Introduction

Antiphospholipid syndrome (APS) is an autoimmune thrombophilic condition occurring due to the presence of antibodies that recognize phospholipid binding proteins ***(Abeysekera et al., 2015).***

APS characterized by arterial and/or venous thrombosis, recurrent pregnancy loss, and persistently positive antiphospholipid antibodies. It could be life threatening as in the case of catastrophic APS where multi organ failure is observed ***(Bittar and Uthman, 2014).***

The disease can be primary or secondary. Primary APS occurs in the absence of any other related disease, secondary APS occurs with other autoimmune diseases, such as systemic lupus erythematosus ***(Ioannidis et al., 2014).***

Primary APS can be a difficult diagnosis in the absence of typical clinical features. The presentation can vary, mimicking many other medical conditions ***(Stichlberger et al., 2014).*** Regarding secondary APS , Up to 40% of patients with systemic lupus erythematosus have test positive for APS antibodies, but only half of these patients go on to develop overt thrombosis or miscarriages ***(Giles and Rahman, 2009).***

Obstetric APS and recurrent pregnancy losses were believed to be the result of different mechanisms acting on placental cells and endometrial tissues. Thrombosis, inflammation, and immunomodulations are thought to affect placental cells ***(Marchetti et al., 2013).***

The 2006 revised APS classification criteria remarked on some clinical features as non-criteria features, which included cardiac valve involvement, livedo reticularis, thrombocytopenia, APS nephropathy and non thrombotic central nervous system manifestations ***(Gómez et al., 2014).*** APS antibodies can be detected in so called asymptomatic positive carriers who display the persistent presence of medium to high levels of antibodies but in whom no clinical events can be documented ***(Meroni et al., 2014).***

APS antibodies are autoantibodies directed against phospholipid bound proteins, particularly the β2-glycoprotein I (β2GPI). It is believed that thrombosis in APS follows a two hit hypothesis where the first hit disrupts the endothelium and the second hit potentiates thrombus formation ***(Bittar and Uthman, 2014).***

Anti-cardiolipin (aCLs), anti-β_2 glycoprotein I (anti-β_2GPI) and lupus anticoagulant (LA) assays are not only the formal diagnostic and classification laboratory tools but

also parameters to stratify the risk to develop the clinical manifestations of the syndrome ***(Meroni et al., 2014).***

Data on anti-β_2GPI antibodies are more controversial, maybe because the assay is less standardized. Recent studies have demonstrated that patients with multiple positive test results (that is: LA, aCLs and anti-β_2GPI autoantibodies particularly of the IgG isotype) display a much higher risk for developing clinical complications ***(Pengo et al., 2013).***

The management of APS thrombosis constitutes either primary thromboprophylaxis or secondary thromboprophylaxis. Primary thromboprophylaxis represents treating APS antibodies positive patients with no previous thrombosis, while secondary thromboprophylaxis represents treating APS patients with previous thrombotic events. The mainstay of treatment is currently anticoagulation, though multiple immunomodulatory therapies are on the rise ***(Bittar and Uthman, 2014).***

Aim of the Study

To study the demographic, clinical and immunological characteristics in a cohort of patients of Antiphospholipid Syndrome (APS).

Section 1: Basic Aspects

Definition:

Antiphospholipid syndrome (APS) is the association of vascular thrombosis and/or pregnancy morbidity with one or more pathogenic autoantibodies that are collectively referred to as antiphospholipid antibodies (aPLs) namely lupus anticoagulant (LA), anticardiolipin antibodies (aCL), or anti-β2 glycoprotein-I (β2GPI) antibodies. A patient must have at least one clinical criterion and one laboratory criterion to be classified as having APS ***(Miyakis et al., 2006).***

APS has been described as primary APS when it occurs in the absence of any features of other autoimmune disease, and as secondary in the presence of other autoimmune diseases, mainly systemic lupus erythematosus (SLE) ***(Sciascia et al., 2015).***

Historical Background:

The association of thrombosis, recurrent fetal losses and thrombocytopenia with the LA phenomenon was observed in early publications in the 60's, but it was not clear until 34 years ago when Graham R.V. Hughes linked major cerebral disease (e.g. recurrent strokes) with

abortions and the LA in an editorial published in the British Medical Journal ***(Hughes, 1983).***

A major advance came in the early 1990s with the simultaneous recognition by different groups that aPL required a plasma protein cofactor to bind cardiolipin on ELISA plates. B2GP1 was identified as this cofactor. Since then, a number of cofactors including prothrombin, have been described ***(McNeil et al., 1990)***.

