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CASE REPORT

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Lupus anticoagulant associated with low grade B-cell lymphoma and IgM paraproteinaemia with lupus cofactor phenomenon on DRVVT and SCT assays - a possible novel association

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Abstract

Background Lupus anticoagulant (LA) is an in vitro phenomenon with prolongation of a phospholipid-dependent coagulation test which is not due to an inhibitor specific to a coagulation factor. Occasionally, addition of normal pooled plasma to patient plasma with lupus anticoagulant potentiates the inhibitory effect of lupus anticoagulant in the mixture, resulting in a paradoxical prolongation instead of shortening of clotting time. The phenomenon has been termed the "lupus cofactor effect". Lupus anticoagulant are known to be associated with lymphoma and immunoglobulin M (IgM) paraproteinaemia. Cases of lymphoplasmacytic lymphoma with concomitant IgM paraproteinaemia and lupus anticoagulant demonstrating lupus cofactor phenomenon on activated partial thromboplastin time (APTT) assay has been reported previously. However, to our best knowledge, there were no reported cases of low grade B-cell lymphoma with positive LA results and lupus cofactor effect demonstrated on dilute Russell's viper venom time (DRVVT) and/or silica clotting time (SCT) assays in the literature.

Case presentation We report two cases of low grade B-cell lymphoma associated with monoclonal IgM paraprotein, high levels of anti-cardiolipin IgM antibody and presence of lupus anticoagulant with lupus cofactor phenomenon on DRVVT and/or SCT assay.

Conclusions Our cases demonstrate a possible novel association between low grade B-cell lymphoma, IgM paraproteinaemia, high levels of anti-cardiolipin IgM antibody and the presence of lupus cofactor effect on DRVVT and SCT assays. The DRVVT assay in the first patient and SCT assay in second patient were falsely negative in the neat sample or less diluted sample, and the lupus anticoagulant activities were only revealed on dilution of samples with normal pooled plasma. This highlights the potential importance of dilution of samples with normal pooled plasma while evaluating the LA status of low grade B-cell lymphoma patients with a markedly prolonged APTT and/or

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prolonged PT by DRVVT and SCT assays, especially if there is concomitant IgM paraproteinaemia and a high level of anti-cardiolipin IgM antibody.

Keywords Lupus anticoagulant, Lupus cofactor effect, Low grade B-cell lymphoma, IgM paraproteinaemia

Background

Lupus anticoagulant is an in vitro phenomenon with prolongation of a phospholipid-dependent coagulation test which is not due to an inhibitor specific to a coagulation factor. This phenomenon is caused by autoantibodies against phospholipid binding proteins [1].

Occasionally, addition of normal pooled plasma to patient plasma with lupus anticoagulant potentiates the inhibitory effect of lupus anticoagulant in the mixture, resulting in a paradoxical prolongation instead of shortening of clotting time. The phenomenon has been termed the "lupus cofactor effect" [2].

We report two cases of low grade B-cell lymphoma associated with monoclonal immunoglobulin M (IgM) paraprotein, high levels of anti-cardiolipin IgM antibody and presence of lupus anticoagulant (LA) with lupus cofactor phenomenon on dilute Russell's viper venom time (DRVVT) and/or silica clotting time (SCT) assay.

Case presentation

Case 1

A 66-year-old Chinese woman had history of low grade B-cell lymphoma with IgM paraproteinaemia diagnosed in 2018. She was treated with 6 cycles of bortezomib, dexamethasone and rituximab (BDR). The disease was in remission after treatment. At her routine follow-up visit in 2023, she presented with splenomegaly. She was otherwise asymptomatic, with no bleeding or thrombosis. The complete blood count results were as follows: haemoglobin concentration 8.6 g/dL, white blood cell 2.88×10^9/L, platelet 131×10^9/L. Computer tomography scan showed multiple enlarged lymph nodes in clusters over the left cervical, left supraclavicular, porta hepatis, paraaortic and aortocaval regions, together with mild hepatomegaly and massive splenomegaly. Biopsy of the left supraclavicular lymph node showed low grade B-cell lymphoma with plasmacytic differentiation. Bone marrow biopsy showed marrow involvement by low grade B-cell lymphoma. Molecular study for MYD88 p.L252P mutation was negative.

