

## Case Report

# Purpura Fulminans Triggered by the Formation of Anti-Endothelial Autoantibodies in a Patient with Chronic Lymphocytic Leukemia

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## Abstract

Purpura fulminans (PF), a life-threatening disorder characterized by cutaneous microvascular thrombosis with secondary hemorrhagic infarction, is a rare complication of sepsis and may also be caused by severe protein C or protein S deficiency.

Here, we describe the case of a 58-year-old man who developed PF in close association with the onset of chronic lymphocytic leukemia (CLL). PF initially manifested with an ecchymosis of the right upper leg in the absence of sepsis or disseminated intravascular coagulation. PF was likely triggered by anti-endothelial IgG autoantibodies functionally interfering with the anticoagulant protein C-protein S-thrombomodulin system on EA.hy926 endothelial cells in a modified thrombin generation assay. Although plasma exchange and immunosuppressive therapy with cyclophosphamide were temporarily effective, PF eventually progressed and the patient died from septic shock during treatment-associated neutropenia.

In rare occasions, CLL may be associated with autoimmune-mediated PF requiring prompt diagnosis and aggressive multimodal therapy.

**Keywords:** Chronic lymphocytic leukemia; Purpura fulminans; Anti-endothelial antibodies; Plasma exchange therapy

## Abbreviations

AIC: Autoimmune Cytopenia; AIHA: Autoimmune Hemolytic Anemia; ANA: Antinuclear Antibodies; APS: Antiphospholipid Syndrome; ASA: Acetylsalicylic Acid; BID: Twice Daily; BTK: Bruton's Tyrosine Kinase; CLL: Chronic Lymphocytic Leukemia; CRP: C-Reactive Protein; CT: Computed Tomography; DIC: Disseminated Intravascular Coagulation; EPCR: Endothelial Protein C Receptor; HIT: Heparin-Induced Thrombocytopenia; ISTH: International Society on Thrombosis and Haemostasis; ITP: Immune Thrombocytopenia; MBL: Monoclonal B-Cell Lymphocytosis; MRI: Magnetic Resonance Imaging; OD: Once Daily; PC: Protein C; PET: Plasma Exchange Therapy; PF: Purpura Fulminans; PNH: Paroxysmal Nocturnal Hemoglobinuria; PS: Protein S; SSC: Scientific and Standardization Committee; sTM: Soluble Thrombomodulin; TF: Tissue Factor; TM: Thrombomodulin

## Case Presentation

A 58-year-old man (169cm, 80kg) was referred to our hospital with the rapid onset of a singular erythema-surrounded ecchymosis with central hemorrhagic bullae and necrotic lesions located at the right upper leg (Figure 1A, left image). The patient had several cardiovascular comorbidities, including atrial fibrillation complicated by a stroke, coronary artery disease, and arterial hypertension. In addition, he had a history of chronic bowel disease and not further specified mild thrombocytopenia and leukocytosis. At hospital admission, he was on oral anticoagulation with apixaban 5mg twice

daily (BID) and antiplatelet therapy with acetylsalicylic acid (ASA) 100mg once daily (OD).

A skin punch biopsy revealed thrombotic vasculopathy with occlusions of cutaneous microvessels by hyaline thrombi (Figure 1B). Muscular and osseous involvement was ruled out by magnetic resonance imaging (MRI). Ultrasound analysis of the lower limbs was unremarkable with no evidence of arterial or venous thrombosis. In addition to Coombs-positive hemolytic anemia (hemoglobin, 10.8g/dL) and thrombocytopenia (platelet count,  $41 \times 10^9/L$ ), laboratory workup revealed elevated inflammatory markers with a leukocyte count of  $13.4 \times 10^9/L$  and a C-reactive protein (CRP) of 70mg/L (Table 1). The absolute lymphocyte count was normal, and extensive analysis of coagulation parameters was unremarkable except for slightly elevated plasma D-dimers (0.96mg/L) (Table 1). The disseminated intravascular coagulation (DIC) score according to the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) [1] was 4. Spontaneous heparin-induced thrombocytopenia (HIT), systemic vasculitis, connective tissue diseases, antiphospholipid syndrome (APS), and paroxysmal nocturnal hemoglobinuria (PNH) were excluded (Table 1), and hence, thrombotic vasculopathy was attributed to a purpura fulminans (PF). Overt malignancy was ruled out by computed tomography (CT) scanning of chest, abdomen and pelvis, but cytological and immunophenotypic evaluation of bone marrow and peripheral blood (Supplementary Figure 1) revealed a high-count monoclonal B-cell lymphocytosis (MBL) without

