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False-negative coagulation factor activity results due to the presence of antiphospholipid antibodies in a case of autoimmune hemolytic anemia

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Abstract

An 88-year-old female was admitted with autoimmune hemolytic anemia (AIHA). Coagulation test revealed severe prolongation of activated partial thromboplastin time (APTT). APTT cross-mixing test with patient plasma and normal plasma demonstrated an inhibitory pattern. Several intrinsic coagulation factor activities, particularly factor IX, showed remarkable decreases, and the inhibitor titers for coagulation factors VIII and IX were elevated. Although AIHA with existing antiphospholipid (aPL) antibodies was diagnosed initially, purpura developed on extremities intermittently during the clinical course. Considering the possibility of coexisting acquired hemophilia, APTT cross-mixing test with patient's plasma and equal amount of the recombinant factor VIII product instead of normal plasma was performed. The APTT value on equal mixing samples with patient plasma and recombinant factor VIII product was decreased to within the normal range, and coagulation factor IX activity was restored. These results indicate that the recombinant factor VIII product had a neutralizing effect on aPL antibodies. We concluded that recombinant factor VIII product may lead to the repair of incorrect results from the APTT-dependent diagnostic system in the presence of aPL antibodies.

Keywords:
antiphospholipid antibody, anticoagulant inhibitor, APTT cross-mixing test, acquired hemophilia

Introduction

The two major autoimmune diseases demonstrating activated partial thromboplastin time (APTT) prolongation and normal prothrombin time (PT) on coagulation screening are antiphospholipid (aPL) antibody syndrome (APS) and acquired hemophilia. APTT cross-mixing test demonstrates an inhibitory pattern in both diseases, and the diagnoses are made according to clinical findings and the measurement of aPL antibodies or coagulation factor activities. Several cases of coexistent APS and acquired hemophilia have been reported; however, the mechanisms underlying the concurrent development of both diseases are yet to be well clarified. Herein, we report a case of autoimmune hemolytic anemia (AIHA) with aPL antibodies, in which the existence of acquired hemophilia was excluded by APTT cross-mixing test with a recombinant factor VIII product.

Case report

An 88-year-old female presented to the outpatient clinic in August 2013. Her chief complaint was a 2-month history of stridor on walking. She had a medical history of mucosa-associated lymphoid tissue lymphoma of the lung from 71 years of age and anemia diagnosed at 75 years of age. Plain chest radiography demonstrated cardiomegaly and pericardial effusion. At the time of admission, generalized pallor and anemic conjunctivae were noted. Slight lower limb edema was also detected. No other abnormalities were observed. She was not administrated heparin or anticoagulant medications. Laboratory testing at this time revealed the following: white blood cell (WBC) count, 2.78 × 10^9/l; hemoglobin (Hb), 6.4 g/dl; mean corpuscular volume (MCV), 109.9 fl; reticulocytes, 7.2 × 10^4/ml (normal range, 2-8 × 10^4/ml); platelet count, 110 × 10^9/l. These tests also demonstrated the existence of macrocytic anemia with normal reticulocyte count. Serological testing was abnormal as follows: lactose dehydrogenase (LD), 402 IU/l (normal range, 119-229 IU/l); and haptoglobin, < 10 mg/ dl. Immunological testing results were as follows: immunoglobulin (Ig)G 1459 mg/dl (870-1700 mg/dl); IgA, 348 mg/dl (110-410 mg/ dl); IgM, 306 mg/dl (35-220 mg/dl); CH50, 14 U/ml (32-58 U/ml); C3, 27 mg/dl (65-135 mg/dl); and C4, 2 mg/dl (13-25 mg/dl). These findings were indicative of accelerated IgM antibody production and hemolysis with consumption of complements. Direct antiglobulin test was positive for C3d and negative for IgG. These results indicated AIHA. Autoantibody testing demonstrated an antinuclear antibody titer of 1:80 and the absence of anti-DNA antibodies. Coagulation testing revealed the following: APTT, 86 s (24-39 s); PT, 14.0 s (10.5-14.0 s); fibrinogen, 244 mg/dl; and antithrombin, 76.2%. These findings demonstrated severe prolongation of APTT. APTT cross-mixing test with patient plasma and normal plasma demonstrated an inhibitory pattern (Fig. 1 A). Antibody test results related to aPL were as follows: lupus anticoagulant (LAC) by diluted Russell's viper venom time (dRVVT), 2.18 (< 1.3); anticardiolipin antibody (IgG), ×10 titer (< 10); and anticardiolipin b2GPI antibody, 11.2 U/ml (< 3.5 U/ ml). We performed the platelet neutralization procedure (PNP) as a confirmatory test for phospholipid dependence, i.e., an aliquot of lysed platelets in the frozen condition, after washing with normal saline, was added to patient plasma to neutralize the effect of aPL

