Immunological Causes Associated to Foetal Death: An Update

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Abstract: Up to 5% of couples at reproductive age suffer from at least two consecutive miscarriages, and around 1% experience three or more consecutive gestational losses, most of them during the first ten weeks of pregnancy, resulting in a significant personal, social and economic public health burden. The pregnancy losses occurring after the twentieth gestational week, namely foetal death or stillbirth, are even more emotionally and psychologically devastating for the couple. In almost half of these cases there is not any known cause of foetal death. In the last years, significant progress in the identification and treatment of the risk factors associated to foetal death has been made: advanced maternal age, obesity, pre-existing maternal diseases or acquired infections during pregnancy; and associated immunological disorders. Among the latter, the most common cause is the obstetric antiphospholipid syndrome, autoimmune disease that can present with placental infarction, thrombosis in the utero-placental circulation and foetal loss. Here we review the evidence of other immunological disorders that have been associated with intrauterine foetal death: non-diagnosed celiac disease, peripheral expansion of natural killer cells and diverse autoimmune or inflammatory abnormalities. The detection and control of such abnormalities in women with history of prior foetal death may lead to a successful subsequent pregnancy.

Keywords: Foetal loss, antiphospholipid syndrome, immunological causes.

INTRODUCTION

Definition of Foetal Death: Incidence

The diverse existing definitions of the term foetal death have contributed to the wide variation in the incidence among countries and over time. The American Academy of Pediatrics classified foetal death as “early” when it occurs from 20 to 27 weeks gestation and “late” at/after 28 weeks gestation. The term stillbirth is also used to describe foetal death at 20 or more weeks gestation [1]. Intrauterine foetal death refers to babies with no signs of life *in utero*. Likewise, the WHO defines late foetal death as third trimester stillbirths with ≥1,000g birthweight or ≥28 completed weeks of gestation or ≥35 cm body length [2].

Foetal death is common, with reported rates of 1 in 200 babies born dead [3]. In a recent publication, it was estimated that the global number of foetal deaths worldwide was 2.64 million in 2009, compared to 3.03 million in 1995. Thus, the overall rate of foetal death was reduced by 14.5%, from 22.1 per 1,000 births in 1995 to 18.9 per 1,000 births in 2009. Hence, the estimated foetal death rate has fallen about 1.1% per year from 1995 to 2009, but still represents an significant public heath problem. In Spain, between 2007 and 2009, there were a total of 1,450 foetal deaths among 500,310 pregnancies, with a foetal death rate of 2.9 per 1,000 births [4].

Moreover, the miscarriages occurring after the 20th gestational week are emotionally and psychologically devastating for the couple. In almost half of these cases there is not any known cause. In this regard, stillbirth is a serious complication of pregnancy, which carries a risk of depression, anxiety and post-traumatic stress in the following months.

IMMUNOLOGICAL CAUSES OF FOETAL DEATH

In the last years, a significant progress in the identification and treatment of the risk factors associated to foetal death has been made. High maternal age, obesity, pre-existing maternal diseases or acquired infections during pregnancy, as well as associated autoimmune and immune-based disorders are known risk factors contributing to foetal death. Among the latter, the most common cause is the obstetric antiphospholipid syndrome (APS), autoimmune disease that can present with placental infarction, thrombosis in the utero-placental circulation and foetal loss. Here we review the current evidence of other immunological disorders that have been associated with intrauterine foetal death, namely, undiagnosed celiac disease, peripheral and uterine expansions of natural killer cells and diverse autoimmune or inflammatory abnormalities. The detection and control of such abnormalities in women with history of prior foetal death may favour a

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successful outcome of a subsequent pregnancy both for the mother and the foetus.

