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CASE REPORT

Two Cases of Pseudoelevation of Plasma Thrombin Time Levels

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SUMMARY

Background: **Thrombin is a protein involved in the clotting process and binds to free fibrinogen turning it into insoluble fibrin, causing blood to clot. Thrombin time (TT) is a test for the conversion of fibrinogen to fibrin on a common pathway and a screening test for the ability of a subject's plasma to convert fibrinogen to fibrin.**
Methods: **We reported two cases of abnormal extension of TT due to different reasons. We used magnetic bead method to detect thrombin time and used time unit sec to represent TT detection data.**
Results: **In the two cases, we found the TT results of patients taking dabigatran are abnormally high, and normal plasma contaminated with heparin can also cause abnormally high TT results.**
Conclusions: **The influence of heparin on TT was the greatest, followed by activated prothrombin time (APTT), but the influence on prothrombin time (PT) and fibrinogen (FIB) was not obvious. TT is highly sensitive to dabigatran in blood, so TT is not suitable for monitoring dabigatran. The normal value of TT can be used to confirm whether dabigatran has drug residues. In addition, idarucizumab can be used to reverse the anticoagulant activity of dabigatran when dabigatran causes life-threatening bleeding or other high-risk bleeding surgeries.**
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KEYWORDS

thrombin time, heparyn, dabigatran, pseudoelevation

INTRODUCTION

In clinical coagulation mechanism detection, the most common tests include prothrombin time (PT), partially activated prothrombin time (APTT), thrombin time (TT), fibrinogen (FIB), and D dimer. Thrombin time (TT) is the time required for thrombin to be added to plasma at 37°C to convert fibrinogen into fibrin [1]. The abnormality is not related to the deficiency of exogenous coagulation factor, but mainly related to thrombin and fibrinogen. Common causes of TT elongation are, on the one hand, low fibrinogen and abnormal fibrinogen levels and, on the other hand, anticoagulants such as heparin and heparinoids which inhibit the action of thrombin. We now report two clinical cases of abnormal TT elevation, the specific cases are shown below.

Case Report accepted September 20, 2024

CASE PRESENTATION

Case 1

The patient, an 82-year-old female, was admitted to the emergency department of our hospital on July 12, 2024. Due to "lung infection and respiratory failure" and was admitted to the hospital, during the examination, we completed the coagulation function test. The results are shown in Table 1.

From the coagulation test results, we can see that APTT and TT of the patient were significantly prolonged, while PT was only slightly prolonged, and FIB was not reduced. Such coagulation results were consistent with the coagulation characteristics of heparin anticoagulation. Did the patient use heparin for treatment, or was the specimen contaminated heparin? Looking at other laboratory tests, it was found that the patient had also had a blood gas specimen taken at the same time, and the blood gas specimen was a heparin anticoagulant specimen. We immediately contacted the clinic. The patient did not use anticoagulant drugs, and asked the nurse to re-sample the blood coagulation function again. The retest results are shown in Table 1. The retest results confirmed our guess that the first sample was interfered by heparin, resulting in an abnormally high TT result.

Case 2

The patient, an 83-year-old female, was "diagnosed with deep venous thrombosis of the left lower extremity 2 months ago" and was followed up in the Department of General Surgery of our hospital on February 3, 2024. No history of hypertension, diabetes, heart, cerebrovascular, lung, kidney and liver diseases, no history of food and drug allergy. The coagulation function test was completed after admission, and the results are shown in Table 2.

