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SHORT REPORT

Antiphospholipid syndrome, monoclonal gammopathy, and cryoglobulinemia overlap leading to recurrent cutaneous microvascular thrombosis: A case report and retrospective cohort study

Alexandra Bohm[1] | Bo Angela Wan[1] | Amir Karin[2] | Lauren J. Lee[3] | Agnes Y. Y. Lee[3,4,5] | Edward M. Conway[1,3,4] | Chieh Min Benjamin Lai[3,4]

[1]Department of Medicine, University of British Columbia, Vancouver, Canada

[2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada

[3]Department of Medicine, Division of Hematology, University of British Columbia, Vancouver, Canada

[4]University of British Columbia Centre for Blood Research, Vancouver, Canada

[5]British Columbia Cancer Agency, Vancouver, Canada

Correspondence
Chieh Min Benjamin Lai, 10th Floor, 2775 Laurel St, Vancouver, BC, V5Z 1M9, Canada.
Email: benjamin.lai@bccancer.bc.ca

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Abstract

Antiphospholipid syndrome (APS), cryoglobulinemia, and monoclonal gammopathies are variably accompanied by thrombotic complications. We describe a patient with recurrent skin microvascular thrombosis, APS, cryoglobulinemia, marginal zone lymphoma, and IgMκ monoclonal gammopathy, responsive to chemoimmunotherapy. The cryoglobulin fraction contained the IgMκ paraprotein, while antiphospholipid antibodies (aPL) were predominantly in the cryosupernatant. A retrospective analysis of aPL-positive patients in our institution showed that 8.1% co-expressed monoclonal gammopathy. These overlap patients had thrombotic complications and most had recurrences. Patients with multiple gammopathies of thrombotic significance may have several autoantibodies and constitute a high-risk group.

KEYWORDS
Antiphospholipid syndrome, cryoglobulinemia, monoclonal gammopathy of undetermined significance, thrombosis

1 | INTRODUCTION

Antiphospholipid syndrome (APS) is a thrombo-inflammatory disorder associated with recurrent thrombosis in the macro- and microvasculature. It is associated with antiphospholipid antibodies (aPL) that activate immune cells, complement, endothelium, and the coagulation cascade [1]. Similarly, cryoglobulinemia is an autoimmune disorder characterized by autoantibodies, inflammatory cell recruitment, occlusive vasculopathy, complement activation, and small vessel thrombosis. Of the 3 types of cryoglobulinemia, type 1 is associated with monoclonal gammopathies. Some have undetermined significance (MGUS), while others are associated with B-cell malignancy [2]. The term "monoclonal gammopathy of thrombotic significance" (MGTS) has been proposed to capture diseases where a thrombogenic paraprotein causes significant or recurrent thrombosis [3].

Studies that examine concurrent APS, MGUS, and cryoglobulinemia are limited. Even fewer investigate the relationship between co-expressed antibodies for each syndrome. Herein, we describe a patient with features of all three syndromes, elucidate the characteristics of the patient's gammopathies, and report a retrospective cohort study to describe the prevalence of patients with concurrent syndromes.

TABLE 1 Laboratory parameters for the case report.

Laboratory parameters	First presentation (Oct 2020)	Repeat aPL (Feb 2021)	First ulcer recurrence (Nov 2021)	Second ulcer recurrence (Feb 2022)	Skin in remission (Apr 2022)	End of study (Aug 2023)
White blood cell (10^9/L)	5.0	3.4	2.8	4.3	3.0	3.8
Hemoglobin (g/L)	125	94	95	110	122	114
Platelets (10^9/L)	209	152	143	192	150	133
Creatinine (μmol/L)	63	79	83	62	53	79
eGFR (mL/min)	101	92	86	100	107	90
Lupus anticoagulant	Negative	Positive[a]	N/A	Negative	N/A	Negative
aCL IgG (GPL'U)	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
aCL IgM (MPL'U)	28	27	21	16	7.4	5.2
aβ2GP1 IgG (U/mL)	<0.8	<0.8	0.8	<0.8	<0.8	<0.8
aβ2GP1 IgM (U/mL)	206	98	56	49	35	15
SPEP IgMκ paraprotein (g/L)	0.7	N/A	10.0[b]	0.4	<1.0	Negative
Cryocrit	Absent	N/A	0.95	N/A	Absent	Absent

Abbreviations: aβ2GP1, anti-beta-2-glycoprotein-1; aCL, anti-cardiolipin; eGFR, estimated glomerular filtration rate; SPEP, serum protein electrophoresis.
[a]Patient was on dalteparin.
[b]SPEP performed at 37°C.

