Received: 15 September 2023 | Revised: 27 November 2023 | Accepted: 18 December 2023

DOI: 10.1002/ccr3.8400

CASE REPORT

Clinical Case Reports Open Access WILEY

A nonneutralizing antibody as cause of prothrombin deficiency in a patient with follicular lymphoma

Cecilia Augustsson[1] | **Knut Taxbro**[2,3] | **Karin Strandberg**[1] | **Eva Zetterberg**[4,5]

[1]Division of Laboratory Medicine, Coagulation, Department of Clinical Chemistry and Pharmacology, University and Regional Laboratories Region Skåne, Malmö, Sweden

[2]Department of Anaesthesia and Intensive Care Medicine, Ryhov County Hospital, Jönköping, Sweden

[3]Department of Biomedical and Clinical Sciences, University of Linköping, Linköping, Sweden

[4]Centre for Thrombosis and Haemostasis, Skåne University Hospital, Malmö, Sweden

[5]Department of Translational Medicine, Lund University, Malmö, Sweden

Correspondence
Eva Zetterberg, Centre for Thrombosis and Haemostasis, Skåne University Hospital, Malmö, Sweden.
Email: eva.zetterberg@med.lu.se

Funding information
Regional funds

Key Clinical Message

Acquired inhibitors of blood coagulation are rare but of clinical importance. Prothrombin is a vitamin K-dependent protein, and acquired antibodies toward prothrombin are often associated with the presence of lupus anticoagulant. We describe a previously healthy 70-year-old man presenting with both hemorrhage and thrombosis as well as a prolonged prothrombin time. At arrival at the hospital, he was diagnosed with deep venous thrombosis, and an enlarged lymph node in the left groin was noted (revealed as follicular lymphoma grade 1 by biopsy). Prothrombin activity and antibody titer were followed for 5 months with 15 sampling time points to monitor the treatment outcome of the patient. Diagnostic work-up identified prothrombin deficiency as cause of bleeding. A nonneutralizing calcium-dependent antiprothrombin antibody was found, suspected to increase the clearance of prothrombin, which has previously only occasionally been reported. Lupus anticoagulant was ruled out and thrombosis was judged to be caused by a combination of malignant disease and stagnant venous flow following enlarged lymph nodes in the groin. This report illustrates how investigation of prolonged global coagulation tests, triggered the diagnosis of a rare but critical condition, immune-mediated prothrombin deficiency. The diagnosis is challenging and involves proper differential diagnosis.

KEYWORDS

hemostasis, immunology, prothrombin

1 | INTRODUCTION

Although acquired inhibitors of blood coagulation are rare, they can have a major clinical impact.[1] Acquired inhibitors of factor VIII are often neutralizing, binding to the active site of the clotting factor. In contrast, acquired antibodies of prothrombin often occur in the presence of lupus anticoagulant (LA).[2] Multiple studies have demonstrated a significant relationship between the occurrence of thrombotic events or obstetric morbidity and anti-phosphatidylserine/prothrombin antibodies.[3] On contrary, nonneutralizing antibodies can increase the clearance of the clotting factor and may result in bleeding symptoms.[4] Prothrombin is a vitamin K-dependent protein consisting of a C-terminal gla region containing γ-carboxyglutamic acid residues, a region involved in calcium binding.[5]

