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Low Molecular Weight Heparin in Immunological Recurrent Abortion – The Incredible Cure

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The most compelling association between pregnancy loss and autoimmune phenomena has been with the presence of antiphospholipid antibodies (APA)—lupus anticoagulant and anticardiolipin antibody. The 'antiphospholipid antibody syndrome' has been described in women with a history of recurrent pregnancy loss or thrombosis with positive APA or lupus anticoagulant on two occasions. Although several treatments have been advocated, heparin and aspirin treatment is emerging as the treatment of choice for the APA syndrome associated with recurrent pregnancy loss. The rationale for prescribing aspirin in cases of recurrent reproductive failure associated with APA seropositivity is that aspirin may counter APA-mediated hypercoagulability in the choriodecidual space, a situation which if left unaddressed would traumatize the trophoblast and compromise feto-maternal exchange. Heparin on the other hand, through preventing APA from interfering with syncytialization of the early cytotrophoblast and by countering APA interference with phospholipid-decidual reactions that are vital to early implantation, might potentially promote both early implantation and subsequent placentation.

KEY WORDS: Low molecules weight heparin; recurrent abortion; antiphospholipid antibodies.

When you make the finding yourself—even if you're the last person on Earth to see the light—you'll never forget it.

Carl Sagan

INTRODUCTION

Recurrent abortion, defined by the occurrence of three or more spontaneous abortions prior to the 20th week of gestation, occurs in approximately 1 in 300 pregnancies (1) and in up to 1% of gravida three or more women (2). However, the cause is unexplained in up to 60% of studied couples (3). Accumulating evidence suggests that the fetal-placental semiallograft is afforded protection by local immunomodulating factors and that immunologic recurrent abortion may result from an imbalance or breakdown in the mechanisms responsible for immune homeostasis (4). The most compelling association between pregnancy loss and autoimmune phenomena has been with the presence of antiphospholipid antibodies—lupus anticoagulant and anticardiolipin antibody. These autoantibodies are also strongly associated with both venous and arterial thrombosis and thrombocytopenia (5). Thrombosis occurs in 25–33% of people with the lupus coagulant (6) and in over 75% of patients with elevated anticardiolipin antibodies (7). Antiphospholipid antibodies (APA) are a group of organ nonspecific autoantibodies that bind to negatively charged phospholipids. Their presence has been associated with reproductive failure; the most consistently reported phenomenon is the association between recurrent spontaneous abortion and the presence of immunoglobulin (IgG) anticardiolipin and lupus anticoagulant (LAC) (8–10). At present, there is convincing evidence that abnormal autoimmune function

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is an etiological factor in approximately 10% of patients with recurrent pregnancy loss (11–13) and assessment of Antiphospholipid antibodies, namely the Lupus anticoagulant (LA) and Anticardiolipin antibodies (aCL), has become routine in the evaluation of women having recurrent abortion (13–16). A particular subpopulation of anticardiolipin antibodies may be strongly represented in the male partners, the clinical significance of which remains to be established (17).

ANTIPHOSPHOLIPID ANTIBODIES

First of all, it is important to note that Antiphospholipid antibodies are present in virtually every individual. They are so called "natural" antibodies which can be found in females as well as males, though they appear at higher levels in females (18,19). Moreover, puberty and/or exposure to semen appears to affect at least the isotype of Antiphospholipid antibodies produced, if not their quantity (20). Pregnancy per se does not result in increased Antiphospholipid antibody titers. The sexes vary in antibody concentrations and production and autoantibody concentrations increase with age (21). Increased autoantibody concentrations cannot, however, automatically be equated with the presence of a disease state. In fact, it is probably reasonable to assume that a majority of women with raised Antiphospholipid antibody concentrations are perfectly healthy. A good example are relatives of patients with established autoimmune diseases. While first degree relatives of patients with autoimmune diseases have an increased incidence of elevated autoantibodies, and while they also experience an increased risk of autoimmune disease, a majority, even amongst those with elevated autoantibodies, will never develop an autoimmune disease (22). The mere presence of autoantibodies does, therefore, only denote a risk of disease and not necessarily the presence of disease itself. One therefore has to question the notion that the presence of autoantibodies per se causes disease and has to raise the possibility that the presence of abnormal autoantibody concentrations is only indicative of either abnormal B cell production or abnormal antibody clearance, while the truly pathognomonic effect, leading to disease, may be at a completely different level within a complex and intertwined immune system (23). The mere presence of abnormal autoantibodies does therefore not necessarily suggest that those autoantibodies cause concomitantly observed disease phenomena. Association is not necessarily causation, and abnormal autoantibodies may be nothing but an epiphenomenon in such a circumstance (23). It is equally curious that women with abnormally high peripheral values of Antiphospholipid antibodies concentrate these antibodies at incredibly high amounts in follicular fluid, while other immunoglobulins demonstrate standard clinical gradients between blood and follicle (24). In consideration of this observation, one has to wonder whether many more cases of unexplained infertility than are usually expected may not be due to an immunological cause. In fact, one can almost conclude that this is the case. A picture seems thus to emerge that suggests that the immune system can cause infertility. Maybe more importantly, however, if we outwit the immunological cause of infertility and succeed in nevertheless establishing a pregnancy, this pregnancy is at considerable excessive risk. This risk involves an increased chance of pregnancy loss (25,26), intrauterine growth retardation (27–31), and increased perinatal morbidity as well as mortality (32–35).