Antiphospholipid Syndrome

APS is an autoimmune systemic disease characterized by vascular thrombosis (arterial or venous), thrombocytopenia and complications during pregnancy that include miscarriages, foetal death, pre-eclampsia and intrauterine growth restriction (Evidence Level 1++). In clinical practice, the following antibodies are evaluated: anti-cardiolipin (aCL), anti-β2 glycoprotein I (β2GPI), both IgG and IgM antibodies, and lupus anticoagulant (LA). Antiphospholipid antibodies (aPL Ab) are a heterogeneous group of antibodies binding to several phospholipids (phosphatidylserine, cardiolipin, phosphatidylinositol) and phospholipid-protein complexes. The proteins, also called cofactors involved are: β2GPI, prothrombin and activated protein C. In particular, β2GPI and prothrombin account for more than 90% of all the antibody binding activity [5]. There are other antigens identified in APS patients: plasminogen-activator inhibitor-1, plasmin, annexin A2 and thrombin. Annexin 2 (A2) is a profibrinolytic receptor on the surface membrane of endothelial cells and monocytes that acts as a cofactor for plasmin generation [4,6].

The two mayor risk factors for foetal death are high levels of anti-cardiolipin IgG Ab and a previous history of foetal loss. These patients have more than 80% risk for subsequent foetal death [7].

In the last years, the β2-GPI role has become more significant in clinical practice since it has been identified as the most important antigen in APS. It is a highly glycosylated protein present in the plasma, and belongs to the complement control protein (CCP) superfamily. Normally, β2-GPI in plasma circulates in a circular conformation with a low affinity for anionic surfaces and the epitope for the antibodies is shielded from plasma [8, 9]. When it encounters cells that expose anionic phospholipids on their surface like endothelial cells, monocytes, platelets and trophoblasts cells, β2-GPI will bind to these phospholipids, making a conformational change. This change exposes the epitope for the antibodies, and stabilizes β2-GPI in its hockey stick-like conformation. The binding of the autoantibodies result in the generation of bivalent complexes that have much stronger affinity for anionic phospholipids expressed on these cells [9]. There are many different receptors that bind β2-GPI: toll-like receptor (TLR)-2, TLR4, annexin 2, apolipoprotein E receptor 2 (ApoER2) and glycoprotein Ib alpha (GPIbα) (Table **1**)[10].

Several mechanisms have been proposed to explain the pathogenic features of aPL Ab. One of them states that they can bind to receptors (ApoER2 and GPIbα) present in the platelet surface and promote platelet activation and aggregation, inducing thrombosis and thrombocytopenia, that are frequent feature of APS (Figure **1**) [5, 11].

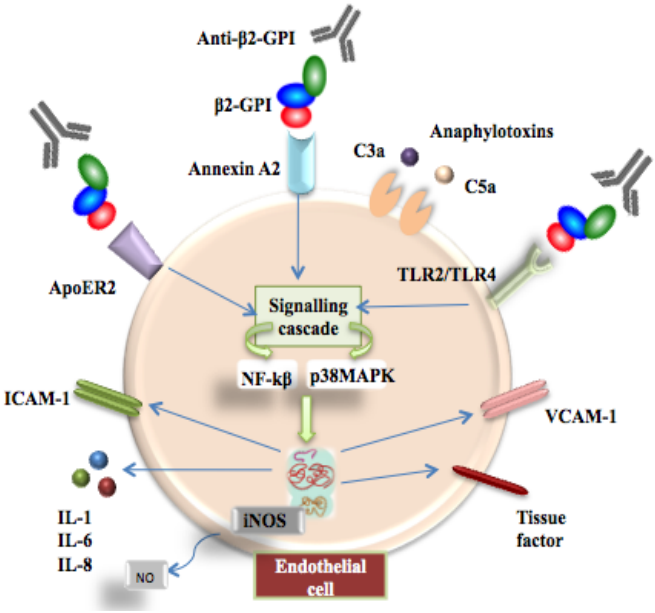

Figure 1: Effects of antiphospholipid antibodies on endothelial cells. Adaptated from [49].

Another mechanism is the interaction with endothelial cells by binding to several receptors as annexin A2, and thus promoting expression of adhesion molecules (ICAM-1 and VCAM-1) and monocyte adhesion. In addition, there is a tissue factor (TF) up-regulation, that could contribute to the prothrombotic effects of aPL Ab (Figure **2**) [5]. Moreover, vascular endothelial growth factor (VEGF) may stimulate TF expression in monocytes, because its expression is increased in monocytes of APS patients when compared with controls [12].