2 | CASE PRESENTATION

A 61-year-old male ex-smoker with a history of dyslipidemia presented in August 2020 with bilateral, painful necrotic ulcers on his shins. He was treated with repeated courses of antibiotics and wound care with no improvement over 3 months. Workup revealed strongly positive anti-beta-2-glycoprotein-1 (aβ2GP1) IgM, weakly positive anti-anticardiolipin (aCL) IgM, and negative corresponding IgG antibodies using an enzyme-linked immunosorbent assay (ELISA, EliA; Thermo Fisher Scientific) (Table 1 and Figure 1A). Lupus anticoagulant (LA) testing was negative (Inova Diagnostics). Serum protein electrophoresis (SPEP) with immunofixation showed a 0.7 g/L monoclonal IgMκ paraprotein. Cryoglobulin was absent. A skin biopsy demonstrated superficial dermal vascular thrombosis with minimal inflammation and prominent fibrin thrombi in the capillary lumen with no evidence of small vessel leukocytoclastic vasculitis. At the same time, he was assessed by vascular surgery and a computed tomography (CT) angiogram with run-off demonstrated extensive atherosclerotic disease, incidental splenomegaly (15.8 cm), and mild pelvic lymphadenopathy (largest measured 1.5 cm). He was started on low-dose aspirin in December 2020 and referred to hematology for the serological, biopsy, and CT findings.

When assessed in December 2020, he continued to have right leg ulcers. Therapeutic dalteparin was added to aspirin due to suspicion of APS. A bone marrow biopsy was non-diagnostic but showed a small population (< 0.3%) of B cells with kappa light chain predominance and otherwise normal immunophenotype. Since there were no other clinical features of lymphoma, a serial imaging approach was adopted. His aPL in February 2021 was persistently positive. LA was positive in the context of receiving dalteparin. Due to worsening claudication and non-healing ulcers, he had a right to left femoral crossover graft with right femoral-popliteal bypass. Post-operatively, he was bridged to warfarin with a target international normalized ratio (INR) of 2.5 (± 0.5) and continued low-dose aspirin. His ulcers healed by May 2021.

In October 2021, he had a recurrence of painful, purpuric, gangrenous lesions on his right foot and shin. His INR was therapeutic and a repeat CT angiogram showed patent arterial vasculature and bypass graft. Conventional CT showed progressed organomegaly (spleen 17.2 cm, largest lymph node 2.3 cm). Lymph node biopsy demonstrated marginal zone lymphoma. SPEP with immunofixation performed at 37°C revealed a 10 g/L IgMκ paraprotein and a diffuse IgAκ band. Cryoglobulin became detectable with a cryocrit of 95%. The cryoglobulin was purified by cryoprecipitation, washes, and resolubilization at 45°C. This fraction was almost exclusively composed of the IgMκ paraprotein by immunofixation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. His aPL IgM antibodies were positive though at lower titer. The aPL titers were measured in neat serum (kept at 37°C), resolubilized cryoprecipitate, and the cryosupernatant using an ELISA that was adapted to various temperatures (Euroimmune). aβ2GP1 IgM was strongly positive in the cryosupernatant and weakly positive in the cryoglobulin fraction while aCL IgM was weakly positive in the cryosupernatant and undetectable in the cryoglobulin fraction (Figure 1B). While awaiting treatment for his lymphoma, the patient continued aspirin and warfarin with an increased INR target of 3.0 (± 0.5). His gangrenous ulcers resolved again by January 2022.