The original concept of the APS, however, has been expanded over the years and now includes diverse complications as heart valve lesions, adrenal insufficiency and even avascular necrosis of bone among many others ***(Petri, 2000).***

Epidemiology:

Prevalence of the aPL in the general population ranges between 1 and 5%. However, only a minority of these individuals develop the APS manifestations. In a literature review focused in the prevalence of APS in the general population with pregnancy morbidity, stroke, myocardial infarction (MI) and deep vein thrombosis (DVT), The authors estimated that APS are positive in approximately 13% of patients with stroke, 11% with MI, 9.5% of patients with DVT and 6% of patients with pregnancy morbidity ***(Andreoli et al., 2013).***

APS is classified as primary or secondary, depending on its association with other autoimmune disorders. Primary APS is diagnosed in patients demonstrating the clinical and laboratory criteria for the disease without other recognized autoimmune disease. Secondary APS is diagnosed in patients with other autoimmune disorders, such as SLE ***(Fischer et al., 2012)***.

Among SLE patients, 30% to 40% of patients have aPL antibodies, but only approximately 10% have APS. In patients with APS, researchers later found that antibodies directed against membrane anionic phospholipids anticardiolipin [aCL] antibody, lupus anticoagulant (LA), or associated plasma proteins (usually beta2 glycoprotein 1) are persistently elevated. But, the presence of aCL antibodies increases with age, often with no clinical manifestaions ***(Keeling et al., 2012).***

A true APS diagnosis requires aberrant laboratory work on 2 occasions more than 12 weeks apart plus one or more episodes of vascular thrombosis or obstetrical complication ***(Taraborelli et al., 2012)***.

Catastrophic antiphospholipid syndrome (CAPS), which is characterized by clots in multiple small vascular beds and leads to multiorgan failure with high mortality, develops in a small subgroup of patients in which

histopathological confirmation is sought, thrombosis should be present without evidence of inflammation in the vessel wall ***(Bill et al., 2013).***

The actual frequency of APS in the general population is unknown. One to 5% of healthy individuals have aPL antibodies, aCL antibodies tend to be found more frequently in elderly persons; thus, positive titer results should be interpreted with caution in this population ***(Lockshin et al., 2006).***

APS affects young patients in the most productive years of their life, and the consequences of organic or tissue damage involve a decrease in health-related quality of life (HRQoL). While acute disease manifestations of APS are well known, information on the long-term prognosis and damage in affected patients is still limited ***(Alba et al., 2016).***

Etiology:

Family studies suggest a genetic predisposition to APS. It appears that this genetic predisposition is in part accounted for by the HLA system, the most consistent associations being those with DR4 and DRw53. Furthermore, it appears that LA and aCL antibodies are both associated with the same HLA antigens ***(Sebastiani et al., 2016).***

Population studies suggest that HLA genes have a role in conferring susceptibility to develop primary APS, with some differences in different ethnic groups. Other genes, outside the MHC, give their contribution to the development of this autoimmune syndrome, such as IRF5, STAT4 and those related to inherited thrombophilia - factor V Leiden and G20210A prothrombin polymorphisms ***(Sebastiani et al., 2016).***

B2GPI represents the major antigenic target for aPL, β_2GPI contains five homologous domains, with domain I (DI) being identified as the main antigenic epitope for pathogenic anti-β_2GPI ***(Raimondo et al., 2015).***

It has been reported that APS patients show an elevated amount of total β_2GPI as compared with healthy donors and other autoimmune disease control groups. Moreover, in healthy individuals, β_2GPI mainly exists in its biochemically reduced form whilst the post-translational oxidized structure (fish-hook linear configuration) is elevated in patients with APS ***(Raimondo et al., 2015).***

Antibodies targeting β_2GPI-DI (aDI) represent a key pathogenic sub-population of aPL, thus their detection may allow the identification of patients at highest clinical risk ***(Raimondo et al., 2015).***

Some investigators showed that primary antiphospholipid syndrome (PAPS) is characterized by a deficiency in immunoregulatory pathways, a phenomenon recently implicated in the pathogenesis of autoimmune diseases. PAPS patients with high titers of aPL IgG antibodies were characterized by decreased systemic levels of TGF-β_1 and its impaired production in vitro, suggesting impaired immunoregulation and enhanced adaptive autoimmune responses leading to the production of aPL antibodies ***(Jakiela et al., 2015).***