Serum protein electrophoresis (SPE) revealed IgM kappa paraprotein measuring 38 g/L. Coagulation studies was performed on Sysmex CS-5100 coagulation analyzer (Sysmex Corporation, Kobe, Japan) (similarly hereinafter unless otherwise specified), the reagents for prothrombin time (PT) and activated partial thromboplastin time (APTT) were Thromborel S (Siemens Healthineers, Erlangen, Germany) and ActinFSL (Siemens Healthineers, Erlangen, Germany) respectively (similarly hereinafter unless otherwise specified). Thromborel S reagent contains thromboplastin from human placenta [3]. ActinFSL reagent uses ellagic acid as activator. The reagent is lupus anticoagulant sensitive [4]. The tests showed a prolonged APTT of 75.3 s (s) (25.8–33.8 s) and PT of 20.2 s (10.7–13.1 s). PT and APTT had been repeated by the ACL TOP750 CTS coagulation analyzer (Instrumentation Laboratory, Bedford MA, USA). The reagents for PT and APTT were HEMOSIL RecombiPlasTin 2G (Instrumentation Laboratory, Bedford MA, USA) (based on recombinant human tissue factor [5]) and HEMOSIL SynthASil (Instrumentation Laboratory, Bedford MA, USA) (a lupus anticoagulant sensitive reagent which uses colloidal silica activator [6]) respectively. The APTT and PT were 29.2 s (9.9–12.3 s) and 56.3 s (29.2–38.4 s) respectively. APTT was further tested upon serial dilution of sample. The specimen was diluted by HemosIL LA Negative Control (Instrumentation Laboratory, Bedford MA, USA) (similarly hereinafter). The HemosIL LA Negative Control is a lyophilized preparation using human citrated platelet-poor plasma to make a pooled normal plasma with added buffer [7]. It has been determined to be negative for LA in accordance with guidelines from International Society on Thrombosis and Haemostasis [1]. There was further prolongation of APTT on the 1:8 and 1:16 diluted sample when compared with the neat sample. This phenomenon was not present in the PT assay. The thrombin time (TT) and fibrinogen levels were 13.3 s (14.0–21.0 s) and 2.80 g/L (1.50–3.60 g/L) respectively. Mixing study of PT and APTT systems showed presence of an immediate-acting inhibitor. There was non-parallelism in factor assays performed on neat sample. Parallelism had been achieved by dilution of sample. Factor II, V, VII, VIII, IX, X, XI, XII levels were within reference intervals. (Table 1)

DRVVT test was performed using LA1 Screening Reagent and LA2 Confirmation Reagent (Siemens Healthineers, Erlangen, Germany) (similarly hereinafter). On the neat sample, the DRVVT (confirm) failed due to a "terrace" coagulation curve error of the Sysmex CS-5100 coagulation analyzer. The coagulation curve was reviewed and an abnormal flat part was noted. LA was not detected on 1:2 diluted sample. Interestingly, further dilution to 1:4 ratio showed the presence of LA. The results were as follows: DRVVT (screen) 47.4 s (28.4–38.2 s), DRVVT (confirm) 33.8 s (27.3–33.2 s), normalized LA ratio 1.28 (<=1.24). The DRVVT (screen) result was further prolonged upon serial dilution up to 1:64, leading to a progressive increase in normalized LA ratio.

Table 1 Basic coagulation assay, mixing study and factor assay results of case 1 and 2