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antibodies. APTT changed its value from 90.2 s to 64.5 s by the PNP process, the result thus supporting the influence of aPL antibodies on APTT-based assay. The dRVVT was positive at 4 months after the diagnosis, confirming that her condition met the laboratory criteria of aPL antibody syndrome without clinical manifestation [1]. Specific factor assay for coagulation factors VIII, IX, XI, and XII were performed by the one-stage APTT-based assay. Factor XIII and von Willebrand factor were measured by chromogenic assay and macroscopic platelet agglutination assay, respectively. To conduct the one-stage APTT-based assay, coagulation factor-deficiency plasma for factors VIII, IX, XI, and XII (HemosIL® series, Instrumentation Laboratory, Bedford, MA, USA) and APTT reagent (HemosIL® Synthasil APTT, Instrumentation Laboratory) were used. Coagulation factor VIII and IX inhibitors were measured by the Bethesda method by the outsourcing company SRL, Inc. (Tokyo, Japan). The results of coagulation factor activity were as follows: factor VIII, 7%; factor IX; < 1%; von Willebrand factor, 119%; factor XI, 3%; factor XII, 4%; and factor XIII, 70%. Inhibitory coagulation factors were additionally tested, with results as follows: factor VIII inhibitor, 16 biological units (BU)/ml; and factor IX inhibitor, 23 BU/ml. These results indicated a high level of both coagulation factor inhibitors. The prothrombin antibody titer was 8 U/ ml (< 10 U/ml), and von Willebrand factor-cleaving protease (ADAM-TS13) activity was 74%. Bone marrow aspiration testing demonstrated no specific abnormalities. Brain magnetic resonance imaging showed brain atrophy corresponding with her age, with no obvious infarction. According to these results, AIHA with laboratory APS was diagnosed [1]. LAC hypoprothrombin syndrome (LH-HPS) was excluded due to the absence of prothrombin antibody.

The patient's clinical course is shown in figure 2. Therapeutic intervention for AIHA was performed, with oral prednisolone (PSL) therapy (15 mg/day) started in August 2013, considering her advanced age. Diuretics were initiated to treat heart failure. After starting PSL therapy, hemolysis and anemia gradually improved. In order to avoid side effects associated with PSL, the dose of PSL was reduced gradually to 8 mg/day by replacing with azathioprine. At the beginning of 2014, the hemoglobin level had increased to > 8 g/dl without red blood cell transfusion; however, the complement titer, coagulation factor IX activity, and inhibitor level of coagulation