The pathophysiology of thrombosis in APS and CAPS remains incompletely understood. Whether the binding of aPLs is primarily a cause or a consequence of endothelial cell activation is unclear, but complement cascade activation with subsequent C5b-9 deposition leads to the generation of a potently pro-thrombotic environment ***(Nester and Brophy, 2013).***

In APS, large vessel thrombosis can be seen, but in CAPS, most of the vascular thrombosis involves the microvasculature, analogous pathologically to prototypic pauci inflammatory microthrombotic syndromes such as thrombotic thrombocytopenic purpera (TTP) and a hemolytic uremic syndrome (HUS) ***(Nester and Brophy, 2013).***

In addition, membrane attack complex (MAC) at sublytic concentrations stimulates endothelial cells to express adhesion molecules and enhances tissue factor (TF) procoagulant activity primarily via the inactive terminal C complex. Furthermore, sublytic C5b-9 deposition induces apoptosis by up-regulating the interferon regulatory factor-1 caspase 8/caspase 3 pathways, an event that leads ultimately to endothelial cell detachment and subsequent activation of the indirect clotting pathway via exposure of collagen ***(Liu et al., 2012).*** Inhibition of the MAC assembly has been shown to protect against thrombosis in mouse models of immune mediated thrombotic microangiopathy ***(Nester and Brophy, 2013).***

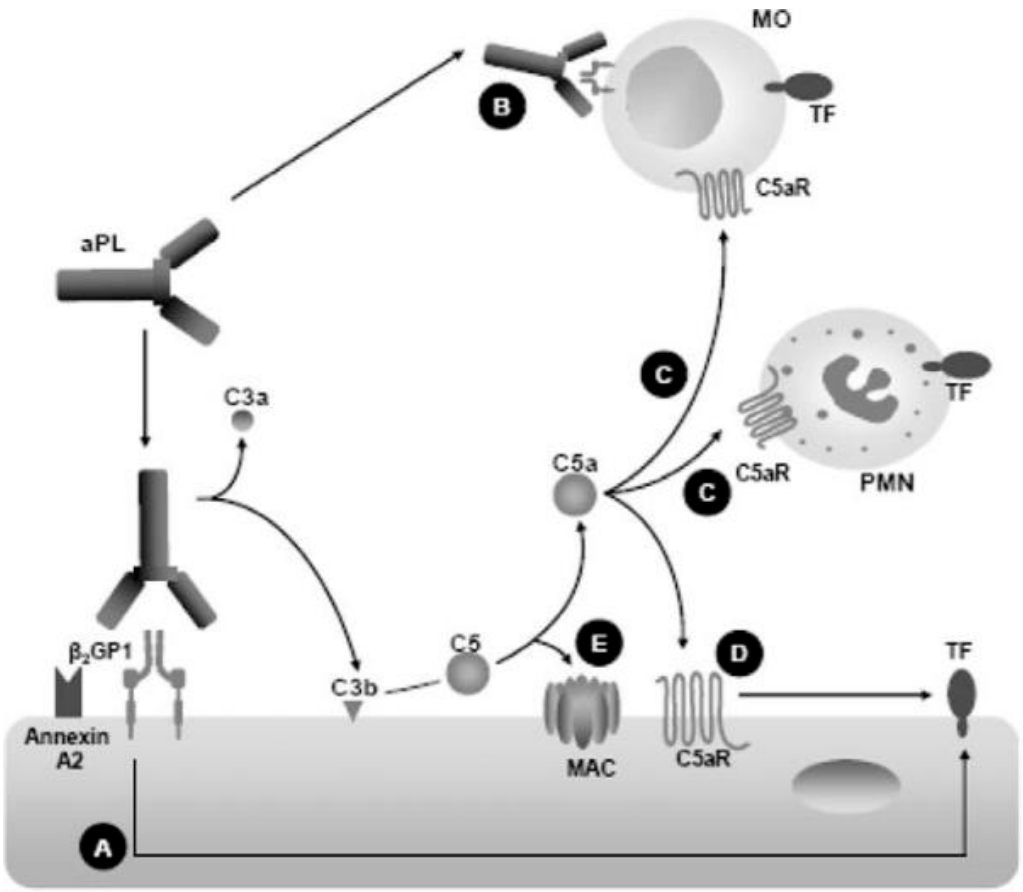

Figure (1): *Different mechanisms explaining aPL-induced TF expression. Antibodies against β_2GPI induce TF expression on endothelial cells (A) and on circulating blood monocytes (B). The activation of complement can cause an increase in the expression of functionally active TF. Complement split product C5a induces TF expression in leukocytes (monocytes and neutrophils) and on endothelial cells (D). The terminal or membrane attack complex (MAC) activates endothelial cells resulting in expression of TF (E)* ***(Girardi and Mackman, 2008).***