	Case 1	Case 2	Reference interval
Basic coagulation assay by Sysmex CS-5100 coagulation analyzer			
PT (s)	**20.2**	**16.2**	10.7–13.1
APTT (s)	**75.3**	**84.0**	25.8–33.8
TT (s)	13.3	15.4	14.0–21.0
Fibrinogen (g/L)	2.80	2.90	1.50–3.60
PT and APTT assay by ACL TOP750 CTS coagulation analyzer			
PT (s)		Not performed due to insufficient sample	
➢ Neat	**29.2**		9.9–12.3
➢ 1:2	20.3		NA
➢ 1:4	16.9		NA
➢ 1:8	15.1		NA
➢ 1:16	14.9		NA
APTT (s)			
➢ Neat	**56.3**		29.2–38.4
➢ 1:2	52.2		NA
➢ 1:4	56.0		NA
➢ 1:8	57.9		NA
➢ 1:16	60.7		NA
Mixing study on APTT system			
1:1 mix of patient with normal plasma (immediate) (s)	60.9	83.2	NA
1:1 mix of patient with normal plasma (2 h, 37 degrees) (s)	63.4	89.2	NA
Mixing study on PT system			
1:1 mix of patient with normal plasma (immediate) (s)	15.6	15.9	NA
1:1 mix of patient with normal plasma (2 h, 37 degrees) (s)	15.4	17.2	NA
Factor assay			
Factor II level (%)	77	**58**	70–120
Factor V level (%)	66	120	50–200
Factor VII level (%)	50	58	50–200
Factor VIII level (%)	191	**269**	50–200
Factor IX level (%)	91	100	40–160
Factor X level (%)	82	81	50–200
Factor XI level (%)	101	70	40–160
Factor XII level (%)	38	**19**	30–150

* The abnormal test results are bolded. Abbreviations: PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; NA, not applicable

SCT assay was performed using HEMOSIL SCT confirm and HEMOSIL SCT screen reagent (Instrumentation Laboratory, Bedford MA, USA) (similarly hereinafter). No definite LA had not been demonstrated by SCT assay, but the SCT (screen) result was also progressively prolonged upon serial dilution up to 1:64, resulting in an increasing SCT ratio which approached the upper limit of reference interval. (Table 2) Further dilution after 1:64 was not performed despite a suggestion that the peak titre had not been reached. Overall features were suggestive of the presence of LA with lupus cofactor effect demonstrated on DRVVT assay. In addition, high levels of anti-cardiolipin immunoglobulin G (IgG) antibody and IgM antibody were detected. Their levels were >100.0 GPL U/mL (<=13.3 GPL U/mL) and >120 MPL (<12.5 MPL) respectively. The anti-β2-glycoprotein1 IgG antibody level was within reference interval.

The patient is currently under treatment. She had complete 2 cycles of bortezomib, rituximab and dexamethasone combination. The latest SPE showed IgM kappa paraprotein of 5 g/L. PT and APTT were shortened to 14.0 s and 54.7 s respectively. LA was still detected but no definite lupus cofactor effect had been demonstrated.

Case 2

A 76-year-old Chinese woman with good past health presented with fever and splenomegaly. The complete blood count results were as follows: haemoglobin concentration 8.5 g/dL, white blood cell 3.4×10^9/L, platelet 120×10^9/L. Bone marrow examination with flow cytometry showed marrow involvement by low grade B-cell lymphoma. Molecular study for MYD88 p.L252P mutation was negative. The left axillary lymph node biopsy was inadequate for diagnosis. Positron emission tomography-computed tomography showed huge

Table 2 DRVVT and SCT results of case 1 and 2

Sample	DRVVT (screen) (s) (28.4–38.2)	DRVVT (confirm) (s) (27.3–33.2)	Normalized LA ratio (< = 1.24)	SCT (screen) (s) (36.2–56.4)	SCT (confirm) (s) (34.7–53.4)	SCT ratio (< = 1.27)
Case 1						
Neat	36.2	Failed due to "terrace" coagulation curve error	-	**63.7**	**63.2**	0.81
1:2	**41.7**	32.5	1.15	**61.8**	**54.1**	0.92
1:4	**47.4**	**33.8**	**1.28**	**62.2**	51.7	0.96
1:8	**52.5**	**33.7**	**1.40**	**62.7**	49.5	1.02
1:16	**57.5**	**33.7**	**1.53**	**66.8**	47.2	1.14
1:32	**61.4**	**33.9**	**1.62**	**70.1**	46.0	1.22
1:64	**61.6**	**33.4**	**1.65**	**71.0**	45.7	1.25
1:128	Not performed			**71.5**	45.9	1.25
Case 2						
Neat	**84.8**	Failed due to slow reaction error	-	**71.9**	**61.5**	0.94
1:2	**106.8**	Failed due to slow reaction error	-	**76.4**	50.9	1.20
1:4	**96.3**	**40.2**	**2.15**	**86.5**	46.4	**1.50**
1:8	**89.9**	**37.4**	**2.15**	**99.3**	45.2	**1.76**
1:16	**84.5**	**35.7**	**2.12**	**111.2**	46.2	**1.95**