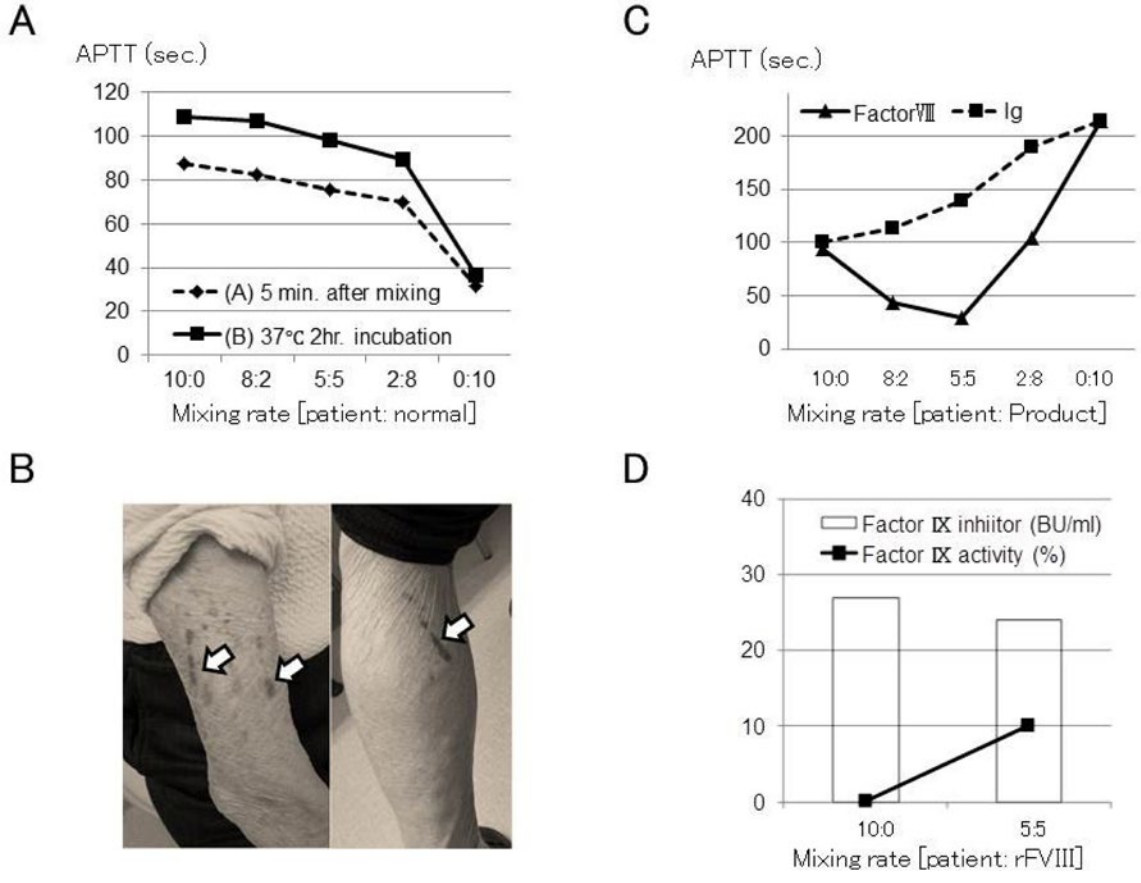

Fig. 1.
A. APTT cross-mixing test with patient plasma and normal plasma. X-axis shows mixing proportions. Patient, patient plasma. Normal, normal plasma.
B. Purpura affecting the extremities (arrow).
C. APTT cross-mixing test with patient plasma and product. The result from patient plasma mixed with recombinant factor VIII product is shown by the dotted line, and the result from patient plasma mixed with the immunoglobulin product is shown by the solid line. X-axis shows mixing proportions. Factor VIII, recombinant factor VIII product. Ig, immunoglobulin product.
D. Factor IX activity (solid graph) and the inhibitor titer of factor IX (bar graph). Original patient plasma, or patient plasma and recombinant factor VIII product (5:5) were tested. Coagulation Factor IX activity increased to 10% when patient plasma was mixed with the recombinant factor VIII product; rFVIII, recombinant factor VIII product

factor IX remained unchanged during the patient's clinical course (Fig. 2). Purpura developed on the right upper arm and left legs in February 2014 (Fig. 1 B), with similar purpura intermittently observed on the extremities. Accordingly, a hemostatic agent was used to continuously treat her purpura.