**Table 1:** Laboratory workup of the patient.

	Value	Reference Range
<b>Blood counts</b>		
Haemoglobin, g/dL	10.8	14.0 - 17.5
Leukocytes, $1 \times 10^9/L$	13.4	3.8 - 11.0
Neutrophils, $1 \times 10^9/L$	9.1	1.5 - 7.7
Lymphocytes, $1 \times 10^9/L$	2.7	1.1 - 3.4
Monocytes, $1 \times 10^9/L$	1.5	0.2 - 0.9
Platelets, $1 \times 10^9/L$	41	150 - 350
Schistocytes, %	< 0.1	0 - 0.1
<b>Clinical Chemistry</b>		
Creatinine, mg/dL	1.16	0.6 - 1.3
CRP, mg/L	70	0 - 5
LDH, U/L	338	87 - 241
<b>Coagulation parameters</b>		
Prothrombin time, %	70.3	80 - 130
INR	1.17	0.85 - 1.15
APTT, sec	37	25 - 38
Thrombin time, sec	19.3	16 - 22
Fibrinogen, g/L	5.59	1.8 - 4.0
D-dimer, mg/L	0.96	< 0.5
Antithrombin, %	68.1	70 - 130
Factor VIII:C, %	236.4	60 - 160
PC activity, %	70.4	70 - 140
Free PS antigen, %	86.3	75 - 145
VWF:Ag, %	315.5	60 - 200
VWF:GPIbM, %	304.1	61 - 179
Plasminogen, %	78.9	75 - 140
Plasmin inhibitor, %	109.8	80 - 120
<b>Immunoglobulins</b>		
IgG, g/L	12.1	7.0 - 16.0
IgM, g/L	0.6	0.4 - 2.3
IgA, g/L	2.8	0.7 - 4.0
<b>Autoantibodies</b>		
ANA	1:320	< 1:80
p-ANCA	Negative	< 1:20
c-ANCA	Negative	< 1:20
ds-DNA, U/mL	< 0.1	< 10
IgM-aCL, U/mL	< 0.1	< 10
IgG-aCL, U/mL	0.2	< 10
IgM-anti- $\beta_2$ GPI, U/mL	< 0.1	< 7
IgG-anti- $\beta_2$ GPI, U/mL	< 0.1	< 7
LA	Negative	Negative
Anti-CCP, U/mL	0.2	< 7
CIC	Negative	Negative
Cold agglutinins	Negative	Negative
Cryoglobulin	Negative	Negative

**Abbreviations:** aCL: Anti-Cardiolipin; ANA: Antinuclear Antibodies; anti- $\beta_2$ GPI: Anti- $\beta_2$ -Glycoprotein-I; anti-CCP: Anti-Cyclic Citrullinated Protein; APC: Activated Protein C; APTT: Activated Partial Thromboplastin Time; c-ANCA: Cytoplasmic Anti-Neutrophil Cytoplasmic Antibody; CIC: Circulating Immune Complexes; CRP: C-Reactive Protein; ds-DNA: Double Stranded DNA; INR: International Normalized Ratio; LDH: Lactate Dehydrogenase; LA: Lupus Anticoagulant; p-ANCA: Perinuclear Anti-Neutrophil Cytoplasmic Antibody; PC: Protein C; PS: Protein S; VWF:GPIbM: Von Willebrand Factor Activity; VWF:Ag: Von Willebrand Factor Antigen.

evidence of bone marrow failure. The karyotype was normal, and quantitative immunoglobulins were within the respective reference ranges (Table 1) with no evidence for a monoclonal paraprotein in serum electrophoresis. In light of co-existing autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP), diagnostic criteria for manifest chronic lymphocytic leukemia (CLL) were fulfilled, and an autoimmune pathogenesis of PF was suspected.