* The abnormal test results are bolded. Abbreviations: DRVVT, dilute Russell's viper venom time; SCT, silica clotting time

splenomegaly with prominent diffuse 18-fluorodeoxyglucose (FDG) uptake and hypermetabolic right supraclavicular, left axillary, upper abdomen and left external iliac lymph nodes.

SPE showed IgM kappa paraprotein with a concentration of 6 g/L. Both the APTT and PT were prolonged at 84.0 s (25.8–33.8 s) and 16.2 s (10.7–13.1 s) respectively. PT and APTT had not been repeated with another reagent due to insufficient sample. The TT was 15.4 s (14.0–21.0 s) and the fibrinogen level was 2.90 g/L (1.50–3.60 g/L). Immediate-acting inhibitor had been demonstrated by mixing study of PT and APTT systems. Factor II and XII levels were mildly reduced, measuring 58% (70–120%) and 19% (30–150%) respectively, the low factor levels could be false low values due to interference by strong lupus anticoagulant. Factor VIII level was mildly increased at 269% (50–200%). Factor V, VII, IX, X and XI levels were within reference intervals. (Table 1)

DRVVT and SCT assays were performed to test for LA on Sysmex CS-5100 coagulation analyzer. The DRVVT (confirm) failed on neat sample and 1:2 diluted sample due to a slow reaction error of the Sysmex CS-5100 coagulation analyzer, which is defined by a reaction time in the segment of the coagulation curve that is longer than the preset limit of the coagulation analyzer. The coagulation curve was reviewed and there were no other significant abnormalities in the coagulation curve. LA was demonstrated on 1:4, 1:8 and 1:16 diluted sample by DRVVT assay, with no significant changes in normalized LA ratio on serial dilution. On SCT assay, the SCT ratio of neat sample and 1:2 diluted sample were within reference interval, signifying negative results for LA. However, the SCT (screen) results were progressively prolonged upon serial dilution, causing an increasing SCT ratio. LA had been detected on 1:4, 1:8 and 1:16 diluted sample. (Table 2) Overall features were suggestive of lupus cofactor effect demonstrated on SCT assay. Further dilution after 1:16 was not performed, and the peak lupus anticoagulant titre may not have been reached. The anti-cardiolipin IgM antibody level was >120 MPL (<12.5 MPL). The anti-β2-glycoprotein1 IgG antibody level was not increased.

The patient had been treated with cyclophosphamide, doxorubicin, vincristine and prednisone (CEOP) chemotherapy regimen. Reassessment was performed after 3 cycles of CEOP. SPE showed a reduction of IgM kappa paraprotein level to 3 g/L. PT and APTT were shortened to 15.8 s and 68.3 s respectively. LA was still demonstrable with presence of lupus cofactor effect on SCT assay.

Discussion and conclusions

We report two low grade B-cell lymphoma cases with similar coagulation and immunological abnormalities, including markedly prolonged APTT, prolonged PT, presence of lupus anticoagulant with lupus cofactor phenomenon demonstrated by DRVVT or SCT assays on serial dilution of patient's plasma with normal pooled plasma, IgM paraproteinaemia, and also very high levels of anti-cardiolipin IgM antibody. Moreover, lupus cofactor effect on APTT assay was also present for case 1. To our best knowledge, the association between low grade B-cell lymphoma and the presence of lupus cofactor effect on DRVVT and/or SCT assays has not been reported previously.

