CASE REPORT

Apparent Factor IX Inhibitor

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ABSTRAK

Keputusan ujian jangkamasa yang berpanjangan di dalam ujian 'activated partial thromboplastin time' (APTT) dan jangka masa normal untuk ujian 'prothrombin time' (PT) adalah kemungkinan disebabkan samada kehadiran kepincangan dalam faktor pembekuan yang melibatkan faktor VIII, IX, XI atau XII, ataupun kehadiran penghalang pembekuan. Kemungkinan penghalang pembekuan ini adalah spesifik terhadap sesuatu faktor pembekuan atau ia adalah penghalang pembekuan tidak spesifik seperti 'lupus anticoagulant' yang memberi implikasi terapeutik yang tidak sehaluan. Kami melaporkan satu pesakit yang telah dirujuk kepada kami untuk rawatan dimana pendiagnosanya daripada hospital terdahulu melaporkan ia mempunyai penghalang faktor pembekuan IX dengan keabnormalan jangkamasa yang berpanjangan di dalam ujian APTT. Hasil lanjutan siasatan makmal kami telah menunjukkan pesakit ini mempunyai penghalang pembekuan 'lupus anticoagulant' melalui ujian faktor 'phospholipid-dependent'. Ujian pencairan, ujian kromogenik dan neutralisasi fosfolipid dapat membezakan penghalang pembekuan 'lupus anticoagulant' dengan yang faktor pembekuan yang lain. Interpretasi terhadap ujian faktor dengan kehadiran penghalang pembekuan 'lupus anticoagulant' perlu dilakukan dengan berhatihati untuk mengelakkan diagnosis yang tidak tepat dan pemberian rawatan yang tidak sesuai.

Kata kunci: APTT, penghalang, lupus anticoagulant, faktor pembekuan IX

ABSTRACT

The causes of an isolated prolonged activated partial thromboplastin time (APTT) with a normal prothrombin time (PT) are either a deficiency of clotting factors VIII, IX, XI or XII or the presence of an inhibitor. The inhibitor may be specific to an individual clotting factor or it may be a non-specific inhibitor like the lupus anticoagulant which has opposite therapeutic implications. We report a patient referred to our hospital for treatment that was previously diagnosed at another medical institution as an acquired factor IX inhibitor following an investigation for a prolonged APTT. On further testing this turned out to be a potent lupus anticoagulant which interfered with the phospholipid-dependent factor assays. The use of dilution studies, chromogenic assays and phospholipid neutralization can help differentiate these inhibitors. Great

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care must be taken in the interpretation of factor assays in the presence of lupus anticoagulant to avoid misdiagnosis and inappropriate treatment.

Key words: APTT, inhibitor, lupus anticoagulant, acquired factor IX inhibitor

INTRODUCTION

Coagulation tests are usually requested by clinicians for a variety of reasons. It could be part of an investigation for a patient with bleeding tendencies or a routine test prior to a surgical procedure. With the increasing trend of routine coagulation testing, the haemostasis laboratory is often burdened with the task of investigating an isolated prolonged APTT. In most cases, these turned out to be inconclusive or due to factor XII deficiency which is of no clinical significance. In this case report we illustrate a patient with chronic myelomonocytic leukaemia who presented with multiple subcutaneous abscesses. The initial investigation showed a prolonged APTT, a low factor IX level and the presence of an inhibitor. She was diagnosed as an acquired factor IX inhibitor. On further testing this turned out to be a lupus anticoagulant. We discuss the investigation of a prolonged APTT and how to differentiate a specific factor inhibitor from a lupus anticoagulant. This is the first case report that demonstrates correction of an apparent low factor level with phopholipid mixing studies in the presence of a lupus anticoagulant.

CASE REPORT

A 51 year-old lady was diagnosed with chronic myelomonocytic leukaemia in August 2004 when she presented with epigastric pain and was noted to have an enlarged liver and spleen with monocytosis. She remained well following treatment with low dose cytarabine until three years later when she presented with multiple large abscesses over the buttocks, thigh and abdomen. She was seen by the orthopaedic surgeon and was planned for drainage and saucerisation of the abscesses. She had no bleeding symptoms. Laboratory results included a haemoglobin of 10.4g/dL, a white blood cell count of $5.6x10^9$/L, and a platelet count of $41x10^9$/L. Prothrombin time was 12.2 seconds (normal, 10.2-13.2 sec); APTT, 72.2 seconds (normal, 21-32 sec) and fibrinogen, 362 mg/dL (normal 150-450 mg/dL). Factor VIII:C activity was 72.9%; factor IX:C activity was 4.6%, with factor IX inhibitor of 4.0 Bethesda units (BU). In view of the results, she was diagnosed as having an acquired factor IX inhibitor. She was treated with recombinant activated factor VII at 90mcg/kg and underwent the surgery without any bleeding or thrombotic complications. She was also given a course of antibiotics. However, three weeks later she returned with recurrence of the abscesses in the left axilla and back.