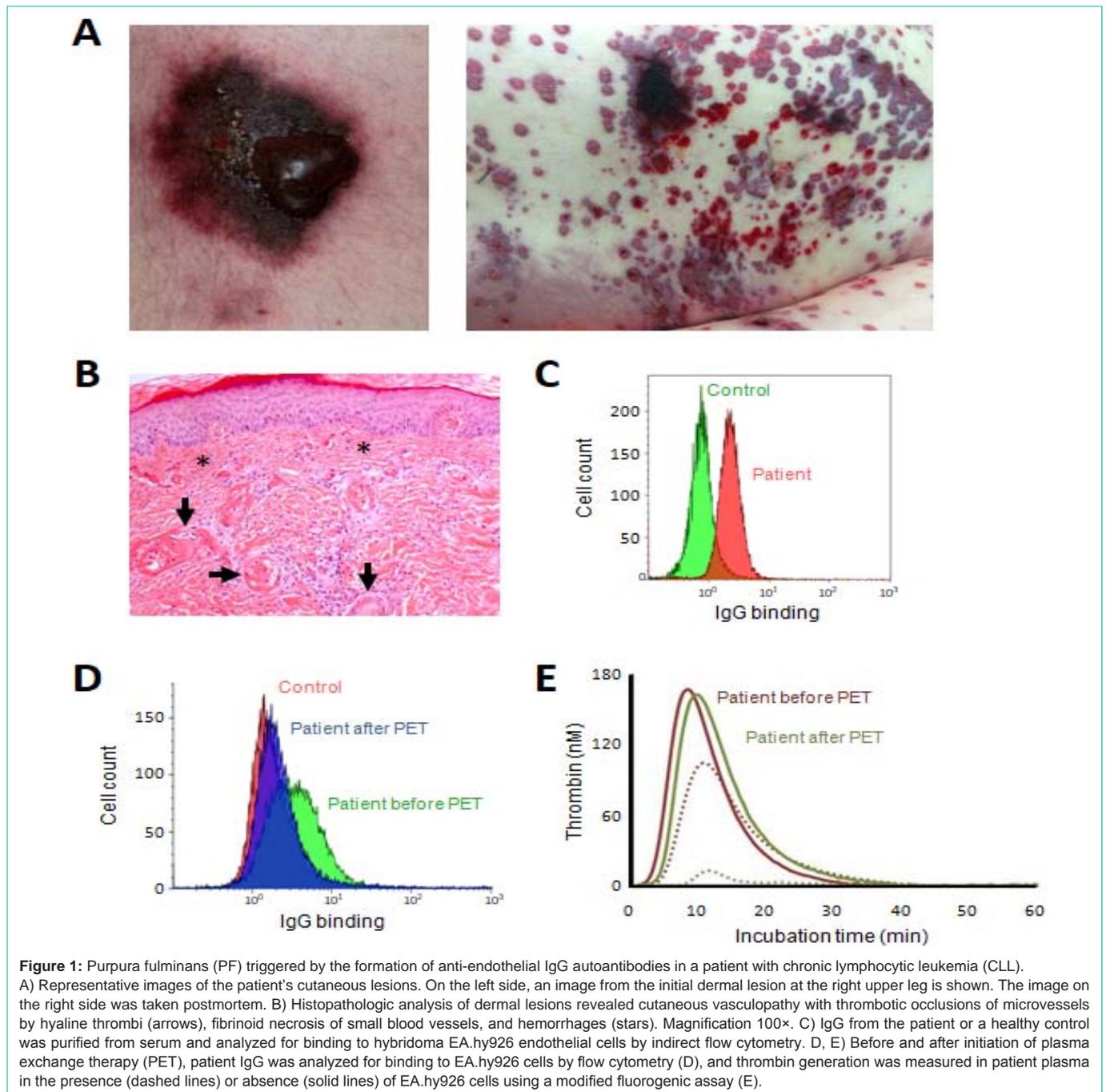
While specific CLL treatment was not indicated [2,3], the patient received high-dose prednisone (80mg OD), local debridement and antibiotic therapy with ampicillin/sulbactam. ASA was stopped and anticoagulation continued with enoxaparin in a platelet count-adapted dose of 60mg daily. Following consolidation of dermal lesions and normalization of inflammatory markers and platelet counts, the patient was discharged home from hospital.