Lupus cofactor effect was first described by Loeliger in 1959 in a patient with lupus anticoagulant hypoprothrombinemia syndrome [8]. Loeliger proposed that the phenomenon could be caused by an unknown cofactor which is required for lupus anticoagulant to exert its effect on clotting time assays, and the cofactor could be prothrombin [8]. This hypothesis has been further confirmed by Pengo et al. [9] Others have demonstrated that β2-glycoprotein I is also a cofactor for lupus anticoagulant [10], and therefore may also be responsible for the cofactor effect. The term "lupus cofactor effect" was initially used to describe abnormalities of APTT assays, but similar phenomenon on DRVVT assays has been described in recent reports [11]. A recent study has shown that the prevalence of lupus cofactor effect in lupus anticoagulant positive cases was 5.9% [12]. Since the prothrombin (factor II) level in case 1 was normal and the level in case 2 was only mildly reduced, the strong lupus cofactor effects demonstrated in these two cases were unlikely to be caused by hypoprothrombinaemia. β2-glycoprotein I could be responsible for the lupus cofactor effects detected in our cases, unfortunately our laboratory did not have the assays to measure β2-glycoprotein I levels, and hence we could not confirm this hypothesis.

Lupus anticoagulant are known to be associated with lymphoma. Lechner et al. have analyzed 61 cases of lupus anticoagulant associated with lymphoma. Majority of cases were described in lymphoplasmacytic lymphoma and splenic marginal zone lymphoma, but there were also cases described in other subtypes of B-cell and T-cell lymphomas. Concomitant paraproteinaemia and elevated anti-cardiolipin antibody levels were frequently found in the cases described by Lechner et al. In most of those cases, the paraprotein and the anti-cardiolipin antibody isotype were IgM [13].

The association between IgM paraproteinaemia and LA was first reported by Thiagarajan et al. [14] Wu et al. has reported a case of IgM monoclonal gammopathy of undetermined significance (MGUS) accompanied by a marked increase of anti-cardiolipin IgM antibody and the presence of LA, with an extensively decreased in coagulation factor activity. They also demonstrated by in-vivo experiments that the IgM paraprotein could bind to cardiolipin and interfere with assays of coagulation, and therefore was responsible for the positive LA results [15]. Three cases of lymphoplasmacytic lymphoma with concomitant IgM paraproteinaemia and lupus anticoagulant demonstrating lupus cofactor phenomenon on APTT assay has been reported previously by Ciaudo et al. [16] However, to our best knowledge, there were no reported cases of low grade B-cell lymphoma with positive LA results and lupus cofactor effect demonstrated on DRVVT or SCT assays in the literature.

Our cases demonstrate a possible novel association between low grade B-cell lymphoma, IgM paraproteinaemia, high levels of anti-cardiolipin IgM antibody and the presence of lupus cofactor effect on DRVVT and SCT assays. The DRVVT assay in Case 1 and SCT assay in Case 2 were falsely negative in the neat sample or less diluted samples, and the lupus anticoagulant activities were only revealed on dilution of samples with normal pooled plasma. This highlights the potential importance of dilution of samples with normal pooled plasma while evaluating the LA status of low grade B-cell lymphoma patients with a markedly prolonged APTT and/or prolonged PT by DRVVT and SCT assays, especially if there is concomitant IgM paraproteinaemia and a high level of anti-cardiolipin IgM antibody. Since nowadays, LA is commonly evaluated by DRVVT and SCT in laboratories, it is useful for haematologists and laboratory scientists to note this association in order to properly evaluate the LA status and to avoid misdiagnosis of other coagulation disorders in low grade B-cell lymphoma patients with abnormal coagulation findings. We recommend the use of higher dilutions in these cases in order to avoid the possibility of false negative lupus anticoagulant results.

Abbreviations

LA	Lupus anticoagulant
IgM	Immunoglobulin M
IgG	Immunoglobulin G
PT	Prothrombin time
APTT	Activated partial thromboplastin time
TT	Thrombin time
DRVVT	Dilute Russell's viper venom time
SCT	Silica clotting time

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Author contributions
THSL: analyzed and interpreted the patient's data, conceptualized the study, wrote the manuscript. PLY: treated the patient and wrote the manuscript. HYC: treated the patient and wrote the manuscript. HKSC: performed and interpreted the coagulation studies. WSW: analyzed and interpreted the patient's data.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
None of the patients' personal identifications were mentioned in the manuscript. Ethical approval is not required.

Consent for publication
Written informed consent were obtained from the patients.

Competing interests
The authors declare no competing interests.

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