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# Diagnosis of catastrophic anti-phospholipid syndrome in a patient tested negative for conventional tests

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## ABSTRACT

*Catastrophic antiphospholipid syndrome (CAPS) is a severe variant of APS, characterised by clinical evidence of multiple organ involvement developing over a very short period of time, histopathological evidence of multiple small vessel occlusions and laboratory confirmation of the presence of aPL (lupus anticoagulant and/or anticardiolipin antibodies and/or anti-Beta2-glcyoprotein I antibodies). Here we report a case of a 39-year-old woman patient who developed a CAPS which was negative to the conventional aPL but positive for aPL in thin layer chromatography immunostaining and vimentin/cardiolipin antibodies by ELISA test. The patient was treated with high doses of glucocorticoids, intravenous immunoglobulins plasma exchange and immunoadsorbent apheresis with a significant improvement of the ischaemic lesions of the hands even though the necrosis of the feet progressively worsened. As a result, the patient underwent partial surgical amputation of the feet. To our knowledge, this is the first ever reported case of CAPS diagnosed by means of thin layer chromatography immunostaining and vimentin/cardiolipin antibody ELISA test.*

## Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterised by the presence of clinical features such as arterial or venous thrombosis and pregnancy morbidity associated to persisent positive antiphospholipid antibody (aPL) tests: anti-cardiolipin (aCL) antibodies, anti-β2 glycoprotein I antibodies (aβ2GPI), and lupus anticoagulant (LA) (1). The diagnosis of seronegative (SN-) APS has been suggested for patients with clinical manifestations indicative of APS but with persistently negative results in the commonly used assays to detect aCL, aβ2GPI, and LA (2). Catastrophic antiphospholipid syndrome (CAPS) is a severe variant of APS, characterised by small-vessel thrombosis developing over a short period of time in multiple organs, resulting in multiorgan dysfunction and often failure, with very high mortality rates (3). We report here the first case ever described of CAPS diagnosed by thin layer chromatography (TLC) immunostaining and vimentin/cardiolipin antibody ELISA test.

## Case report

A 39-year-old Philippine woman with a two-week history of pain in her limbs, cyanosis, and ischaemic skin lesions of the toes was admitted to our Department. Her past medical history included autoimmune thyroiditis and a foetal loss beyond the 10$^{th}$ week of gestation one year before. The patient presented peripheral cyanosis predominantly localised in the fingers and painful ischaemic skin lesions of the fifth right toe and third and fifth left toes. Feet and hands were extremely cold. Laboratory tests showed leucocytosis (35×10$^9$/L, Neutrophils 93%) and ANA with a speckled pattern, whereas anti-dsDNA, anti-ENA, p-ANCA, c-ANCA, aCL IgG/IgM, aβ2GPI IgG/IgM antibodies, and LA, C3 and C4 fractions were in normal range, and cryoglobulins undetectable; thrombophilic screening showed no mutation of MTHFR, Factor II, and Factor V; antithrombine III, homocysteine, protein S, and protein C were in the normal range. Infections from HBV, HCV, CMV, EBV, HIV, and syphilis were ruled out.

During hospitalisation, despite treatment with enoxaparin sodium, and methylprednisolone (20 mg *i.v.* daily) a rapid progression of the necrosis was observed: more severe changes involved the toes. CT angiography showed occlusion (3 cm) of the right interosseous artery, segmental stenosis of the left popliteal artery (10 cm) and distal occlusion of anterior tibial artery (Fig. 1). A chest CT displayed thrombotic deposits in the lower lobar arteries and in the segmentary and sub-segmentary branches (Fig. 1). Perilesional toe skin biopsy displayed thrombosis occlusions of superficial cutaneous and subcutaneous vessels and deep vessels without evidence of vasculitis.

Since all known causes of thrombophilia were ruled out, a SN-CAPS was hypothesised. In our patient, serum TLC immunostaining was performed and aCL and anti-phosphatidylethanolamine antibodies were found (Fig. 2)

