluminescence method for quantifying TAT levels in human plasma. Performance validation analysis demonstrated that the kit exhibits robust performance and overall stability, showing strong correlation with Sysmex's methodology. The kit was utilized in clinical research employing the developed technique, yielding detection results consistent with patient conditions. It fulfills clinical requirements effectively. Furthermore, this study implemented an automated chemiluminescence approach to measure t-PAIC, TM, TAT, and PIC levels in plasma while assessing the diagnostic value of each biomarker in cervical cancer patients experiencing venous thrombosis. The method facilitates convenient sample collection, necessitates only a minimal sample volume of 30 μL of venous plasma per test, and provides results within 20 min. This approach is suitable for large-scale rapid testing in laboratory settings. The performance characteristics of the method established herein are comparable to those of Sysmex's system; it meets precision and accuracy standards required by clinical laboratories while offering lower costs and reduced instrument footprint-effectively addressing bedside testing needs within clinical departments.

Related studies have indicated that surgery is the primary treatment modality for most localized tumors and serves as an additional risk factor for thrombosis in patients. This study did not examine the variations in thrombosis incidence associated with different surgical techniques. Future research should consider expanding the sample size to investigate the likelihood and implications of thrombosis in cervical cancer patients resulting from various surgical approaches, which could inform clinical nursing interventions tailored to patients undergoing different surgical procedures.

Conclusion

A TAT chemiluminescence immunoassay detection method was established in this study, which showed a high correlation ($R=0.9995$) with the measured value of the TAT test kit from Sysmex. The lowest detection limit was 0.048 ng/mL. Clinical performance validation demonstrated an accuracy with a relative deviation within 5%, precision within 3%, and an accelerated stability deviation of less than 10%, meeting the standards for clinical application. The TAT test can now be conducted at the bedside, thanks to the small footprint of the kit developed in this study. This makes it suitable for use in oncology departments, chest pain centers, ICUs, and other departments. By avoiding the need to send the test to the hospital, doctors can quickly determine a patient's condition and save valuable time. Ultimately, this benefits patients by providing them with more time for treatment.

The fluctuations in the TAT index serve as more reliable predictors of thromboembolic risk in patients with cervical cancer compared to other indicators. Furthermore, a combined diagnostic approach utilizing t-PAIC, TM, TAT, and PIC demonstrates superior efficacy over individual tests. By analyzing changes in thrombosis markers, it becomes feasible to conduct early screening for cervical cancer patients at elevated risk of venous thrombosis, thereby emphasizing prevention and treatment strategies that enable patients to proactively utilize prophylactic medications and identify optimal timing for therapeutic interventions.

Future research should explore the feasibility of TAT detection in whole blood samples, utilizing TAT indicators to assess thrombosis risk in patients and

formulate long-term monitoring and care strategies. This endeavor may necessitate interdisciplinary collaboration between diagnostic testing and patient management, with the objective of enhancing the overall medical experience for patients.

Abbreviations

VTE	Venous thromboembolism
DVT	Deep vein thrombosis
PE	Pulmonary embolism
CAT	Cancer-associated thrombosis
t-PAIC	Tissue plasminogen activator inhibitor complex
TAT	Thrombin-antithrombin III complex
PIC	Plasmin-α2-plasmin inhibitor complex
TM	Thrombomodulin
D-D	D-Dimer
FDP	Fibrin degradation products
RLU	Relative light units
CLSI	Clinical and Laboratory Standards Institute
M	Mean
SD	Standard deviation
LOD	Limit of detection
QCs	Quality controls
CV	Coefficient of variation
ROC	Receiver operating characteristic
AUC	Area under the curve

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Conceptualization and experimental design—ZXL, XH; Methodology, ZXL, XH, SH, YRQ, YJ; Sample collection and experimentation—YRQ, YJ; Data processing and analysis—SH, YRQ, YJ; Writing—preparation of the original manuscript, YRQ, YJ; Writing—review and editing, ZXL, XH, SH; Project management, ZXL, XH; Supervision, ZXL, XH, SH; Funding acquisition, XH, SH.

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

Declarations

Ethics approval and consent to participate
All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki. The study was approved by Medical Ethics Committee of The Affiliated Hospital of Jiangnan University (Wuxi, Jiangsu, China) (No. LS2023059), and written informed consent was obtained from all participants before enrolment in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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