In February 2022, painful, necrotic ulcers recurred in his right leg despite a therapeutic INR (Figure 2). aPL antibody titers (EliA; Thermo Fisher Scientific) were unchanged. Warfarin was switched to dalteparin and he started chemoimmunotherapy with bendamustine

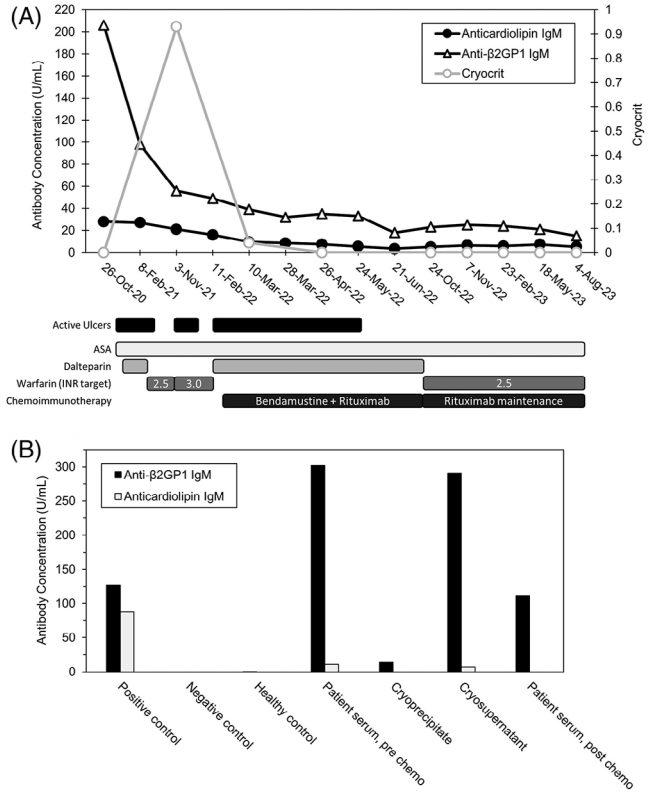

FIGURE 1 (A) Antiphospholipid antibody and cryoglobulin testing over time. Anticardiolipin IgM and anti-β2GP1 IgM antibody concentrations by ELISA (EliA, Thermo Fisher Scientific), left Y-axis. Manufacturer recommended cut-off for anticardiolipin IgM weak positive 10–40 U/mL, positive > 40 U/mL, anti-β2GP1 IgM equivocal 7–10 U/mL, positive > 10 U/mL. Cryocrit, right Y-axis. Periods of active leg ulcers, anti-thrombotic, and chemoimmunotherapy treatments corresponding to dates are listed under the X-axis. (B) Antiphospholipid antibody testing of different serum fractions. Anticardiolipin IgM and anti-β2GP1 IgM antibody concentrations by ELISA in neat serum at 37°C (Euroimmune) before and after six cycles of bendamustine plus rituximab. Antibody titers in the cryoprecipitate fraction and cryosupernatant fraction pre-treatment were also shown. The manufacturer recommended a cut-off for aCL IgM positive ≥12 U/mL and anti-β2GP1 IgM positive ≥20 U/mL.

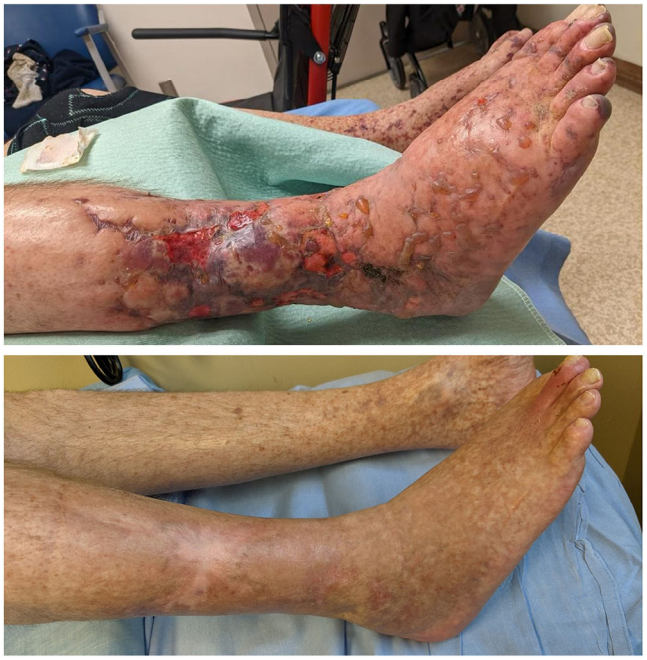

FIGURE 2 Patient leg ulcers before (March 2022, top) and after chemoimmunotherapy (October 2022, bottom).