Holers *et al.* proposed that platelet and endothelial cell activation alone cannot explain foetal loss or growth retardation [13]. They pointed out that complement activation is also necessary, because it increases the production of mediators such as C3a, C5a, and C5b-9 MAC, which promote further platelet

and endothelial cell activation, leading to inflammation, tissue damage, and finally, foetal loss [13].

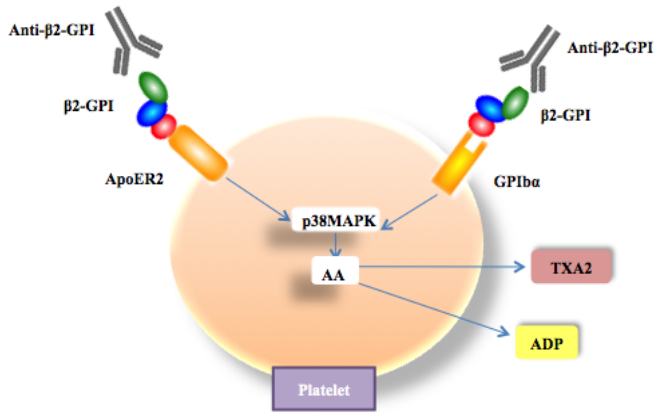

Figure 2: Platelet activation by anti-β2 antibodies. Adaptated from [49].

From a clinical point of view, aPL Ab are reported as the most frequent acquired risk factor for recurrent pregnancy loss. Different pathogenic mechanisms have been suggested to play a role in APS-associated obstetrical manifestations (Table **2**): first, the occurrence of thrombotic events. Intraplacental thrombosis with maternal–foetal blood exchange impairment was suggested to be the main pathogenic mechanism in recurrent pregnancy loss. aPL Ab may induce a procoagulant state in the placenta through several mechanisms. One of them is the breakage of the anticoagulant annexin A5 shield on trophoblasts by aPL Ab, mainly by anti-β2-GPI antibodies. Rand *et al.* reported that women with aPL have significantly lower distribution of annexin A5 covering the intervillous surfaces of their placentas in comparison with normal controls [14, 15]. Second, defective placentation is a relevant pathogenetic mechanism in APS. In addition to thrombosis, aPL Ab binding to trophoblasts leads to cellular injury, apoptosis, inhibition of proliferation and syncytia formation, decreased human chorionic gonadotrophin (hCG) production and defective invasiveness. The high expression of β2-GPI on the trophoblast cell membranes that binds to phosphatidylserine, may explain the aPL/anti- β2-GPI antibody placental tropism. And third, inflammatory local events also take place. Changes in the maternal immune response occur during natural pregnancy in order to adapt and tolerate the foetus. There is evidence for the predominance towards Th2 and regulatory (Treg) responses and for a balance towards anti-inflammatory mediators. In this regard, it is widely accepted that acute inflammatory events are generally responsible for a negative pregnancy outcome [16, 17].

Table 1: Cells and Receptors Involved in APS Pathophysiology

Cell	Receptor	Mechanism
Platelet	ApoER2, GPIbα	Platelet activation, aggregation and thrombosis
Endothelial cells	Annexin 2, TLR2/TLR-4, ApoER2	Express higher amounts of cellular adhesion molecules, tissue factor and von Willenbrand factor.
Monocytes	Annexin 2, TLR2/TLR-4	Increase of tissue factor and VEGF.

Table 2: Pathogenic Mechanisms of aPL-Mediated Foetal Loss

Abnormal placentation	
Decidua	Decreased angiogenesis (↓VEGF, ↓MMP) Abnormal spiral artery transformation and maturation Abnormal endometrial differentation
Trophoblast	Decreased invasiveness Abnormal intertrophoblast fusion Abnormal implantation (↓EGF-like GF) Decreased hCG secretion
Thrombotic mechanisms	
Interference with natural anticoagulants Interference with activation of anticoagulation protein C Interference with annexin A5 Inhibition of fibrinolysis Endothelial cell perturbation Induction of tissue factor expression on circulating monocytes Platelet activation	
Inflammation	
Complement activation Neutrophil and macrophage recruitment and activation (TF upregulation, oxidative damage) Proinflammatory decidual phenotype and cytokine production	