Pathogenesis:

Thrombosis is the major disease mechanism, enhanced mainly by activating endothelial cells, monocytes, platelets, coagulation and complements pathways in addition to inhibiting fibrinolytic and anticoagulation pathways. However, vasculopathy enhanced mainly by severe intimal hyperplasia is the major contributor to arterial stenotic lesions and can also play a role in vascular occlusions and pregnancy morbidity ***(Merashli et al., 2015).***

Such vasculopathy has been recognized as a major contributor to large artery occlusion rather than thrombosis or vasculitis. These lesions are described in many vascular territories, including the coronary, carotid or mesenteric arteries ***(Canaud et al., 2015).***

A study performed on patients with primary and secondary APS nephropathy, which is mainly mediated by vasculopathy rather than thrombosis, revealed that the activation of mammalian target of rapamycin complex (mTORC) enzyme stimulates intimal hyperplasia, leading to the formation of the chronic vascular lesions as seen in APS. In vitro, aCL and anti-b2GPI activated the phosphatidylinositol 3-kinase (PI3K) – AKT pathway and

subsequently mTORC, leading to proliferation of vascular endothelial cells ***(Canaud et al., 2014).***

The development of intimal hyperplasia is associated with the activation of both mTORC1 and mTORC2 in the endothelial cells leading to their proliferation and the proliferation of the surrounding vascular smooth muscle actin cells (VSMC). The strongest activation was detected in the vessels with the most prominent lesions from catastrophic antiphospholipid syndrome (CAPS) autopsy cases ***(Canaud et al., 2014).***

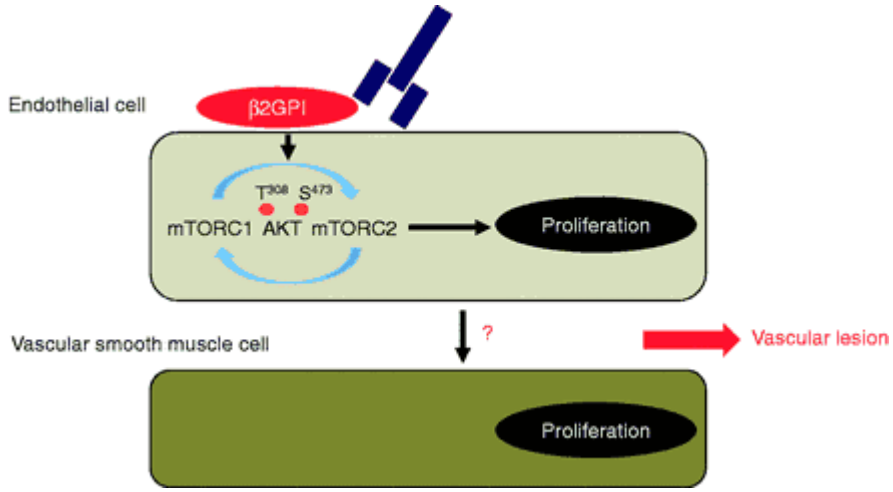

Figure (2): *Antiphospholipid antibodies recruit the AKT/mTORC pathway in endothelial cells that leads in turn to endothelial cell proliferation, synthesis of one or several mitotic factor(s) targeting the surrounding vascular smooth muscle cells* ***(Canaud et al., 2015).***

The physiopathology of the thrombosis in APS patients is thought to be the consequence of a ‘two-hit’ model. Indeed, the ‘first hit’ is an injury that disrupts the endothelium, and the ‘second hit’ potentiates thrombus formation in patients with APS ***(Meroni et al., 2012).***

The two-hit hypothesis fits well with the clinical observation that thrombotic events occur only occasionally despite the persistent presence of aPL. Consistently, most APS patients experiencing a thrombotic event present concomitant cardiovascular risk factors ***(Chighizola et al., 2015).***

In particular, hypertension has emerged as an independent predictor for a first thrombotic event in aPL carriers. Infections have also been found to precede APS onset, and their frequency can be as high as 24% in CAPS patients. Consequently, a careful assessment of cardiovascular status should be accomplished in all aPL-positive individuals: age, diabetes, arterial hypertension, dyslipidemia, obesity, smoking, sedentary lifestyle, hyperhomocysteinemia, protein C, protein S, and ATIII deficiency, Factor V Leiden and prothrombin mutations, prolonged immobilization, surgical procedures, and oestrogen use ***(Chighizola et al., 2015).***