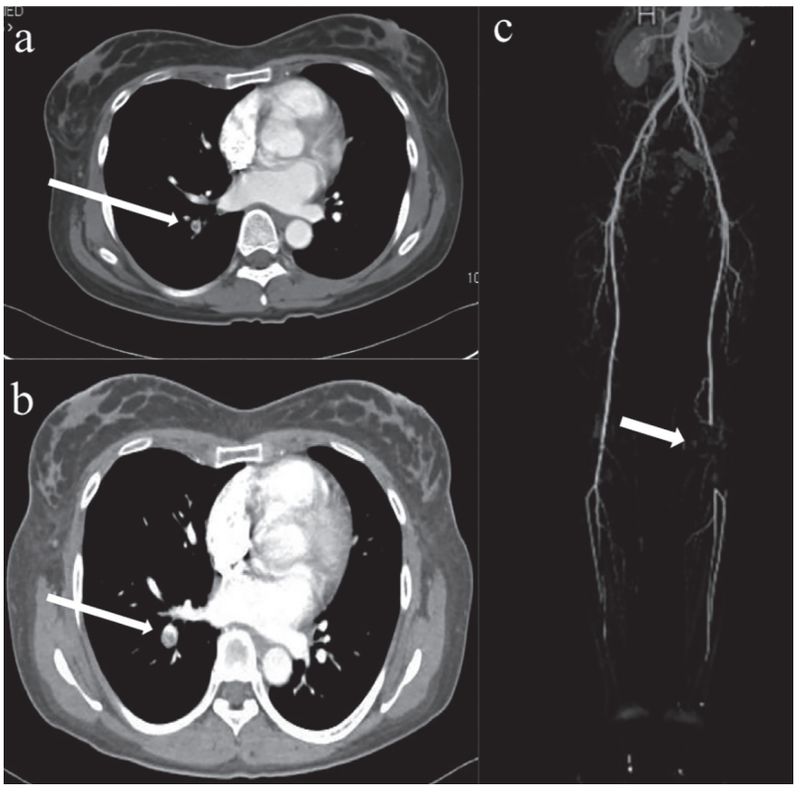


**Fig. 1.** Chest computed tomography (CT) and CT angiography of lower extremity.
**a-b**. Chest CT displayed thrombotic deposits in the lower lobar arteries and in the segmentary and sub-segmentary branches.
**c**. CT angiography of lower extremity showed segmental stenosis of the left popliteal artery (10 cm) and distal occlusion of anterior tibial artery (*arrows*).

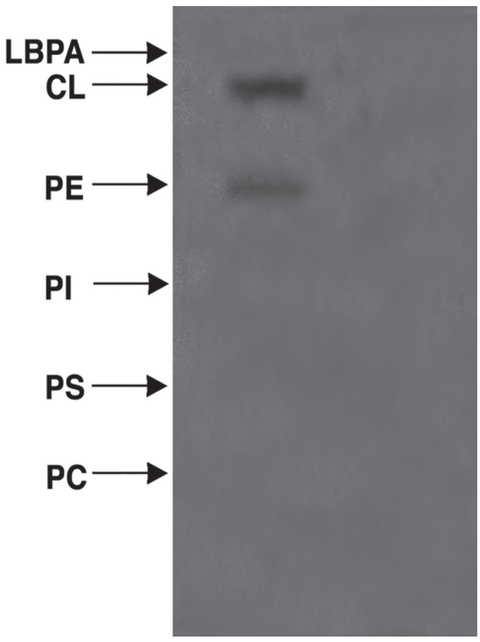


**Fig. 2.** TLC Immunostaining CL, Lyso(bis)phosphatidic acid (LBPA), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylcholine (PC) were run on aluminium-backed silica gel 60 high performance thin layer chromatography (HPTLC) plates. Chromatography was performed in chloroform: methanol: CH3COOH: water (100:75:7:4) (v:v:v:v) (5). The dried chromatograms were soaked for 90 sec in a 0.5% (w:v) solution of poly(isobutyl methacrylate) beads dissolved in hexane. After blocking, the plate was incubated with patient's serum diluted 1:100 in 1% BSA/PBS. After washing, bound antibodies were visualised with HRP-conjugated anti-human IgG and immuno reactivity was assessed by the ECL Western blotting system. CL and PE were detected.