2 | CASE PRESENTATION

A previously healthy 70-year-old man presented to his primary healthcare physician with a 2-week history of unilateral warmth, tenderness, and erythema to the lower left leg as well as several spontaneous ecchymosis on the torso and arms. He was admitted to the hospital 2 days later following epistaxis and melena. On arrival at the hospital, he was diagnosed with deep venous thrombosis (DVT) engaging left popliteal and fibular veins. Because of the history of bleeding and prolonged prothrombin time, treatment with a reduced dose of tinzaparin (4500 Units o.d. subcutaneously) was initiated and increased to 3500 Units t.d. the day after corresponding to 108 units/kg/day to improve treatment of thrombosis. During the initial admission, he received endoscopic treatment of a duodenal angiodysplasia, and an enlarged lymph node in the left groin was noted. Computer tomography revealed extensive lymph adenopathy in the groins and abdomen. A biopsy of a lymph node and the insertion of a tunneled central venous catheter (CVC) was performed under local anesthesia after the administration of tranexamic acid (10 mg/kg body weight) and four-factor prothrombin complex concentrate (2000 Units Ocplex® Octapharma). Prior to definitive hematological diagnosis, treatment with intravenous gamma globulin (1 g/kg body weight) and prednisolone (1 mg/kg) were started on the suspicion of malignancy-associated coagulation factor deficiency. Despite treatment, clinical bleeding from the groin and CVC persisted. Results from lymph node biopsy revealed follicular lymphoma grade 1. As levels of prothrombin activity were still low, treatment with rituximab was started. The patient profile is outlined in Figure 1A.

3 | INVESTIGATIONS

Coagulation parameters were assayed on an automated analyzer, Atellica COAG 360 (Siemens Healthineers, Erlangen, Germany) with reagents and methods explained previously.[6] LA was measured using Siemens dilute Russell viper venom time (dRVVT) reagents. Mixing studies were performed using hepes-buffered normal plasma (Precision Biologic, Dartmouth, Canada). Fibrinogen was measured on Sysmex CS 5100 using Siemens Dade® thrombin reagent. Anti-Cardiolipin IgG antibodies were quantified using immunoassay (Orgentec by Sebia, Mainz, Germany) and β2-glycoprotein I (GPI) IgG antibodies on Acustar (Werfen, MA, USA). Antiprothrombin neutralizing antibody titer was calculated according to the Bethesda assay with Nijmegen modification. Antiprothrombin-binding (nonneutralizing) antibody titer was calculated