The patient was now referred to our hematology department for further management. She had no bleeding symptoms. A repeat coagulation test was done. The results showed PT was 14.7 seconds (normal, 11.0-14.5 sec), APTT was 120.0 seconds (normal, 28-40 seconds) and the mixing studies with normal plasma showed a persistent prolongation of the APTT at 96.5 seconds suggesting the presence of an inhibitor. Factor VIII:C was 22% (normal, 60%-150%), factor IX:C was <1.0% (normal, 60%-150%), factor XI was <1.0% and factor XII <1.0%. Serial dilutions for factor levels were assayed to look for specificity of the inhibitor (Table 1). A chromo-

genic factor VIII assay was also done and this showed a factor VIII level of 120%. To demonstrate that the low factor levels were due to the presence of a lupus anticoagulant (LA), phospholipid was added to the factor assays. The factor levels rose confirming that the apparent low factor levels were due to the interference by the LA (Table 2). The platelet count was $80x10^9$/L with the presence of giant platelets and platelet clumps. Further testing for LA was carried out. The dilute Russell's viper venom test (DRVVT) was prolonged at 88.2 seconds (normal range 26-36 seconds) and corrected to 42.3 seconds with the addition of phospholipid, giving a normalized DRVVT ratio of 2.6 (normal range up to 1.2) (Table 3). The hexagonal II phase phosphatidylethanolamine assay (Staclot-LA), showed a positive test for LA at 54 seconds (negative ≤ 8 sec) (Table 4). The diagnosis of acquired factor IX inhibitor was therefore revised and the prolonged APTT was attributed to the presence of a LA. The patient underwent a second surgical drainage and saucerisation of the abscesses, this time without any factor cover. There was no bleeding complication. Her blood cultures were negative but the indirect fluorescent antibody titer for meloidosis was positive (1:160). She responded to treatment with intravenous ceftazidime and oral cotrimoxazole. She has had no recurrence of the abscesses and is on long-term treatment with co-trimoxazole. A repeat test 6 months later showed persistence of the LA.

MATERIALS AND METHODS

The PT, APTT and factor assays were determined by using an automated coagulation analyzer (ACL 9000, Instrument Laboratory, USA). The PT reagents include fibrinogen HS and calcium thromboplastin (Rabbit-brain tissue), the APTT reagents consist of phospholipids (synthetic derived for optimal platelet lite activity) and calcium chloride (0.020M). All these reagents were supplied by the company from the Instrument Laboratory Sdn. Bhd. Mixing studies were performed using pooled plasma from 50 healthy normal individuals recruited from our hospital. The factor deficient plasma was commercially obtained from the Instrument Laboratory (USA). The presence of LA was detected by the dilute Russell's viper venom test (DRVVT) and the Staclot

Table 1: Factor assays at serial dilutions. Low factor level was observed for all factor assays which could due to nonspecific inhibitor.

Dilution	Neat	1/5	1/10	1/50	1/100
Factor VIII:C %	22	25	25	<1.0	<1.0
Factor IX:C %	<1.0	<1.0	<1.0	<1.0	<1.0
Factor XI:C %	<1.0	<1.0	<1.0	<1.0	<1.0
Factor XII:C %	<1.0	<1.0	<1.0	<1.0	<1.0

Table 2: Normal Control and Patient's Plasma for Phospholipid mixing studies

Factors %	Normal Plasma		Patient's Plasma	
	without phospholipid	with phospholipid	without phospholipid	with phospholipid
VIII	88	48	22	82
IX	97	42	< 1.0	89
XI	87	42	< 1.0	88
XII	89	54	< 1.0	89

Table 3: Dilute Russell Viper Venom Test

Test	Results (Seconds, sec)	
LA-screen	88.2	Normal range: 31- 42 sec
LA-confirm	42.3	Normal range: 30 – 37 sec
Normalized ratio	2.6	Normal ≤ 1.2

Table 4: Hexagonal II phase phosphatidylethanolamine assay (Staclot-LA)

Test	Results (Seconds, sec)
Tube 1 (- phospholipid)	123 s
Tube 2 (+ phospholipid)	69 s
Correction	54 s (positive >8 sec)

LA. The phospholipid from the Staclot LA was used in the phospholipid mixing studies.