Table 3: Immunological Tests Recommended in Women with History of Foetal Loss

Lab tests
Complete blood count
Chemistry panel
Urinalysis
C3, C4, CH50
Anti-dsDNA antibodies
Anti SSB/Ro anti SSA/La antibodies
Anti-cardiolipin IgG and IgM antibodies
Thyroid antibodies: Thyroperoxidase antibody (anti-TPO), Thyroglobulin Antibody (TgAb).
NK cells (CD16+ CD56+)

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease that mainly affects women of reproductive age. The frequency of pregnancy loss in SLE has decreased in the last 40 years from 43% in 1960s to 17% in the 2000 decade. Around 19% of pregnancies affected by SLE finish with a miscarriage or stillbirth [18]. About 55% of pregnancy losses occur as stillbirth in the second trimester [19].

Clowse *et al.* identified four risk factors for the occurrence of foetal loss that take place in the first trimester: proteinuria (>500mg/dL), secondary APS, thrombocytopenia and hypertension [19, 20].

The worsening of lupus during pregnancy has been generally associated with changes in sex hormone levels. Oestrogen levels increase in the maternal circulation throughout pregnancy and enhance autoimmune reactivity. Increasing oestrogen levels during pregnancy lead to a high risk of lupus flare up [21].

The poor gestational outcomes are due in part to placental pathology, including decidual vasculopathy, decidual blood clots and extensive infarction [22]. In addition, placental weight is generally reduced.

At the onset of pregnancy, to perform baseline laboratory tests: lupus activity tests (C3, C4, CH50, anti-dsDNA Ab) should be recommended, risk for neonatal lupus (anti-SS-A/Ro and anti-SS-B/La Ab, and risk for foetal loss (aPL Ab, (Evidence Level 3) [23].

Anti-Ro/SSA and anti-La/SSB Ab have been associated with hydrops, endomyocardial fibro-elastosis or AV node calcification at postmortem. In addition to complete blood test and test of renal function (Table **3**) [19]. A 25% decrease of serum complement levels during pregnancy may be considered indicative of a lupus flare [24].

To ensure a successful outcome in pregnant women with SLE, a correct pre-gestational check-up, appropriate monitoring of risk factors and close monitoring of both mother and foetus during gestation should be warranted.

Pre-Eclampsia

Pre-eclampsia is a multisystemic disorder specific to pregnancy, which usually occurs after the 20th week of gestation, appearing in about 2-4% of pregnant women. Pre-eclampsia represents one of the most important causes of maternal-foetal morbidity and mortality [25]. Its causes are still unknown and induction of labour remains the only definitive treatment.

Many pathophysiological processes contribute to this syndrome, being involved several signalling pathways that lead to endothelial system dysfunction, hypertension and proteinuria [26]. This disorder has been considered as an immune-mediated syndrome. Women who developed pre-eclampsia during pregnancy are exposed to a exaggerated production of inflammatory cytokines, with poor angiogenesis due to increased levels of soluble Flt-1 and endoglin; as well as autoantibodies. In this regard, anti-receptor angiotensin I Ab have been reported with a very high frequency (70-90%) in women with pre-eclampsia and they are detected prior to the clinical onset and remain in some women postpartum [26].

In addition, recent studies suggest that diverse inflammatory mediators, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, may induce the development of this syndrome by generating anti-receptor angiotensin I Ab, and the activation of Toll-like receptors, also involved in innate response [27, 28]. An imbalanced differentiation of Treg and Th17 cells has been postulated [29].

In recent years, it has been found that the factor Bb, produced by complement activation, has been detected in the serum of pregnant women during the first trimester and is considered a strong predictor of the subsequent development of pre-eclampsia [30]. A maternal inflammatory response against foetal alloantigens, exposed on the trophoblast cells surface, offers a possible explanation for the elevated levels of factor Bb observed and supports the hypothesis of a poor immune adaptation in pre-eclampsia [31].

In pre-eclampsia, there is also an abnormal expression of cell adhesion molecules and the endothelial growth factor (VEGF) and its receptor on trophoblast cells, driving a utero-placental vascular insufficiency that leads to release nitric oxide, endothelin and prostaglandins at the placenta. These changes induce platelet aggregation, endothelial dysfunction and hypertension [32]. Pre-eclampsia is associated to severe systemic inflammatory changes.