ANTIPHOSPHOLIPID SYNDROME

The "antiphospholipid antibody syndrome" has been described in women with a history of recurrent pregnancy loss or thrombosis with positive APA or lupus anticoagulant on two occasions (36). Although several treatments have been advocated, heparin and aspirin treatment is emerging as the treatment of choice for the APA syndrome associated with recurrent pregnancy loss (37–40). However, the significance of APA in a woman without a prior pregnancy or in the absence of prior thromboembolic phenomena is unclear (41). Antiphospholipid antibodies (aPL) are associated clinically with thrombocytopenia, recurrent thrombosis, repeated pregnancy losses, or a combination of these events (42). Patients whose pregnancies last beyond the middle of the second trimester may have a variety of collateral obstetric complications, such as early and severe pregnancy-induced hypertension and intrauterine growth retardation (36). Although the specific antibodies most commonly detected in these patients are against cardiolipin- and phosphatidylserine dependent antigens, current advances in the field suggest that phospholipid-binding plasma proteins, such as beta-2-glycoprotein I (beta2-GPI), human prothrombin, proteins C and S, and annexin V are involved in the binding of sera from patients with the antiphospholipid syndrome (APS) to anionic phospholipids (43).

Previously, the "reproductive autoimmune failure syndrome" was described in women with increased

autoantibodies, endometriosis and infertility, leading to a recommendation for immunological testing of women with infertility and endometriosis (25). Recently, several investigators have advocated testing women undergoing in vitro fertilization (IVF) for APA (44,45). The theoretical rationale for the role of APA and the potential benefits of anticoagulation therapy for women undergoing IVF is based on several observations. Firstly, phospholipids function as adhesion molecules during the formation of syncytiotrophoblasts (46). Secondly, the attachment of APA to surface phospholipids on trophoblasts may result in direct cellular injury. Moreover, inhibition of syncytiotrophoblast formation may cause indirect damage via intravascular thrombosis (47). More recently, some investigators have recommended treatment of all APA-positive women with heparin and aspirin (48,49). Exogenous heparin has been shown to inhibit binding of APA with phospholipids (50), and endogenous heparin manufactured by trophoblasts should function in the same fashion. The antithromboxane effects of aspirin on inhibition of platelet aggregation are thought to work in concert with heparin to promote and enhance implantation (51,52).