DISCUSSION

The activated partial thromboplastin time (APTT) is a non-specific screening test of the intrinsic system while the prothrombin time (PT) is a screening test of the extrinsic system. A prolonged APTT with a normal PT indicates a possible deficiency of factors VIII, IX, XI or XII or the presence of inhibitors. These inhibitors are either specific to these factors or are non-specific, the most common of which is the lupus anticoagulant (LA) (Chng et al. 2005). No single test is definitive for a LA. As a result, a variety of tests and also the clinical history are used in an attempt to establish the diagnosis (Lupus anticoagulant working party 1991). In the present case, a misdiagnosis of factor IX inhibitor was made based on the initial test showing a low factor IX with normal factor VIII levels. The clinical history is very important to help us decide what further tests need to be done. A specific factor inhibitor usually has bleeding manifestations. In this case the patient had no bleeding symptoms, so one should suspect the presence of a LA. Lupus anticoagulant can affect the apparent level of coagulation factors by interfering with the phospholipid-dependent one-stage assay based on APTT. In our experience factor IX and XI assays are affected markedly than factor VIII. To overcome this, dilution assays on the patient's plasma should have been carried out to dilute out the effect of the lupus inhibitor (Kasper 1991).

In our laboratory, we found multiple factor deficiencies which made it easier as we then knew we were most likely dealing with a nonspecific inhibitor such as a LA. The dilution assays however did not show a rise in the apparent factor VIII, IX, XI and XII levels as would be expected in the presence of a LA with factor levels remaining low in the presence of a specific inhibitor (Kasper 1991). This could be explained by the interference of a very strong lupus inhibitor. The factor VIII level did rise a little but fell to < 1.0% at dilutions above 1/50, most likely due to dilution of the factor itself. To further prove that the apparent low factor levels were due to the presence of the LA, we added phospholipid to the patient's plasma and repeated the factor assays. Addition of phospholipid neutralizes the effect of LA and will correct the clotting times in the APTT-based factor assays (Tripodi 2007). This was clearly shown by the normalization of the apparent low factor levels with addition of phospholipid. It should be noted that addition of phospholipid to normal plasma produces a small dilution effect reducing the factor level slightly. We also did the chromogenic assay for factor VIII and this was normal. Chromogenic factor assays are based on chromogenic substrates specific for the factor assayed and are not phospholipid-dependent, hence will not be affected by the LA (Kazmi et al. 1998). The DRVVT and Staclot tests confirmed the presence of a LA in our patient. The DRVVT is very specific for LA and is not prolonged in the presence of a specific

inhibitor to factor VIII or IX as the DRVVT directly activates factor X (Tropodi et al. 2005).

This patient was treated initially with recombinant activated factor VII when she was misdiagnosed with factor IX inhibitor. This was unnecessary and inappropriate. Not only was it expensive, it could have been dangerous to the patient as she could have had a thrombosis. Patients with LA have an increased risk for thrombosis and infusion of activated factor concentrates will increase this risk further. This case report illustrates the importance of correct interpretation of factor assays in the presence of a LA to avoid misdiagnosis and inappropriate treatment. In summary, a specific factor inhibitor can be differentiated from a lupus anticoagulant by: (1) the clinical history: patients with a specific factor inhibitor usually bleed, while patients with LA are usually asymptomatic or they may present with a thrombosis; they rarely bleed; (2) dilution assays: factor levels with a specific inhibitor should not rise with serial dilutions of patient's plasma; (3) DRVVT: a specific factor inhibitor to factor VIII or IX will not prolong the DRVVT; (4) mixing with phospholipids: a specific factor inhibitor should not be corrected with phospholipids and (5) chromogenic factor assay: factors assayed by the chromogenic method will remain low in the presence of a specific factor inhibitor (Douglas 1994).

CONCLUSION

A lupus anticoagulant can affect the apparent levels of the APTT-based factor assays leading to a misdiagnosis of a specific factor inhibitor. It is important to distinguish LA interference from factor deficiency or the presence of a specific factor inhibitor to avoid misdiagnosis because the implications and the treatment differ between these groups of patients.

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