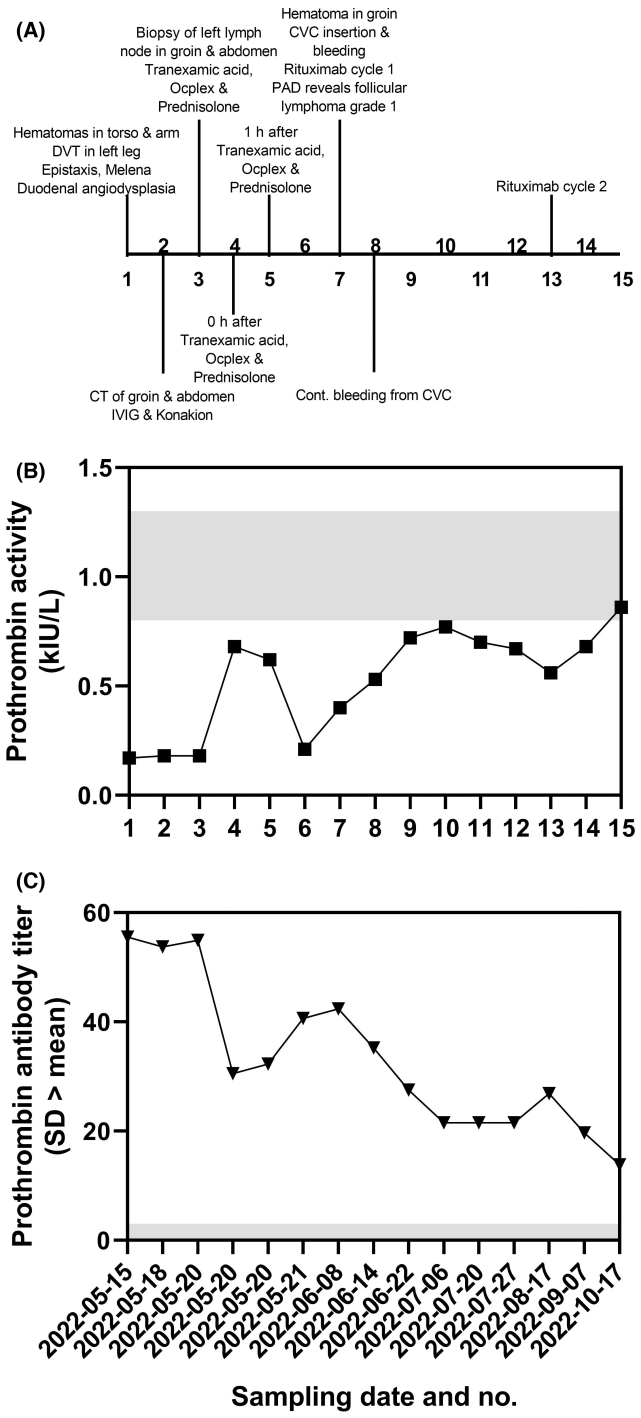

FIGURE 1 Patient's profile demonstrating inverse correlation between prothrombin and antibody level. Clinical presentation (A) and protein profile (B, C) in patient's plasma at different sampling numbers shown as sampling no. 1–15 with corresponding dates. (A) Clinical presentation, (B) prothrombin was followed by activity measurements using one-stage assay. Gray area indicates the reference range (0.80–1.30 kIU/L). (C) Nonneutralizing-binding antibody titer was followed by prothrombin as coat in ELISA and shown as standard deviations (SD) above the mean from analyzing plasma samples from healthy donors. The gray area indicates the reference range (<3 SD).

with ELISA immunoassay using prothrombin, prothrombin with gla domain removed (Enzyme Research Laboratories, South Bend, USA), or Factor IX Benefix® (Pfizer, NY, USA) as coat at 5 μg/mL.[4] Patient plasma was added in 1:50 dilution in quench buffer.

4 | OUTCOME

Prolonged activated partial thromboplastin clotting time (APTT) and prothrombin time (PT, Owren, and Quick) were noticed on the first and second sampling time points (see Table 1). A correction of APTT was obtained by mixing patient's plasma with normal plasma; APTT mixing indicated a factor depletion rather than LA or an inhibitor. Prolongations of dRVVT screen and confirm were seen but the ratio screen/confirm was below our local cut-off value, thus not diagnostic for LA. No anticardiolipin or β2-GPI IgG antibodies were detected, however, other immunoglobulin forms (i.e., IgM/IgA) were not measured. LA is known to inversely affect factor assays using APTT-based reagents. Using this reagent, factor XI activity was measured within the reference range, indicating no such assay interference and thus, a low probability of LA. Due to prolonged PT Owren, an extended diagnostic panel revealed factors

TABLE 1 Routine and special coagulation assays in patient plasma indicate low prothrombin levels.

Analysis	Sampling no. 1	Sampling no. 2	Reference interval (2.5 and 97.5 percentile) or cutoff (99th percentile)
APTT (s)	48 (H)	47 (H)	21–30
APTT mixing (s)	26	26	<32
PT (Owren) (INR)	1.6 (H)		0.9–1.2
PT (quick) (s)	13 (H)		6–10
dRVVT screen (s)	87 (H)	78 (H)	<42
dRVVT screen mixing (s)	38 (H)		<38
dRVVT confirm (s)	72 (H)	62 (H)	<37
dRVVT ratio (screen/confirm)	1.2	1.3	<1.4
Anti-cardiolipin IgG (kU/L)	<5		<10
Anti-β2-glycoprotein I IgG (U/mL)	<6.4		≤20
Anti-factor Xa (LMWH) (kIU/L)	<0.10	<0.10	<0.10
Fibrinogen (g/L)	4.7 (H)		2.0–4.0
Factor VIII CSA (kIU/L)	2.57 (H)		0.55–1.17
Factor IX CSA (kIU/L)	1.34		0.71–1.58
Factor VII OSA (kIU/L)	0.85		0.60–1.60
Prothrombin OSA (kIU/L)	0.17 (L)		0.80–1.30
Factor X OSA (kIU/L)	1.00		0.70–1.40
Factor XIII (kIU/L)	0.93		0.83–1.77
VWF:Ag (kIU/L)	2.32 (H)		0.58–1.65
VWF:GP1bM (kIU/L)	2.81 (H)		0.47–1.81
Factor XI OSA (kIU/L)		1.43	0.83–1.48
Prothrombin neutralizing antibody (kBU/L)	<0.4		<0.4

