RESEARCH ARTICLE

Diagnostic value of plasmin-α2-plasmin inhibitor complex in patients with malignant tumor and venous thromboembolism

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Abstract

Objective: We aimed to evaluate in this study the diagnostic value of the plasmin-α2-plasmin inhibitor complex in patients with malignant tumors and venous thromboembolism (VTE).

Methods: A total of 58 patients with confirmed malignant tumors and VTE were selected, and their plasma samples were collected within 24 hours after VTE diagnosis. We also selected 60 patients with malignant tumors who were hospitalized at the same time and did not have VTE following imaging examination. Their plasma samples were collected within 24 hours after admission and were compared to those of the VTE group concerning the levels of plasmin-α2-plasmin inhibitor (PIC), thrombin-antithrombin complex (TAT), tissue-type plasminogen activator-inhibitor complex (tPAI-C), and thrombomodulin (TM). We used the receiver operator characteristic (ROC) curve to evaluate the diagnostic efficacy of each index regarding malignant tumors accompanied by VTE.

Results: PIC, TAT, and tPAI-C were significantly higher in the group with malignant tumors and VTE compared to the group with malignant tumors without thrombosis ($p = 0.010$, $p = 0.001$, and $p = 0.003$, respectively). In contrast, we found no significant difference in TM levels between the two groups ($p = 0.483$). The area under the curve (AUC) of PIC, TAT, and tPAI-C regarding patients with malignant tumors and VTE was 0.852, 0.636, and 0.655, respectively, demonstrating diagnostic values for those cancer patients suffering VTE. PIC had the highest diagnostic efficiency in those patients with malignant tumors and VTE, while the AUC of TM was 0.537, so its diagnostic value for VTE-complicated malignant tumors was limited.

Conclusion: PIC has a sufficient value for the early diagnosis of VTE in patients with malignant tumors.
HIPPOKRATIA 2023, 27 (2):37-40.

Keywords: Venous thromboembolism, malignant tumors, plasmin-α2-plasmin inhibitor complex, thrombin-antithrombin complex, tissue-type plasminogen activator-inhibitor complexes, thrombomodulin

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Introduction

Venous thromboembolism (VTE) includes deep vein thrombosis (DVT) and pulmonary embolism (PE). It is a common complication in cancer patients and is often associated with poor prognosis[1]. The leading causes of VTE include damage to blood vessel walls, slow blood flow, and hypercoagulability. VTE can significantly affect patients' quality of life and increase mortality[2]. Recent studies showed the annual VTE incidence rate in cancer patients to be 0.5 %, compared to 0.1 % in the general population[3]. Untreated or residual cancer account for 20 % of VTE incidences[4]. VTE is the second leading cancer-related cause of death, after cancer itself[5]. The causes include leukocytosis, increased platelets, raised tissue factors, phospholipids, and inflammatory markers; this change sequence influences patients suffering from malignant tumors, provoking a state of susceptibility to thrombosis[2,6]. Without effective prevention, the incidence of DVT, especially proximal DVT, is as high as 40-80 % and 10-20 %, respectively, in patients undergoing surgery for tumors. The incidence of PE is 4-10 %, and the incidence of fatal PE is 1-5 %[2]. The clinical manifestations of VTE in malignant tumor patients include limb swelling, pain, tenderness in the back of the calf or inside the thigh, etc. However, many patients have atypical symptoms and are prone to misdiagnosis and missed diagnosis. When clinically suspicious VTE symptoms appear, D-dimer is the first choice to exclude or confirm the diagnosis, while in uncertainty, imaging is performed to facilitate differential diagnosis. However, D-dimer elevation frequently occurs in oncologic patients, so it has limited diagnostic value in excluding VTE in the presence of tumor[7]. Currently, the gold standard for VTE diagnosis is performing imaging[8]. However, it is often passively detected when there is clinical suspicion of VTE, which is of limited contribution to the early VTE diagnosis. Furthermore, it is more expensive and inconvenient for patients with mobility difficulties. Therefore, there is an imperative

need for more convenient, noninvasive, and economical diagnostic indicators for early detection and diagnosis of tumor-related VTE and in-time treatment.