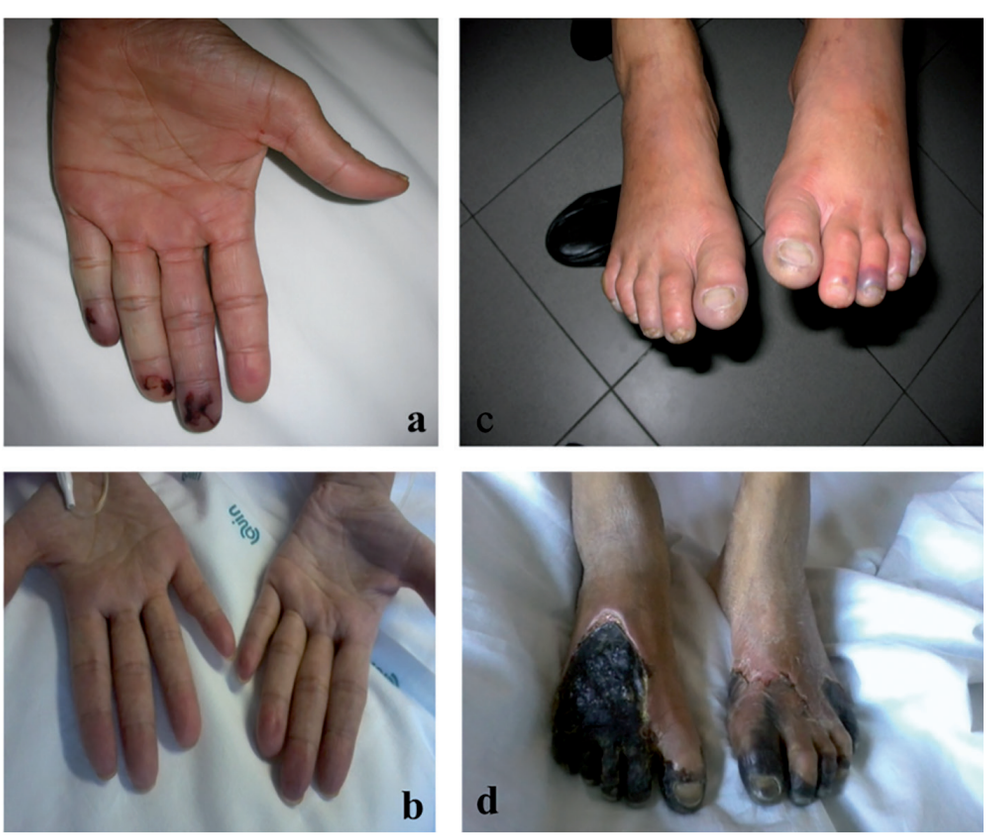


**Fig. 3.** The progression of ischaemic lesions.
**a-b.** Improvement of ischaemic lesions of the hands during hospitalisation.
**c-d.** Progressive worsening of the ischaemic lesions of the feet during hospitalisation.

with the results being confirmed twelve weeks apart. In addition, antibodies against vimentin/cardiolipin were positive tested by ELISA. The patient was then treated with intravenous methylprednisolone (1 g daily for three days) followed by 80 mg daily. Therapeutic angiography with urokinase was then performed with no improvement. Subsequently, she was treated with five cycles of plasma exchange followed by high doses of intravenous IgG and later by five cycles of immunoadsorbent apheresis. After immunoadsorbent apheresis treatment a significant improvement of the ischaemic lesions of the hands was obtained even though the necrosis of the feet progressively worsened (Fig. 3). As a result, the patient underwent partial surgical amputation of the feet. On discharge, aspirin and oral anticoagulant were prescribed. Currently, after a three-year follow-up no recurrent thrombosis has been observed and she tested positive again for aCL by TLC immunostaining and for vimentin/cardiolipin antibodies.

## Discussion

Antiphospholipid syndrome is an autoimmune disorder characterised by pregnancy losses and recurrent arterial and/or venous thrombosis in association with the presence of aPL; different clinical features have been suggested to be associated to a specific autoantibody subset (1, 4). CAPS is a severe, sometimes fatal, variant of APS, characterised by clinical evidence of multiple organ involvement developing over a very short period of time, histopathological evidence of multiple small vessel occlusions and laboratory confirmation of the presence of aPL (3). Patients with CAPS represent less than 1% of all patients with APS (3). Early and correct diagnosis and prompt treatment are needed to reduce mortality rate.

On the basis of the revised Sapporo criteria, patients with clinical manifestations highly suggestive of APS but persistently negative for consensus aPL antibodies cannot be classified as having APS: Hughes *et al*. have proposed the term of seronegative APS (2).

Previous obstetric history of our patient would suggest an APS and the new onset multi-organ involvement supported the diagnosis of CAPS but she was persistently negative for conventional aCL, aβ2GPI ELISA and LA; however, aPL reactivity (*i.e*. aCL, anti-phosphatidylethanolamine antibodies) was detected by TLC immunostaining and ELISA (vimentin/cardiolipin antibodies) (5, 6).

Recently, we showed that SN-APS represents a serological mosaic, in which antibodies against different antigenic targets may be detected using "new" antigenic targets (mainly vimentin/cardiolipin in 45% of patients) or a different antigen exposure by methodological approaches different from traditional techniques (mainly TLC immunostaining in 54.2% of patients) (7).

In our patient, the positivity of aPL in TLC immunostaining and vimentin/cardiolipin ELISA test allowed us to perform a diagnosis of CAPS and consequentially to start treatment.

In conclusion, this case confirms that TLC immunostaining and anti-vimentin/cardiolipin antibody test can identify the presence of aPL in patients with clinical features suggestive of APS not ascertained by traditional tests for aPL; such identification could have a major impact on the prognosis and therapeutic approach.

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