PATHOPHYSIOLOGY IN THE CLINICAL CONTEXT

A large body of evidence is emerging which suggests that a series of complex immune mechanisms modulates implantation. It has been demonstrated that increased concentrations of prostaglandins (PGE_2 and PGF_2alpha) at the site of embryo implantation increase vascular permeability prior to implantation and are critical to the process (53,54). Platelet activation factor (PAF), an ether-linked phospholipid, is produced by the blastocyst, by invading trophoblast and by adjacent decidua for a few days around the time of implantation (55–57). PAF facilitates implantation by increasing local consumption of thrombocytes and by promoting the release of PGE_2 (58,59). Phospholipids function as adhesion molecules in the formation of myoblasts and syncytiotrophoblasts (46,60). Exposure of surface phospholipids (especially phosphoserine and phosphoethanolamine in the hexagonal phase II form) creates an immunogenic state leading to delayed syncytialization of the trophoblast. This mechanism could play an important role in the pathogenesis of recurrent spontaneous abortion (47). PAF promotes local production of early pregnancy factor, an immunosuppressive glycoprotein (59). Conceivably, antibodies to surface phospholipids and to this glycoprotein could reduce the efficiency of implantation and promote autoimmune rejection of the conceptus. It has been postulated that in situations of local or systemic tissue damage, cellular surface phospholipids convert from a bilaminar configuration to a hexagonal phase II structure. In this isomeric form phospholipids combine with lipoproteins to become antigenic and lead to APA production (61). These antibodies have been identified in a number of autoimmune disorders (including but not limited to systemic lupus erythematosus, scleroderma, and Hashimoto's thyroiditis) that are known to be associated with a high incidence of pregnancy wastage (32). Infertility associated with pelvic inflammatory disease, endometriosis and postsurgical pelvic adhesions is likewise associated with a high prevalence of APA seropositivity. This phenomenon could explain the reduced implantation rate per embryo as compared with implantation rates in women without these pathologies and following the transfer of embryos to a third party (IVF/ovum donation and/or IVF/surrogacy) who does not have pelvic pathology (62). APA have been shown to be transiently produced during ovarian stimulation and/or as a consequence of oocyte retrieval with subsequent disappearance within several weeks (63). This might explain the reduced implantation rate per embryo that occurs following embryo transfer in cases of ovarian stimulation, as well as explain the increased miscarriage rate that occurs in spontaneous pregnancies that immediately follow failed cycles of IVF/embryo transfer (64,65). When present, APA bind with surface phospholipids on the trophoblast and result in direct cellular injury, inhibit syncytia formation and cause indirect damage through intravascular thrombosis (47). Heparin, whether endogenous (manufactured by trophoblast) or exogenously administered inhibits binding of APA with phospholipids, protecting the trophoblast from injury (66). Aspirin on the other hand, exerts an antithromboxane effect and inhibits platelet aggregation (67). It is postulated that Heparin–Aspirin (H-A) therapy facilitates and promotes implantation through these mechanisms. While it is reasonable to link recurrent reproductive failure in APA seropositive women (in whom pregnancy has already been diagnosed) to failed implantation, it is difficult to attribute a failure to conceive following IVF/embryo transfer (where pregnancy has not yet been diagnosed) to a similar mechanism. The prevalence of APA seropositivity in the general population ranges from 5 to 17%, while in patients who

experience recurrent spontaneous abortion it is as high as 59% (68). Some studies indicate that the prevalence of APA seropositivity in patients undergoing IVF/embryo transfer due to organic pelvic disease is similar to that seen in women who suffer from recurrent spontaneous abortion. In contrast, patients undergoing IVF/embryo transfer cycles in the absence of female pelvic pathology demonstrate similar APA seropositive rates to the general population (14%). Damage to pelvic organs from endometriosis, infection or iatrogenic trauma may induce the production of APA, and these antibodies may contribute to a woman's inability to conceive naturally or via IVF.

Fisch *et al.* (63) have shown that there is a transient rise in APA titers in women undergoing ovarian stimulation. Thus, it is possible that some IVF/embryo transfer failures in the precycle APA seronegative women are indeed due to antibody induction and/or transient rises in titers previously below detection. However, the mechanism by which aPL might cause recurrent miscarriages remains the subject of research. Fetal losses have been attributed to thrombosis of the uteroplacental vasculature and placental infarction (69,70). Although thrombosis is observed frequently in the decidua and placentas of aPL-positive patients, this observation is not universal, nor present in a sufficient degree to account for the pregnancy loss associated with this syndrome. An alternative hypothesis proposed that aPL have a detrimental effect on the trophoblastic layer of the human placenta (71). In line with the recent idea that several pathogenic mechanisms can be present simultaneously in the same patient, monoclonal aPL have been shown to prevent placental human chorionic gonadotrophin (hCG) and human placental lactogen (HPL) secretion (72).

Though recurrent pregnancy loss involves the loss of clinically recognized (postimplantation) pregnancies, it has been postulated that the same immunological dysfunction that may lead to some cases of recurrent pregnancy loss could also affect earlier unrecognized pregnancies as well, leading to heretofore unexplained infertility (13,73).