For all above mentioned, it is considered important to perform and adequate clinical assessment of blood pressure, renal functions tests and routine monitoring,

during and after pregnancy, in order to prevent maternal and foetal complications.

Gestational Diabetes Mellitus

Poorly controlled gestational diabetes during pregnancy may confer higher risk of preterm birth, gestational complications, and foetal malformations, such as polyhidramnios, macrosomia and foetal death. Causes of foetal death related to diabetes mellitus include placental abruption, maternal and foetal infection, antepartum haemorrhage and pre-eclampsia (Evidence Level 3). Rarely, a non diabetic woman could have incidental glucose type 1 diabetes mellitus, usually with severe ketosis (Level Evidence 2+). Women with gestational diabetes mellitus return to normal glucose tolerance within a few hours after late IUFD has occurred.

HELLP Syndrome

HELLP syndrome is a multisystem thrombotic microangiopathy, associated with pregnancy complications, high maternal-foetal mordibidity and pre-eclampsia. It is characterized by Haemolysis, ELevated liver enzyme tests levels and Low Platelet count. It is occurs in 0.17 to 0.85% of all pregnancies, being more frequent in multiparous and older women. The disorder is diagnosed ante-partum in 70% of the cases and in 30% of them, postpartum. The risk of recurrence in a subsequent pregnancy is around 19-27% [33].

In approximately 60% of cases stillbirth occurs, in 30% intrauterine growth retardation and 25%, thrombocytopenia. The probability of death of the mother varies between 1% and 24%, mainly due to coagulation disorders, hemorrhagic complications, or cardiopulmonary, central nervous system, hepatic and gastrointestinal disorders. Cerebral haemorrhage is the most severe complication, and this is fatal in 50-65% of cases. Thrombocytopenia is the main early effect of alteration of coagulation due to vascular endothelial damage, alteration of prostacyclin production and increased fibrin deposits in the vascular wall. Also, accelerated platelet destruction, as well as an increase in activation and production of megakaryocytes have been reported [33].

High HLA-DR plasma levels are found in this syndrome, and the assessment of HLA-DR levels have been proposed to identify patients with pre-eclampsia and HELLP syndrome during pregnancy [34]. Likewise, T lymphocytes that regulate trophoblast invasion of the endometrium play an important role in this pathology. An alteration of the gene that expresses the TNFRSF, protein that belongs to the receptor family of TNF expressed on T lymphocytes, leads to an increased capacity of the maternal lymphocytes to recognize and destroy the trophoblast cells during invasion of the uterine wall and the spiral arteries, leading to an increased risk of HELLP syndrome [35].

In summary, HELLP syndrome is mainly due to a generalized microangiopathy, which normally occurs in the third trimester of pregnancy, especially in multiparous old women including hepatic involvement, haemolysis and thrombocytopenia. The early diagnosis of HELLP syndrome and the prompt initiation of early therapy are essential to ensure the favourable outcome of both mother and foetus [33].

Immune Imbalance: NK and Th17 cells: Immunoregulation Defects

Multiple mechanisms are involved in the maintenance of immune tolerance during pregnancy. For instance, a shift towards Th2 and Treg in normal pregnancy, the expression of Fas ligand by trophoblast cells and the inhibition of complement activation, are crucial to ensure the tolerance at the maternal-foetal interface. Treg are key factors in the maintenance of immune homeostasis, they suppress effector immune responses and thus prevent autoimmune processes [36]. Treg constitutes an important subsets of T CD4 cells that constitutively express CD25, the high-affinity α-chain of the interleukin 2 receptor (IL2R) and specifically express the transcription factor FoxP3 [37]. Treg cells are indispensable for immune tolerance to self and foreign antigens.

In physiological pregnancies, CD4+CD25+FoxP3+ Treg are increased, playing a critical role in maternal immune tolerance confronting to a semi-allogeneic fetal antigens and in embryo implantation [37]. Given the decidua is the fetal-maternal interface and the likely site for fetal antigen encounter, the proportion of Treg is increased in the decidua during pregnancy compared to peripheral blood [38]. Somerset et al demonstrated that the median proportion of Treg was higher in pregnant women than in nonpregnants [39]. Other studies suggested that a defect of Treg may be leading to pregnancy failure and women with spontaneous abortion were found to have low proportions of these cells [40].