To investigate the cause of emerging purpura, we considered the possibility of coexisting acquired hemophilia. We particularly focused on acquired hemophilia B as coagulation factor IX had the lowest titer. We supposed that recombinant factor VIII product, where factor IX did not exist, could bind to aPL antibodies, resulting in the decrease of the aPL antibodies by neutralization in the test tube study. We hypothesized that factor IX activity might emerge by elimination of aPL antibodies with recombinant factor VIII product, while factor IX activity might not increase in the existence of acquired hemophilia B disease in the same study. APTT cross-mixing test [2] with recombinant factor VIII product instead of control plasma was performed, in which we attempted to remove the effect of aPL antibodies. Kogenate® FS Bio-Set® was purchased from Bayer Pharmaceuticals Inc. as the recombinant factor VIII product, and Venilon® was kindly provided by Teijin Pharma Limited (Tokyo, Japan) as the immunoglobulin product. The immunoglobulin product was used as the control mixing agent by comparing with the recombinant factor VIII product. The product containing 250 IU of Kogeneate® FS Bio-Set® was dissolved in 25 ml of water, and 500 mg (1 vial) of Venilon® was dissolved in 100 ml of water. Coagpia® APTT-N (Sekisui Medical, Co., Ltd., Tokyo, Japan) was prepared as the reagent for the APTT test, and APTT was measured with a Blood Coagulation Analyzer Coapresta® 2000 (Sekisui Medical). This analyzer was designed to perform a fully automated coagulation assay based on photo-optical end point detection, possessing a multidiluting function with a maximum seven-point dilution [3]. Automated cross-mixing test was performed using the patient plasma and reagent (with the five concentration ratios of 10:0, 8:2, 5:5, 2:8, and 0:10), the results of which were expressed as a graph pattern, measured using the APTT-N reagent. The result of the APTT cross-mixing test is shown in figure 1 C. The APTT value of her plasma mixed with the immunoglobulin product increased gradually in proportion with the dilution, indicating no neutralization effect by the immunoglobulin product. On the contrary, the APTT value of her plasma mixed with an equal amount of the recombinant factor VIII product decreased to within the normal range, indicating that the recombinant factor VIII product had a neutralizing effect on aPL antibodies in the APTT system. When measuring coagulation factor IX activity and factor IX inhibitor titers by using mixed sample of plasma and the recombinant factor VIII product in equal proportions, factor IX activity increased to 10% level, while the factor IX inhibitor titer did not obviously change (Fig. 1 D). These results indicate that the recombinant factor VIII product had the ability to diminish the false-negative reaction caused by the aPL antibodies on the APTT analysis system. We therefore excluded acquired hemophilia B complication by confirming the emergence of factor IX activity in the test tube study.