Several days later, however, re-admission became necessary due to rapidly progressive necrotic skin lesions on both lower extremities. MRI revealed necrotic fasciitis, and emergency surgery was initiated.

To further investigate a potential autoimmune pathomechanism of PF, IgG was purified from patient serum and analyzed for binding to the endothelial hybridoma cell line, EA.hy926, by flow cytometry in comparison to control IgG. Patient IgG specifically bound to EA.hy926 cells (Figure 1C), a finding consistent with the formation of IgG autoantibodies against endothelial surface antigen(s), and thus further supporting autoimmune-mediated PF. Despite repeated rigorous debridement, therapeutic anticoagulation with enoxaparin and re-initiation of corticosteroid therapy, necrotic skin lesions progressed and became superinfected, eventually involving the whole integument (Figure 1A, right image).

Immunosuppressive therapy was continued with cyclophosphamide, and plasma exchange therapy (PET) was initiated based on the identification of anti-endothelial antibodies. Skin lesions did not further progress, and the patient's clinical condition temporarily improved. Consistently, binding of patient IgG to EA.hy926 cells was markedly decreased following PET (Figure 1D). A few weeks later, however, the patient died from septic shock during cyclophosphamide-induced neutropenia.

We performed additional experiments to further investigate an autoimmune mechanism interfering with the endothelial protein C-protein S-thrombomodulin (PC-PS-TM) system. To this end, we used a modified fluorogenic thrombin generation assay, which assesses thrombin generation in the presence of EA.hy926 cells, as previously described [4]. In this assay, thrombin generation is highly regulated by the EA.hy926 PC-PS-TM system. In patient plasma obtained after PET, addition of EA.hy926 cells almost completely abolished tissue factor (TF)-triggered thrombin generation (Figure 1E). In patient plasma obtained before PET, however, this effect was significantly less pronounced (Figure 1E), suggesting that patient IgG



attenuated the inhibitory effect of EA.hy926 cells on TF-triggered thrombin generation. Interestingly, patient IgG neither bound to immobilized soluble TM (sTM) in an in-house solid-phase ELISA (Supplementary Figure 2A) nor affected the anticoagulant activity of sTM in the thrombin generation assay in the absence of EA.hy926 cells (Supplementary Figure 2B).

## Discussion/Conclusion

PF is a rare, but life-threatening manifestation of DIC and characterized by thrombotic occlusion of cutaneous microvessels with subsequent hemorrhagic tissue necrosis [5,6]. In most cases, PF

is triggered by severe sepsis, but it is also associated with functional defects of the PC-PS-TM system [5,6].

Our patient, however, developed PF most likely triggered by the formation of anti-endothelial IgG autoantibodies, which functionally interfered with the PC-PS-TM system, in close association with the onset of CLL. Autoimmune complications are frequent in this hematological malignancy and affect up to 10% of all patients [2,7,8], but to the best of our knowledge this is the first report of autoimmune-mediated PF in a CLL patient.

In our patient, AIHA, ITP and PF were the initial findings of

the underlying lymphoproliferative disease. It is well-known that autoimmune complications in CLL can manifest at any disease stage [7] and might even precede overt CLL in a minority of patients [8]. Of note, especially non-hematological phenomena are linked to initial disease stages [2]. Nevertheless, vascular complications are rather uncommon in CLL [2], but have been reported in several cases [9-11]. Although the most predominant finding was leukocytoclastic vasculitis [9-11], Yamac et al. reported a case of PF in a CLL patient almost 20 years ago [12].