As for the result of the abnormal increase of TT in the patient, our laboratory immediately analyzed the cause. The instrument’s light source did not exceed the service life, the quality control was in control on the day, the TT test results of other specimens on the day did not appear abnormal, and the cause of instrument failure and reagent was preliminarily eliminated. No hemolysis, jaundice, or clot were found in the specimens. No abnormal blood collection and hematocrit were found. No heparin contamination was found, so we ruled out specimen factors. We contacted the clinic to inquire about the relevant medical history of the patient, and found that the patient took dabigatran regularly for 2 months, and it was reported that Dabigatran could cause TT prolongation. The study proved that there was a linear relationship between the TT value and the concentration of dabigatran, and it was very sensitive to dabigatran. Therefore, the patient's TT was abnormally prolonged. We considered the drug cause, and the clinician stopped dabigatran rivaroxaban for anticoagulation therapy. Due to the short half-life of dabigatran ester, the anticoagulant effect was greatly reduced 12 - 24 hours after the administration [1]. Therefore, after seeking the patient's consent, we continued to monitor the patient's TT within the next week, and the TT results show a downward trend (Figure 1).

DISCUSSION

The reason for TT extension should start from its detection principle. *In vitro*, after adding thrombin solution to the plasma to be tested at 37°C, the fibrinogen in the plasma is directly converted to fibrin, so that the platelet-deficient plasma coagulates, and the coagulation time is the thrombin time [2]. TT detection was not related to the deficiency of internal and external coagulation factors related to PT and APTT upstream of the coagulation waterfall, but only related to thrombin and fibrinogen. Heparin is an indirect thrombin inhibitor, which has no direct anticoagulant effect, but it can cause conformational change of AT by binding with lysine on antithrombin (AT), expose the arginine active center of AT, and bind with the thrombin (FIIa) serine active center to form a complex, which inactivates thrombin. Heparin also has anticoagulant effects on other serine proteases, such as FIXa, FXIa, FXa [3]. In case 1, the FIB results were consistent before and after two times, and were not affected by heparin contami-nation. Although TT and FIB have the same detection principle, the influence of heparin on them is very dif-ferent [4]. First of all, the detection reagent is different. TT reagent adds a standard amount of thrombin and does not contain heparin inhibitors, while FIB reagent contains excessive thrombin and heparin inhibitors. The second is to detect the difference in plasma volume. The plasma used for TT detection was twice the original, and the relative volume concentration of plasma was 33.3% (plasma 50 µL, reagent 100 µL). During FIB detection, the patient's plasma was first diluted 10 times, and the relative volume concentration of plasma was the smallest, accounting for only 6.7% (100 µL after plasma dilution and 50 µL of reagents). However, the purpose of TT was to detect abnormal interfering factors in the reaction process, while the purpose of FIB was to detect the amount of true FIB. The plasma relative volume concentration of PT and APTT was the same, accounting for 33.3%, but the PT reagent contained heparin inhibitors. Therefore, heparin had the greatest effect on TT, followed by APTT, and had no obvious effect on PT and FIB.

In recent years, many new anticoagulant drugs that act on a single target in the coagulation waterfall process and are more effective and safer have been successfully developed for the key links in the coagulation process. Dabigatran is a new direct oral anticoagulant with the advantages of a wide therapeutic window, fixed dose, oral administration and predictable curative effect, and does not require monitoring [5]. Its anticoagulant target is thrombin (IIa), so it is called a direct thrombin inhibitor. Dabigatran ester is the precursor of Dabigatran, and

Table1. Results of two coagulation tests in the patient.

Project	Test results	Retest results	Unit	Reference range
PT	15.80	14.30	Sec	11.00 - 15.00
INR	1.28	1.13	Ratio	0.80 - 1.20
APTT	140.00	32.80	Sec	28.00 - 34.00
TT	> 240.00	17.50	Sec	14.00 - 21.00
FIB DD	1.55 2,150.00	1.52 2,340.00	g/L μg/L	2.00 - 4.00 < 500

Table 2. Coagulation test results on February 25, 2024.