Recently, new molecular markers for vascular endothelial injury, blood hypercoagulability, and fibrinolytic activity, including plasmin-α2-plasmin inhibitor (PIC), thrombin-antithrombin complex (TAT), tissue-type plasminogen activator-inhibitor complex (tPAI-C), and thrombomodulin (TM), have become more convenient and automated for clinical detection. Previous studies have analyzed the value of these four markers in the early diagnosis of sepsis-induced coagulopathy and disseminated intravascular coagulation[9,10]. Still, there are few studies on their value in diagnosing patients with tumors complicated with thrombosis. The purpose of this study was to compare TAT, PIC, tPAI-C, and TM within 24 hours of admission in malignant tumor patients without VTE and within 24 hours of VTE diagnosis in malignant tumor patients complicated with VTE to assist in early diagnosis and treatment of VTE in patients with malignant tumors.

Materials and Methods

We conducted a single-center prospective study in Gansu Provincial Hospital from 01/01/2022 to 29/07/2022. Fifty-eight consecutive patients with confirmed malignant tumors and VTE were prospectively enrolled as the study group, and 60 patients with malignant tumors admitted during the same period who underwent imaging examination to exclude VTE were selected as the control group. The inclusion criteria were initial diagnosis of malignancy by imaging, confirmed by pathology, and not receiving anticoagulant medication for ten days before admission. Exclusion criteria were coinfection or previous thrombotic disease.

The study was approved by the Medical Ethics Committee of Gansu Provincial Hospital (decision No 2021-339, date: 25/01/2022), and informed consent was obtained from all patients.

Specimen processing

In total, 1.8 mL of fasting venous blood with 3.2 % sodium citrate anticoagulant was collected from the control group within 24 hours after admission and from the study group within 24 hours after VTE diagnosis; the samples were delivered to the laboratory within one hour, centrifuged with a centrifugal force of 3,000g for ten minutes, the upper layer of plasma with deficient platelets was taken, and the test was completed within two hours. The TAT, PIC, tPAI-C, and TM levels were detected by the Sysmex HISCL5000 chemiluminescence immunoassay analyzer and its matching reagents (HISCL5000, Sysmex, Japan).

Statistical analysis

The sample size was calculated based on an area under the curve (AUC) of 0.852 for the PIC in the diagnosed malignant tumor in the study group. With a type I error of 0.05, the allowable error δ was 0.1, and the specificity was 0.917; the calculated sample size was 30.

We tested recorded data for normality using the Shapiro-Wilk test, and the data with non-normal distribution are reported as the median, and first and third quartile (Q1, Q3) in brackets. We utilized the Mann-Whitney test to compare the groups and the receiver operator characteristic (ROC) curve to evaluate the diagnostic efficacy of novel thrombus molecular markers for tumors accompanied by VTE. A p-value of <0.05 was considered statistically significant.

Results

Basic clinical features of the two groups

We included in the study 58 patients (33 males, 25 females) diagnosed with malignant tumors complicated and VTE, with a median age of 65 (57.75, 68) years. During the same period, sixty patients (36 males, 24 females) were hospitalized with malignant tumors and no VTE after imaging, with a median age of 64 (56.25, 67) years.

TAT, PIC, tPAI-C, TM level analysis of the two groups

We analyzed the levels of four indexes between the two groups (Table 1). The levels of TAT, PIC, tPAI-C, and TM in the study group were 6.20 (2.8, 8.20) μg/L0.99 (0.65, 1.47) mg/L10.00 (6.43, 16.90) μg/L, and 8.00 (6.38, 10.53) kU/L, respectively, which were higher than those in the control group, and there were significant differences between the two groups (z values were -2.567, -6.655, and -2.921; p values were 0.010, 0.001, and 0.003, respectively). There was no marked difference in TM level between the two groups (p =0.483).

Diagnostic value of TAT, PIC, tPAI-C, and TM in malignant tumor patients with VTE

According to the ROC curve analysis, the AUC of PIC in the study group was 0.852, the diagnostic value was the highest, and the maximum value of the Youden index was 0.566. The sensitivity and specificity of PIC in the study group were 73.3 % and 83.3 %, respectively, which were higher than those of other indexes. The AUC of TAT and tPAI-C were 0.636 and 0.655, respectively, with specific diagnostic significance for malignant tumor patients with VTE. The AUC of TM was 0.537, which is of low diagnostic value for malignant tumors with VTE (Figure 1, Table 2).