and rituximab. After two cycles, the cryoglobulin was undetectable and aPL antibody titers trended downward. His ulcers completely healed by the end of the third cycle. His cryoglobulin and IgMκ paraprotein remained undetectable 3 months after finishing chemoimmunotherapy. Concurrently, his aβ2GP1 and aCL IgM titers were reduced or became undetectable (Table 1). He was then initiated on Rituximab maintenance in November 2022 for his lymphoma. He remained stable with no recurrence of his skin lesions or lymphoma at the end of the study period.

To gain more insight into the prevalence of concurrent APS, cryoglobulinemia, and MGUS, we reviewed the medical records of 118 patients aged 18 or above with at least one positive aPL test in our clinic between January 1, 2019, and October 30, 2023. This retrospective study was approved by the local institutional research ethics board. Cryoglobulin testing was performed within 6 months of aPL testing in 65 patients but only one patient (1.5%) had cryoglobulins. SPEP was performed in 86 patients within 6 months of aPL testing and seven patients (8.1%) had a monoclonal gammopathy. Five of these seven patients had a paraprotein of the same isotype as the APS antibody. All seven patients had thrombotic complications and five had recurrences (Table 2).

3 | DISCUSSION

We describe a patient with recurrent cutaneous microvascular thrombosis and features of APS, cryoglobulinemia, and MGTS. The recurrent episodes may reflect sequential or synergistic disease manifestation. APS may have been responsible for thrombogenesis in the first episode, reflected by high aPL titers, undetectable cryoglobulin, and a skin biopsy showing thrombotic vasculopathy with minimal inflammation. While he initially responded to anti-thrombotics, the ulcers recurred with the emergence of lymphoma-associated monoclonal cryoglobulins. The aPL titers were lower, but still detectable. Hence, both cryoglobulinemia and APS may have contributed to the recurrences. Unsurprisingly, the ulcers kept recurring until he received chemoimmunotherapy which targeted B-cells and potentially changed the natural history of his concurrent syndromes.

There are limited studies that examine the relationship between APS, MGUS, and cryoglobulinemia. APS with MGUS cases have been reported and some demonstrated a high incidence of recurrent thrombosis despite anticoagulation [4–10]. Likewise, APS with cryoglobulinemia has been described, sometimes with concurrent MGUS [11–14]. We performed a retrospective cohort study for patients with

TABLE 2 Patients identified in a retrospective cohort who co-expressed antiphospholipid antibodies and monoclonal gammopathies.

ID	aPL test results (serology in U/mL)	aPL platform	Cryoglobulin	Monoclonal protein	Clinical diagnoses
1	LA negative aβ2GP1 IgG 0.8 aβ2GP1 IgM 56 aCL IgG < 0.8 aCL IgM 21	EliA	Positive	10 g/L IgM kappa	Present case report. Recurrent cutaneous small vessel thrombosis and marginal zone lymphoma
2	LA positive (on heparin) aβ2GP1 IgG 358 aβ2GP1 IgM < 0.8 aCL IgG 48 aCL IgM 5.5	ELiA	Negative	5.5 g/L IgG lambda	Recurrent stroke, finger ischemic ulcer, and monoclonal B-lymphocytosis
3	LA positive (on warfarin) aβ2GP1 IgG 98 aβ2GP1 IgM < 0.8 aCL IgG 80 aCL IgM 1.2	ELiA	Not done	Faint IgG kappa	Recurrent stroke, myocardial infarction, transient ischemic attack, immune thrombocytopenia with purpura, and marginal zone lymphoma
4	LA positive aβ2GP1 IgG > 160 aβ2GP1 IgM 0.3 aCL IgG > 160 aCL IgM 0.3	BioPlex	Not done	0.4 g/L IgG kappa	PE and recurrent DVT
5	LA negative aβ2GP1 IgG < 1.6 aβ2GP1 IgM 3.3 aCL IgG 2.4 aCL IgM 27	BioPlex	Negative	Faint IgA lambda	PE and recurrent DVT
6	LA positive aβ2GP1 IgG 6.3 aβ2GP1 IgM < 0.8 aCL IgG 4.6 aCL IgM 2.3	BioPlex	Negative	2.1 g/L IgG kappa	Stroke and seizure
7	LA positive (on heparin) aβ2GP1 IgG 255 aβ2GP1 IgM 52 aCL IgG 55 aCL IgM 200	EliA	Not done	Faint IgM kappa	PE with chronic thromboembolic pulmonary hypertension, DVT, stroke, immune thrombocytopenia with purpura, moderate aortic regurgitation, moderate aortic stenosis, and chronic kidney disease with suspected APS nephropathy.