Note: Results below or above the reference interval are marked with L (Low) and H (High), respectively. For references of reagents used see Section 3.

Abbreviations: Ag, antigen; APTT, activated partial thromboplastin clotting time; B, Bethesda; CSA, chromogenic substrate assay; dRVVT, dilute Russell viper venom time; GP1bM, gain-of-function mutant glycoprotein 1b; I, International; IgG, Immunoglobulin G; INR, international normalized ratio; LMWH, low molecular weight heparin; OSA, one stage assay; PT, prothrombin time; U, unit; VWF, von Willebrand factor.

VII and X activity within normal range, while prothrombin activity was decreased (0.17 kIU/L) (see Table 1 and Figure 1B). Other factor activities (Factors VIII, IX, XI, fibrinogen, and von Willebrand factor) affecting bleeding tendency were either within or above the normal range. No M-component was detected on serum protein electrophoresis (data not shown). No neutralizing antibody could be detected in patient's plasma (see Table 1). The presence of a nonneutralizing-binding antibody was detected, with a clear inverse correlation between the patient's prothrombin and antibody level (Figure 1C). Treatment with four-factor prothromplex concentrate (Ocplex®, Octapharma) resulted in an increase in prothrombin activity and a decrease in antibody titer measured by ELISA (see sampling numbers 4 and 5, Figure 1). Ocplex® contains prothrombin and will thus increase levels of measurable prothrombin. Since levels of prothrombin activity were still low, treatment with rituximab was started. In the absence of calcium or in the presence of prothrombin with removed gla domain as a coating material, no antibody was detected, indicating that the gla domain (the calcium-binding region of prothrombin) plays an important role in epitope binding. The fact that the antibody showed no neutralizing capacity with concomitantly decreased activity of prothrombin indicates that the antibody, by binding to the gla domain, facilitates clearance of prothrombin from the circulation. Furthermore, no cross-reactivity to other gla domain-containing proteins such as Factor IX was observed.

5 | DISCUSSION

Acquired prothrombin deficiency is often seen in patients with vitamin K deficiency or liver disease, but in rare cases, it is caused by antibodies affecting prothrombin activity and/or level. The most common are prothrombin-binding antibodies in the presence of LA. These antibodies target prothrombin alone or in complex with phosphatidylserine and show a strong correlation to thrombosis.[2–4,7,8] In our case, LA was not detectable, although the presence of DVT suggested the opposite. Lymphomas are among the neoplasias with high risk for venous thromboembolism, including DVT.[9] The DVT in our patient could be explained by a combination of lymphoma and stagnant venous flow following enlarged lymph nodes in the groin. Non-LA, calcium-dependent prothrombin antibodies are ultra-rare but have been reported in a few cases including lymphoma patients,[10–12] and in one report a prothrombin antibody with cross-reactivity toward other gla domain-containing proteins was shown.[13] In these cases, antibodies cause low prothrombin activity, conferring a risk of bleeding. The underlying cause of autoantibody production in our patient was most probably follicular lymphoma, a condition known to be associated with several autoimmune conditions such as autoimmune hemolytic anemia, Evan's syndrome, autoimmune thrombocytopenia as well as the presence of autoantibodies to a variety of self-proteins.[14] Treatment of the underlying disease by repeated doses of Rituximab resulted in remission of lymphoma as well as successfully eradication of the antibody and prothrombin level as well as bleeding diathesis returned to normal.