Interestingly, a very recent report (74) showed that antiphospholipid antibody was not predictive of IVF outcome but the rate of miscarriage was 2-fold higher in the IVF patients positive for antiphospholipid antibodies compared with antibody-negative women. The authors concluded that consideration whether to perform antiphospholipid antibody testing should be given to all patients whose IVF cycle results in a miscarriage after a clinical pregnancy has been documented (74). Another essential criterion applying to antiphospholipid antibodies testing in patients with pregnancy loss, is that only patients with recurrent miscarriage should be tested and considered for treatment (14,15,75). Since repeatedly unsuccessful embryo transfers after IVF are thought to be because of occult abortion (10,13,76), a role for antiphospholipid antibodies in failure of nidation after repeated IVF—embryo transfer could be postulated. Favoring this concept is the fact that all the four studies in the literature investigating antiphospholipid antibodies in patients failing three or more IVF—embryo transfer attempts are in agreement that such patients have a higher incidence of antiphospholipid antibody seropositivity than women becoming pregnant with their first IVF attempt (12,77–79). Therefore, as with the "primary antiphospholipid syndrome," antiphospholipid antibodies testing in IVF patients should be applied according to strict laboratory criteria in those women having repeated failures of implantation/clinical abortion after embryo transfer but not in an infertile general population reaching an IVF program.

RATIONALE OF HEPARIN–ASPIRIN (H-A) THERAPY

Several regimens have been proposed for the treatment of APS, including aspirin alone, prednisone and aspirin, heparin and aspirin and more recently i.v. immunoglobulin (Ig). Recent studies have suggested that aspirin plus heparin may be superior to prednisone or aspirin taken alone for the treatment of aPL associated recurrent pregnancy loss (39,48). This combination of aspirin and heparin may promote successful embryonic implantation in the early stages of pregnancy and protect against thrombosis of the utero-placental vasculature after placentation (80). The rationale for prescribing aspirin in cases of recurrent reproductive failure associated with APA seropositivity is that aspirin may counter APA-mediated hypercoagulability in the choriodecidual space, a situation which if left unaddressed would traumatize the trophoblast and compromise feto-maternal exchange. However, a haemochorial relationship is only established with placentation. (i.e. after establishment of a clinical pregnancy) and accordingly, it is unlikely that aspirin therapy would influence early implantation. Rather, the possible benefit of aspirin therapy could lie in an ability to protect the trophoblast from damage after placentation has been established. Rai and Regan (80) suggested

that low-dose aspirin might improve pregnancy outcome in women with APS by blocking the action of cyclooxygenase in platelets, thereby inhibiting platelet thromboxane synthesis and preventing thrombosis of the placental vasculature.

Heparin on the other hand, through preventing APA from interfering with syncytialization of the early cytotrophoblast and by countering APA interference with phospholipid-induced decidual reactions that are vital to early implantation, might potentially promote both early implantation and subsequent placentation. Furthermore, authors have not only shown a direct interference of heparin in the IgG binding to primary trophoblast cells, but also that heparin treatment is able to restore normal trophoblast invasiveness (81). In a previous study, the same authors identified a failure of placental cells to respond to GnRH after 72 h of incubation in the presence of sera containing aPL(82). Subsequent heparin treatment of cytotrophoblast cells restored the GnRH-induced secretion of HCG (82). They suggested that this failure might be due to reduced syncytium formation and that the morphology and differentiation state of the trophoblast differed between untreated and heparin-treated cultures. In line with this hypothesis, they have now shown that heparin treatment of cytotrophoblast cells is able to restore regular differentiation (81). Different regimens have been proposed for the treatment of APS, including aspirin monotherapy, prednisone and aspirin, or heparin and aspirin. The success of heparin treatment on pregnancy outcome in women with APS stimulated interest on the drug's mechanism of action. McIntyre *et al.* (66) suggested direct binding of heparin to aPL and, using an ELISA, showed a decrease in aPL concentrations with increasing doses of heparin. This was not thought to be due to an electrostatic interaction, as chrondroitin sulphate—which has a negative charge similar to that of heparin—had no effect on aPL concentrations in the ELISA. In addition, LMWH appeared more effective at pharmacological and lower concentrations than did regular heparin (82), suggesting that steric hindrance was not a significant problem.