Discussion

DVT and PE are collectively known as VTE[1]. To establish the diagnosis of DVT in the lower limbs, regardless of whether the clinical manifestations are typical or not, further laboratory and imaging tests are required to confirm the diagnosis and avoid missed diagnosis and misdiagnosis[11]. In recent years, clinicians have increasingly recognized the correlation between cancer and thrombosis, and many autopsy studies have confirmed the increased incidence of thromboembolic death in cancers (including pancreatic mucous cancer, lung cancer,

Table 1: Comparison of plasmin-α2-plasmin inhibitor, thrombin-antithrombin complex, tissue-type plasminogen activator-inhibitor complex, and thrombomodulin levels between a study group of 58 malignant tumor patients with venous thromboembolism (VTE) and a control group of 60 malignant tumor patients without VTE hospitalized at the same time.

Index	Control Group	Observation Group	z value	p-value
Age (year)	64 (56.25, 67.00)	65 (57.75, 68.00)	-1.333	0.182
Male/Female	36/24	33/25	-0.186	0.852
TAT (μg/L)	3.60 (2.18, 6.28)	6.20（2.8, 8.20）	-2.567	0.010*
PIC (mg/L)	0.51 (0.41, 0.64)	0.99 (0.65, 1.47)	-6.655	0.001*
tPAI-C (μg/L)	7.40 (5.23, 11.65)	10.00 (6.43, 16.90)	-2.921	0.003*
TM (kU/L)	8.15 (6.43, 9.58)	8.00 (6.38, 10.53)	-0.701	0.483

Values are reported as the median, and first and third quartile in brackets or number. TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAI-C: tissue plasminogen activator-inhibitor complex, TM: thrombomodulin, *: statistically significant.

Table 2: Evaluation of diagnostic efficacy of plasmin-α2-plasmin inhibitor, thrombin-antithrombin complex, tissue-type plasminogen activator-inhibitor complex, and thrombomodulin levels in patients with malignant tumors complicated with venous thromboembolism.

Index	AUC	SE	p-value	95 % CI	
				upper limit	lower limit
TAT	0.636	0.051	0.010*	0.536	0.736
PIC	0.852	0.034	0.001*	0.785	0.919
tPAI-C	0.655	0.050	0.003*	0.557	0.752
TM	0.537	0.053	0.483	0.433	0.641

AUC: area under the curve, SE: Standard Error, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, tPAI-C: tissue plasminogen activator-inhibitor complex, TM: thrombomodulin, *: statistically significant.

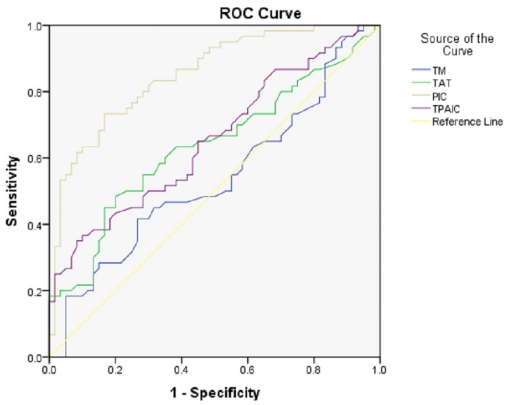

Figure 1: Receiver operator characteristic curve analysis of each index. Thrombin-antithrombin complex: area under the curve (AUC) =0.636, plasmin-α2-plasmin inhibitor complex: AUC =0.852, tissue plasminogen activator-inhibitor complex: AUC =0.655, thrombomodulin: AUC =0.537.