While in the latter case [12], the pathophysiology of PF remained obscure, we identified anti-endothelial IgG autoantibodies (Figure 1C), which functionally interfered with the PC-PS-TM system on EA.hy926 endothelial cells (Figure 1E). Under physiological conditions, the endothelial PC-PS-TM restricts coagulation to the site of vessel wall injury [13], but severe acquired or inherited deficiencies of PC and PS, including formation of inhibitory PC or PS autoantibodies in rare occasions, have already been linked to PF [5,6,14,15]. In these patients, isolated skin manifestations of the lower extremities sparing the acral regions are common [16]. A similar distribution pattern was found in our patient at initial presentation (Figure 1A), further supporting our hypothesis of autoimmune-mediated PF. Moreover, slightly elevated antinuclear antibody (ANA) titers compatible with an autoimmune epiphenomenon were measured (Table 1).

The pathophysiology of PF is complex and likely involves a plethora of mechanisms, including vascular congestion and disturbed endothelial integrity [17]. While severe sepsis and overt DIC are frequently observed [5,6,17], they were absent in the report from Yamac and colleagues [12] and in our patient at initial presentation (Table 1). It is thus tempting to speculate that the pathophysiology of PF in CLL patients might be distinct from other common conditions. Of note, we have recently demonstrated the serious consequences of the formation of a function-blocking anti-PS antibody in a woman with primary APS [18]. The patient suffered from overt DIC, recurrent arterial and venous thrombosis, and a highly painful livid skin erythema triggered by antiphospholipid antibody-mediated coagulation activation. Moreover, a multitude of procoagulant mechanisms have been delineated for anti-endothelial antibodies, such as release of pro-inflammatory cytokines, upregulation of adhesion molecules or induction of TF [19]. Interestingly, elevated von Willebrand factor levels were measured in our patient (Table 1). It is thus at least conceivable that additional IgG-related effects with activation of endothelial cells contributed to the procoagulant state, and that local or low-grade systemic coagulation activation was sufficient to trigger PF.

Although we could not identify the specific target of the anti-endothelial antibodies, we ruled out direct interference with TM (Supplementary Figure 2A). Moreover, the function of both, PC and PS, was intact as evidenced by the inhibitory effect of soluble TM on thrombin generation in patient plasma (Supplementary Figure 2B). In addition, both proteins were in the respective reference ranges (Table 1). Thus, we suggest the formation of anti-endothelial autoantibodies, which functionally interfered with the anticoagulant PC-PS-TM system independently of PS, PC and TM. Interestingly, recent studies have identified an increased risk for venous and arterial

thrombosis in patients with anti-endothelial antibodies directed against the endothelial protein C receptor (EPCR) [20,21]. Moreover, in a 74-year-old woman, the idiopathic development of such an antibody was associated with thrombotic skin necrosis [22]. Routine laboratory assessment of PC, PS and TM was unremarkable in this patient [22]. It is thus tempting to speculate that the EPCR might have been a potential target of the anti-endothelial IgG autoantibodies in our patient.

Current treatment guidelines still recommend corticosteroids in patients with isolated CLL-associated autoimmune cytopenia (AIC) as first line therapy, but specific recommendations for treatment of other CLL-associated autoimmune phenomena are lacking [2,3]. Second-line treatment options include anti-CD20 monoclonal antibodies, such as rituximab, or immunosuppressive agents, like cyclophosphamide [2,3]. Several studies have also indicated promising efficacy and safety for the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, in treatment of both, corticosteroid-responsive and refractory AIC [23-25], but at the time point of our case presentation, these data have not yet been available, and ibrutinib was not approved for treatment-naïve CLL patients. Our patient, however, showed at least temporarily a good clinical response to immunosuppressive therapy with both, corticosteroids and cyclophosphamide, in combination with PET. We also discussed escalation of immunosuppressive therapy by the addition of rituximab, but the patient died before rituximab could be initiated.

Taken together, autoimmune-mediated PF may occur in CLL patients in rare occasions. Rapid identification and initiation of adequate therapy are of utmost clinical importance in these patients. In addition to therapeutic anticoagulation and local wound management, antibody depletion by immunosuppressive therapy and PET might be a promising therapeutic approach.

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