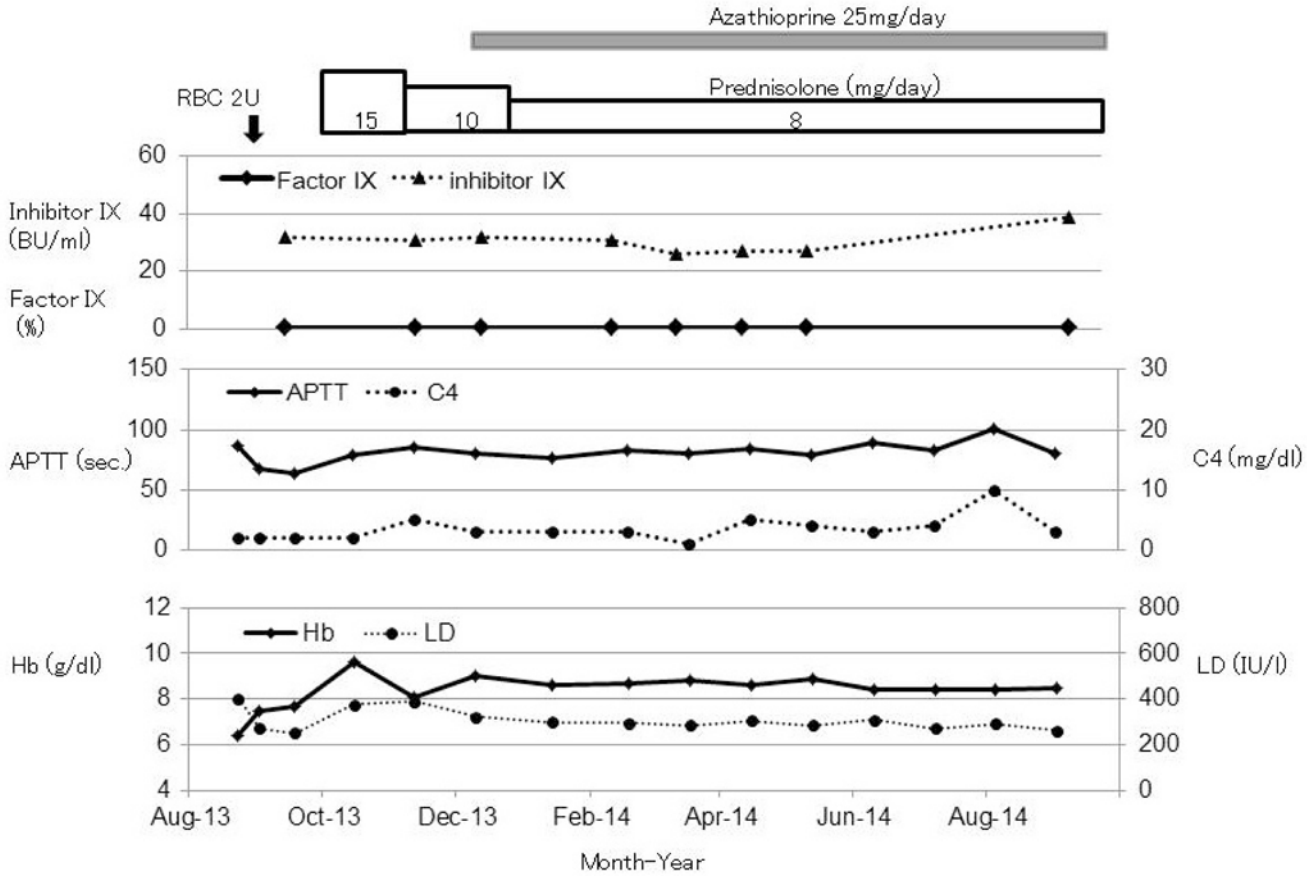

Fig. 2. Clinical course

Discussion

AIHA is an autoimmune disease that may occur as a consequence of another autoimmune disease, termed secondary AIHA, or as a complication of other autoimmune diseases. AIHA accompanied by APTT prolongation was diagnosed in the present case. In general, APTT cross-mixing test is adapted as an initial diagnostic test for cases with prolonged APTT and normal PT. The diagnosis is determined according to the pattern of APTT cross-mixing results, i.e., the correction status of APTT on mixing equal volumes of patient and normal plasma. When no correction is observed, the differential diagnosis, including APS or acquired hemophilia, is determined according to clinical findings, aPL antibody titers, and coagulation factor activity. The clinical evaluation of this patient revealed the presence of aPL antibodies, as the present case had no history of vascular thrombosis or pregnancy but met the laboratory criteria of aPL antibody syndrome [1].