In conclusion, the diagnosis of immune-mediated prothrombin deficiency is challenging and involves proper differential diagnosis in which vitamin K deficiency, LA, and hereditary prothrombin deficiency should be excluded. Our patient presented with both bleeding and thrombosis and a correct diagnosis was vital for optimal treatment.

AUTHOR CONTRIBUTIONS

Cecilia Augustsson: Data curation; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Knut Taxbro:** Data curation; visualization; writing – original draft; writing – review and editing. **Karin Strandberg:** Formal analysis; visualization; writing – original draft; writing – review and editing. **Eva Zetterberg:** Conceptualization; funding acquisition; investigation; supervision; visualization; writing – original draft; writing – review and editing.

FUNDING INFORMATION

This work was supported by regional funds.

CONFLICT OF INTEREST STATEMENT

EZ has received speakers' fee from Octapharma. The other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available upon request.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Cecilia Augustsson https://orcid.org/0000-0001-5587-3712

REFERENCES

1. Macik BG, Crow P. Acquired autoantibodies to coagulation factors. *Curr Opin Hematol*. 1999;6(5):323-328.
2. Galli M, Barbui T. Antiprothrombin antibodies: detection and clinical significance in the antiphospholipid syndrome. *Blood*. 1999;93(7):2149-2157.
3. Vandevelde A, Devreese KMJ. Laboratory diagnosis of antiphospholipid syndrome: insights and hindrances. *J Clin Med*. 2022;11(8):2164.
4. Knobe K, Tedgard U, Ek T, Sandstrom PE, Hillarp A. Lupus anticoagulants in two children–bleeding due to nonphospholipid-dependent antiprothrombin antibodies. *Eur J Pediatr*. 2012;171(9):1383-1387.
5. Hansson K, Stenflo J. Post-translational modifications in proteins involved in blood coagulation. *J Thromb Haemost*. 2005;3(12):2633-2648.
6. Strandberg K, Augustsson C. Evaluation of the Atellica COAG 360 coagulation analyzer in a specialized coagulation laboratory. *J Clin Lab Anal*. 2022;36(3):e24276.
7. Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML. Anti-prothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. *Thromb Haemost*. 2014;111(2):354-364.
8. Bertolaccini ML, Sciascia S, Murru V, Garcia-Fernandez C, Sanna G, Khamashta MA. Prevalence of antibodies to prothrombin in solid phase (aPT) and to phosphatidylserine-prothrombin complex (aPS/PT) in patients with and without lupus anticoagulant. *Thromb Haemost*. 2013;109(2):207-213.
9. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood*. 2008;111(10):4902-4907.
10. Bajaj SP, Rapaport SI, Barclay S, Herbst KD. Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: mechanism and management. *Blood*. 1985;65(6):1538-1543.
11. Alamelu J, McCormick A, Moore GW, Madan B. Acquired prothrombin deficiency in a patient with follicular lymphoma. *Haemophilia*. 2008;14(3):634-636.
12. Lee ES, Hibsman BK, Liebman HA. Acquired bleeding disorder in a patient with malignant lymphoma: antibody-mediated prothrombin deficiency. *Cancer*. 2001;91(4):636-641.
13. Rochanda L, Del Zoppo GJ, Feinstein DI, Liebman HA. Approach to the treatment, characterization and diagnosis of an acquired auto-antibody directed against factors prothrombin, factor X and factor IX: a case report and review of the literature. *Haemophilia*. 2012;18(1):102-107.
14. Miller EB. Autoimmunity and lymphoma: a brief review. *J Rheum Dis Treat*. 2018;4:4.

How to cite this article: Augustsson C, Taxbro K, Strandberg K, Zetterberg E. A nonneutralizing antibody as cause of prothrombin deficiency in a patient with follicular lymphoma. *Clin Case Rep*. 2024;12:e8400. doi:10.1002/ccr3.8400