The mechanisms by which Treg may prevent allorejection are through the secretion of IL-10, TGF-β, haem oxigenasa isoform (HO-1), indoleamine 2,3-dioxygenase (IDO) and leukaemia inhibitory factor (LIF) rather than decrease the Th1 cytokines [37]. In addition, there is also an appropriate balance between inhibitory signals (PD1/PDL1 co-stimulatory pathway, Stat3 and TGF-β1) and co- stimulatory signals (CD80 and CD86), involved in this tolerance. Both NK cells and immature dendrite cells promote expansion of Treg to confer protection to the foetus [41].

It has been reported that alterations of NK cells and increased prevalence in Th17 cells in peripheral blood and decidua in patients with recurrent abortions [41]. Th17 seem to increase in decidua of inevitable abortion, namely, at the progression stage of abortion, which suggest that increased Th17 may indicate more a consequence rather than a cause of foetal loss.

NK cells play an important role in the control of myometrial invasion by trophoblast cells and contribute to the maintenance of a balance between excessive or defective invasion. If this control fail, lead to development of obstetric syndromes such as pre-eclampsia, stillbirth and foetal growth restriction [42].

New strategies for improving pregnancy outcomes and novel approaches for therapeutically use Treg cells memory. One of them, are anti-TNFα blockers to decrease the inflammatory maternal-fetal response related to the Th1 cytokine. Another approach is the administration of recombinant human granulocyte colony-stimulating factors (G-CSF) that appears to increase Treg and tolerogeneic DCs. Treg up-regulation could be hypothesized as a possible future therapeutic strategy in humans for preventing fetal death of immunological cause [37].

Crohn's Disease

Inflammatory bowel disease confers a slightly higher risk of miscarriage, premature delivery and complications of labour and delivery with respect to general population. Crohn's disease has been associated with intrauterine growth restriction and foetal loss [43].

Maternal Thyroid Disease

The presence of anti-thyroid antibodies has been associated with an increased rate of abortion and perinatal death. Thyrotoxicosis increases the risk of abortion by 26% [44]. TSH, FT4 and FT3 as well as anti-thyroid antibodies (anti-thyroglobulin, anti-peroxidase and anti-TSHR) should be recommended to unravel maternal thyroid disease (Evidence Level 3).

Haemolytic Disease of the Fetus

In women who are rhesus D (RhD)-negative should be advised to have a Kleihauer test undertaken urgently to detect large feto–maternal haemorrhage (FMH) that might have occurred a few days earlier (Evidence Level 2). Anti-RhD gammaglobulin should be administered as soon as possible after presentation (level of recommendation C) [45]. If there has been a large FMH, the dose of anti-RhD gammaglobulin should be adjusted upwards and the Kleihauer test should be repeated at 48 hours to ensure the foetal red cells have cleared (level of recommendation C). Since its introduction in the 60s, anti-D immunoglobulin has been highly successful in reducing the incidence of haemolytic disease of the foetus and newborn (HDFN) and achieving improvements to maternal and foetal health [46]. However, without an appropriate screening and treatment program, more than 50% of untreated cases lead to neonatal death [47]. The D antigen is present in foetal blood from the seventh week from this point forward. The administration of anti-D is recommended from 28 weeks of pregnancy, since the anti-D antibodies are developed from this period [48].

CONCLUSIONS AND FUTURE PERSPECTIVES

Foetal death is a terrible event in the life of a couple. It is important emotionally for the parents to have an explanation of the potential causes of the foetal death. Moreover, it represents a challenge for clinicians confronted with a heterogeneous myriad of potential causes to be investigated in order to prevent an adverse outcome in a subsequent gestation and to prompt management of undiagnosed maternal diseases. Clinical assessment and laboratory studies should be directed to rule out preventable causes of foetal loss and maternal morbidity. Until more evidence studies better define the pathogenesis of foetal loss, efforts to translate the results of basic science into development of more targeted drugs and improvement of clinical practice and therapeutic outcome of pregnancy are warranted.

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