Project	Test results	Unit	Reference range
PT	14.9	Sec	11.00 - 15.00
INR	1.20	Ratio	0.82 - 1.15
APTT	64.00	Sec	28.00 - 34.00
TT	> 240	Sec	14.00 - 21.00
FIB DD	4.01 280	g/L μg/L	2.00 - 4.00 < 500

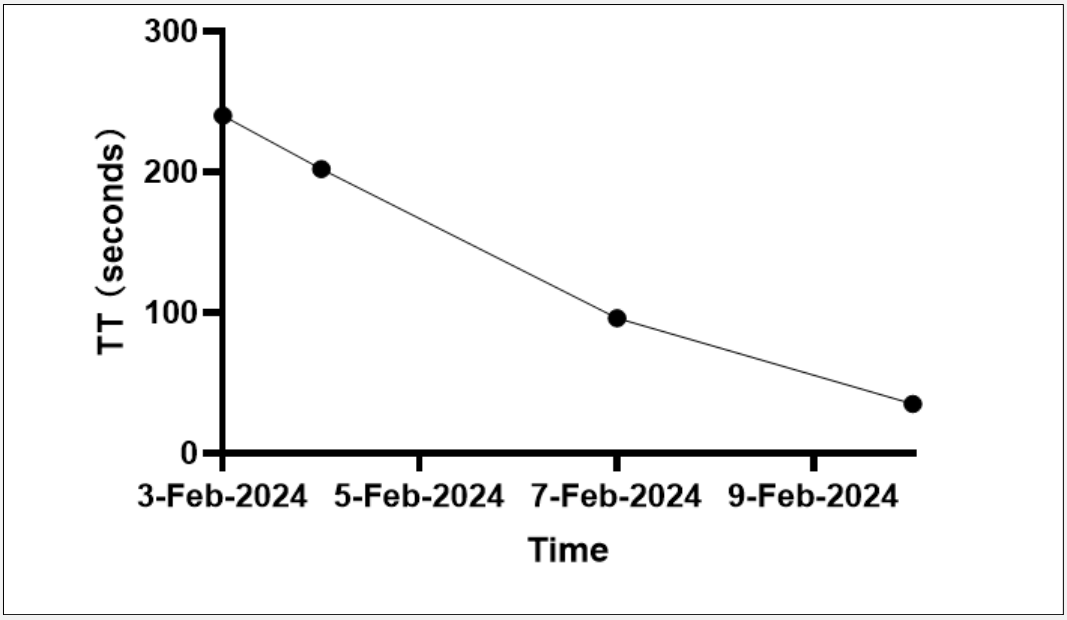

Figure 1. We continuously tested the TT results of patient's plasma.

its active form Dabigatran is hydrolyzed by plasma and liver esterase after oral administration. It can specifically block thrombin and prevent the cleavage of fibrinogen into fibrin, thus blocking the last step of the coagulation waterfall to achieve the purpose of anticoagulation. TT is highly sensitive to dabigatran in blood, and dabigatran at lower than therapeutic concentration can also prolong TT, such as 60 ng/mL of blood concentration often lengthens TT by more than 300 seconds [6]. Therefore, TT is not suitable for dabigatran monitoring, and normal TT helps to confirm the absence of dabigatran residues. Drug calibrated dTT (dilution thrombin time) and ECT (snake venom enzyme clotting time) can be used as suitable methods for rapid quantification of dabigatran [7], but they are not widely used in clinical laboratories. In addition, studies have shown that when dabigatran causes adverse bleeding events, idarucizumab is a monoclonal antibody fragment that rapidly binds to dabigatran and has been approved for the treatment of life-threatening bleeding associated with dabigatran, or patients taking dabigatran require urgent invasive surgery for bleeding [8].

In summary, TT elongation can be seen in the following situations: 1) presence of heparin (cardiopulmonary bypass, hemodialysis, PCI, etc.) and heparin-like anticoagulant substances; 2) direct thrombin inhibitors (dabigatran, hirudin, argatroban and bivalirudin); 3) increased fibrin degradation products (FDP); 4) abnormal fibrinogen or quantity; 5) antithrombin antibodies; and 6) other substances, such as monoclonal immunoglobulin. There are various reasons for TT prolongation. In addition to analyzing whether the detection process is wrong, we should communicate more with the clinic, master the relevant clinical and pharmacological knowledge, and comprehensively consider the specific reasons for TT prolongation.

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Declaration of Interest:
All authors declare that they have no competing interests.

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