ROC: receiver operator characteristic, TM: thrombomodulin, TAT: thrombin-antithrombin complex, PIC: plasmin-α2-plasmin inhibitor complex, TPAIC: tissue plasminogen activator-inhibitor complex.

and gastrointestinal tumors)[12,13]. More and more studies have shown that cancer patients are in a state of persistent subclinical hypercoagulation, the process of fibrin formation and clearance being parallel to the malignant tumor advancement, and fibrin and other clotting products are essential components of thrombosis and tumor progression[14]. Therefore, early diagnosis of tumor-related VTE is critical for timely treatment, improved prognosis, and quality of life of cancer patients. The process of thrombosis and degradation results from a combination of factors related to the coagulation system, the fibrinolytic system, and the endothelial system. Multiple markers can evaluate this process. Much literature recommends the use of D-dimer to exclude thrombosis[9]. However, D-dimer is a product of plasmin degradation after the production of fibrin clots, and the time of the formation is relatively late, which has no advantage for early diagnosis of thrombosis. This study selected TAT, PIC, tPAI-C, and TM to analyze their value in the early diagnosis of tumors associated with VTE formation. TAT can respond sensitively to thrombin production and, thus, can be utilized to predict the early activation of the coagulation cascade. PIC is a sensitive marker to evaluate fibrinolysis activation. The primary function of a tissue-type plasminogen activator (t-PA) is to activate plasminogen to convert it into plasmin and dissolve fibrin. Plasminogen activator inhibitor-1 (PAI-1) is a specific inhibitor of t-PA, and t-PAIC is the product of the combination of t-PA and PAI-1, which is used to reflect the release amount of t-PA in blood. The measurement of t-PAIC can help judge the degree of activation of the fibrinolytic system and relate to the degree of endothelial injury[15,16]. TM is a glycoprotein released by vascular endothelial cells, and its elevation is closely related to vascular endothelial injury[17]. Our research results show that TAT, PIC, and tPAI-C in patients with malignant tumors complicated by VTE are higher than those in patients without thrombosis, which confirms that there is, indeed, activation of the coagulation and fibrinolytic system in the acute phase of tumors accompanied by thrombosis. The study by Anastasiou G et al[18] showed that TAT is one of the reliable markers of thrombosis, which is consistent with the results of this study. Elevated PIC indicates increased fibrinolytic activity and increased risk of bleeding, which also provides

evidence for VTE formation[19]. The study by Bollen et al[20] showed that tPAI-C is one of the best diagnostic indicators of VTE. In our study, there was no significant difference in TM levels between the two groups, suggesting that although patients with tumors themselves had vascular endothelial damage, there was no significant additional vascular endothelial damage during the acute phase of VTE, which is consistent with the research results of Wen et al[21]. Therefore, we speculate that the elevation of tPAI-C is mainly due to the activation of the fibrinolytic system, which is also a marker of thrombus formation and fibrinolytic initiation.

ROC curve analysis showed that PIC had the most significant AUC and the highest diagnostic value. The Youden index was 0.566, with a cut-off value of 0.69, and the sensitivity and specificity were 73.3 % and 83.3 %, respectively, higher than other indexes. PIC is a sensitive index reflecting thrombus formation and fibrinolytic activation and can be used as a sensitive index for early diagnosis of malignant tumors with VTE. Guo et al[22] in their study also proved that plasma PIC level is a valuable biomarker of VTE that can be used to determine whether patients with orthopedic trauma need drug prophylaxis, which is consistent with our findings. The AUC of TAT and tPAI-C were 0.636 and 0.655, respectively, which also had specific diagnostic significance for patients with malignant tumors complicated with VTE. The AUC of TM was 0.537, and the diagnostic value of TM for malignant tumors complicated with VTE was low. However, it has been shown that low TM level is associated with a poor prognosis of malignant tumors[23,24].

This study also has certain limitations: first, the sample size is small; second, due to objective conditions, the patients have not been followed up. The sample size is expected to be expanded in the future to verify the clinical value of this study, and follow-up will be added to study the prognostic value of various indexes for malignant tumors complicated by tumors.

Conclusion

PIC has a satisfactory value for early diagnosis of VTE in patients with malignant tumors. Early detection of VTE could devote time for early treatment and improve the prognosis and patients' quality of life.

Conflict of interest

None of the authors have any financial or scientific conflicts of interest with regard to the research described in this manuscript.

Acknowledgements

This work was supported by the Health Industry Research Project of Gansu Province (grant no.GSWSKY2018-04) and the Internal Medicine Research Project of Gansu Provincial People's Hospital (grant no.18GSSY5-7).

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