Abbreviations: aβ2GP1, anti-beta-2-glycoprotein-1; aCL, anti-cardiolipin; APS, antiphospholipid syndrome; DVT, deep vein thrombosis; LA, lupus anticoagulant; PE, pulmonary embolism.

combinations of these entities. To our knowledge, our cohort is the largest to date that examines the prevalence of overlap syndromes. Our analysis showed that concurrent aPL positivity with cryoglobulinemia is rare while aPL positivity with MGUS is more common. Most patients in this latter group had a monoclonal protein of the same isotype as the aPL antibody.

We further explored the relationship between the antibodies of aPL, MGUS, and cryoglobulinemia. It is conceivable that monoclonal cryoglobulins give rise to an antibody targeting domain 1 of β2GP1, which may be a key pathologic epitope in APS [1]. Hence, a single MGTS can clinically be interpreted as APS or cryoglobulinemia depending on the testing performed. This mechanism has been postulated but previous studies have employed either chromatography or crude precipitation techniques for purification where isolation of the paraprotein from other immunoglobulins of the same isotype is difficult [7–9]. The reversible precipitation of the paraprotein in our patient, however, provided a unique opportunity to elucidate its relationship with the patient's aPL. We showed that the purified cryoglobulin fraction contained the IgMκ paraprotein and was largely depleted of the aPL IgM antibodies, which were mostly in the cryosupernatant fraction. This suggests that the aPL was polyclonal while the cryoglobulin was monoclonal. They are different entities instead of a single MGTS. We postulate that when he expressed both gammopathies, his skin ulcers became more resistant to anti-thrombotics and required immunosuppression. Similarly, severe or refractory cases of APS and cryoglobulinemia have been reported to respond to immunosuppression [2, 15].

Our report has limitations. It is possible that the precipitated immunoglobulins represent multiple clones. We believe this is unlikely, as the precipitated fraction yielded only kappa-restricted IgM. While functional testing of the precipitated and supernatant fractions would

be of interest, it is beyond the scope of this report. Lastly, the prevalence of concurrent syndromes in our retrospective cohort is subject to investigation bias and not everyone in this cohort would satisfy the revised Sapporo APS classification criteria. Nevertheless, it is a moderate-sized cohort and we reviewed test results within 6 months of each other to increase the likelihood of true co-expression.

Taken together, thrombogenic autoantibodies can be co-expressed. Patients may present with refractory thrombosis despite antithrombotics. Clinicians should maintain a high degree of suspicion for gammopathies of thrombotic potential, polyclonal or monoclonal. Identifying these entities may inform alternative treatment strategies such as B-cell targeted immunosuppression. Further research is required to improve the detection and management of these patients.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

I confirm that my article contains a Data Availability Statement even if no data is available.

ETHICS STATEMENT

The authors have confirmed ethical approval statement is not needed for this submission.

PATIENT CONSENT STATEMENT

The participant has consented to the submission of the case report including data and photographs to the journal.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

ORCID

Chieh Min Benjamin Lai https://orcid.org/0000-0002-4130-9413

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