The mechanism by which heparin might bind to aPL has still to be ascertained, though investigators have considered the possibility that heparin either binds to and interferes with recognition of either the aPL—protein complex, or binds directly with the aPL. The 54 kDa serum beta2-glycoprotein (beta2-GPI) appears to serve as cofactor in the recognition of the putative antigens by aPL (43). Either alone or in complex with anionic phospholipid, beta2-GPI may form an antigenic site for these antibodies (83). Findings indicate that beta2-GPI binds to heparin (84), which in turn might interfere with the aPL binding and thus eliminate the requirement for a cofactor in the binding reaction.

The data of Kutteh and Ermel (85) indicated that heparin plus low-dose aspirin provided a significantly better pregnancy outcome than low-dose aspirin alone for aPL-associated recurrent pregnancy losses. Recently, Kutteh (39) also reported that heparin combined with aspirin is as effective as heparin alone for the treatment of pregnancy losses associated with APS.

Dawes *et al.* (86) demonstrated that low molecular weight heparin may be more effective than unfractionated heparin, because it is more effectively absorbed after s.c. administration and has a longer half-life in the circulation. This represents an important role for low molecular weight heparin in the treatment of APS in pregnancy, because it causes less bleeding in both vaginal and abdominal deliveries (87). Antithrombotic therapy during pregnancy is used for the treatment and prophylaxis of venous thromboembolic disease, for the treatment and prevention of systemic embolism associated with valvular heart disease and/or mechanical heart valves, and for the prevention of fetal growth retardation and pregnancy loss in patients with antiphospholipid antibodies. Based on an equal efficacy and safety profile, low molecular weight heparins have replaced unfractionated heparin in the prophylaxis and treatment of patients with venous thromoembolism (88). Compared to unfractionated heparin, LMW heparins have the advantage of an increased half life and improved bioavailability (89,90). These LMW heparins can be administered once daily and have a substantially lower incidence of heparin induced thrombocytopenia and osteoporosis (91,92). As with unfractionated heparin, LMW heparins do not cross the placental barrier and are suitable for use during pregnancy (93).

CURRENT TREATMENT PROTOCOL

Our current treatment regimen is implemented as follows. Patients with APS are encouraged to take low dose aspirin (after a normal platelet count is confirmed) while trying for a pregnancy. We obtain an ultrasound as early as possible (6 weeks+) in order to confirm the presence of a fetal heart beat. Once a fetal heart flicker is identified, we begin subcutaneous low molecular weight heparin. This is started at 3500 IU of Logiparin (NovoNordisk) in the first trimester and

is continued on till the third trimester daily until it is tapered off just before delivery. We monitor these patients with initial weekly activated PTT/thrombin time and platelet counts which are then done monthly once we get four normal weekly readings. At our institute, the heparin dosage is not adjusted based on coagulation testing except if the platelet counts fall to less than 75,000/cumm. We strongly believe that aggressive treatment of patients with aPL and previous pregnancy loss may improve the chance of delivering a viable infant. We have treated 29 pregnancies in women with APS with Aspirin and Low Molecular Weight Heparin. Twenty three patients have delivered a viable infant, with no antenatal/intranatal/postnatal complications like spontaneous abortions, osteoporosis, severe pre-eclampsia, preterm delivery, or antepartum/postpartum hemorrhage. The babies had no abnormalities. We have six patients at varying stages of pregnancy presently on the H-A treatment regimen with no antenatal problems. Our experience with the H-A therapy suggests that the treatment is beneficial (79.31% success rate) and is very well tolerated with almost no side-effects except easy bruisability in 20.68% of the patients on H-A therapy despite normal coagulation parameters.

CONCLUSION

A thorough understanding of the potential immunologic mechanisms underlying recurrent abortion is essential before successful preventive strategies can be discerned. Effective therapy is needed for immunologic recurrent abortion; however, the treatment must be scientifically well founded and more innocuous than the disease. The poor obstetric outlook for women with a history of recurrent miscarriage in association with phospholipid antibodies may be improved with a combination therapy of low dose aspirin and low molecular weight heparin. This combination promotes successful embryonic implantation in the early stages of pregnancy and protects against thrombosis of the uteroplacental vasculature after successful placentation. We believe that future studies should be aimed at refining the protocol used by us to determine the benefits of preconceptual administration of heparin and whether it can be stopped after 13 weeks' gestation without adversely affecting the rate of live births.

All truths are easy to understand once they are discovered; the point is to discover them.

Galileo Galilei

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