Regarding the coagulation test performed in the present case, several intrinsic coagulation factor activities, particularly factor IX, showed remarkable decreases, and the inhibitor titers for coagulation factor VIII and IX were elevated. When detecting decreases in the activity of numerous coagulation factors or the presence of coagulation factor inhibitors, the false phenomenon caused by aPL antibodies due to modulation of the APTT system should be considered [2, 4]. As the APTT system depends on phospholipids, decreases in coagulation factors or the detection of coagulation inhibitors may be attributable to inhibition of phospholipid activity by aPL antibodies. One solution is to use the dRVVT test [5, 6]; however, careful consideration is required to arrive at the correct diagnosis. To confirm the phospholipid dependence, PNP or hexagonal phase phospholipid neutralization procedure (Staclot® LA) is generally used, in which we selected PNP. In terms of the differential diagnoses for aPL syndrome and acquired hemophilia, cases with the coexistence of aPL syndrome and acquired hemophilia have been reported in recent years [7–10], in most of which the bleeding symptom was the major event irrespective of fulfillment of the criteria for aPL syndrome. The identification of the coexistence of aPL and anti-coagulation factor antibodies is limited by the APTT-dependent diagnostic system [11, 12]. The present case demonstrated the presence of aPL antibodies with purpura rather than thrombotic symptoms. The absence of numerous intrinsic coagulation factors, particularly factor IX (< 3% activity), with a high level of coagulation factor IX inhibitor, should be considered when assessing for acquired hemophilia B. If acquired hemophilia is present, an antibody to coagulation factor IX should be suspected as the most likely cause. To determine the presence of factor IX antibodies, we attempted the APTT cross-mixing test with patient serum and a recombinant factor VIII product instead of the control plasma, the result of which demonstrated normalization of the APTT with mixing of equal sample volumes. The APTT cross-mixing test with patient plasma and the immunoglobulin product as a control demonstrated no normalization of the APTT (Fig. 1 C). This result indicates that the recombinant factor VIII product had the ability to normalize the APTT of the patient sample. When using equal volumes of patient plasma and the recombinant factor VIII product, factor IX activity increased to 10%, while the inhibitor titer of factor IX remained at 24 BU/ml in the revised calculation (Fig. 1 D). As the recombinant factor VIII product contained no other coagulation factors, the increasing factor IX activity indicates that the factor IX activity of the initial patient sample was a false-negative result and the recombinant factor VIII product had the ability to correct this false-negative phenomenon. In other words, recombinant factor VIII product may lead to the repair of incorrect results from the APTT-dependent diagnostic system in the presence of aPL antibodies.

We showed no complication of hemophilia B in the present case; on the other hand, we did not exactly show whether hemophilia A was complicated or not. However, acquired hemophilia B was more likely the cause than acquired hemophilia A if considering the complication of acquired hemophilia, due to the fact that the level of coagulation factor VIII remained as 7%, which was higher than the level of factor IX activity, and the inhibitor titer of coagulation factor VIII was less than that of coagulation factor IX.

Chromogenic assay is another method for the evaluation of coagulation factors, including factor VIII and IX. There are several reports on the comparison between one-stage APTT-based assay and chromogenic assay for assessing coagulation factor levels in patients with anti-PL antibodies, in some of which the chromogenic assay is recommended. The one-stage APTT-based assay may be influenced by nonspecific inhibitors [13], and Chandler et al. report that chromogenic assay is better than one-stage APTT-based assay for measuring factor VIII activity in lupus inhibitor-containing samples [14]. In addition, a few cases showed that aPL antibody influenced the one-stage APTT-based assay but not the chromogenic assay [15], implying that the chromogenic assay is an optimal test for evaluating coagulation factor VIII and IX activities in patients with anti-PL antibodies.

We concluded that the present case did not have acquired hemophilia B. To the best of our knowledge, this is the first report of the use of the APTT cross-mixing test using coagulation factor products, including recombinant factor VIII, as a dilution reagent instead of normal plasma. As the present report involves a single case, further studies are required to clarify the effects of autoantibodies in patients with APS.

Author contributions

TS and SO designed and analyzed the data. KM provided support for the data analysis. HH tested the chemical samples. YK suggested the plan about the automated cross-mixing test. YY supervised the analysis.

Conflict of interest

None declared.

Financial support

None declared.

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