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Autoimmunity and pregnancy loss

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Certain autoimmune conditions, such as systemic lupus erythematosus (SLE), are associated with pregnancy loss. Almost 30 years ago, investigators published data that focused on fetal wastage in women with antiphospholipid antibodies (aPLs) and that included fetal loss as one clinical criterion for the diagnosis of antiphospholipid syndrome (APS) [1,2].

After these initial publications, interest in other autoimmune disorders as possible causes of pregnancy loss has increased. This interest prompted an ongoing effort to link the presence of a specific autoantibody or patterns of autoantibodies to recurrent pregnancy loss. A multitude of candidate autoantibodies have been evaluated, and some clinicians routinely perform assays for multiple autoantibodies in the evaluation of women with recurrent pregnancy loss; however, there is only modest evidence to support the concept that most autoantibodies cause pregnancy loss. This article discusses the potential association between several specific autoimmune conditions and recurrent pregnancy loss.

Recurrent pregnancy loss may be caused by a myriad of factors, including anatomic, hormonal, thrombotic, autoimmune, genetic, infectious, or unknown causes. Most women with two or three unexplained, consecutive embryonic losses are otherwise healthy and have no other signs or symptoms that might be considered part of an autoimmune condition.

Terminology

In this article, the following definitions are used: Pregnancy loss refers to the failure to obtain a viable, live born infant. Types of pregnancy loss include anembryonic pregnancy (failure of a detectable embryo to develop), embryonic

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loss or death (fertilization through the ninth postmenstrual week of gestation), fetal death (10 postmenstrual weeks of gestation), and early neonatal death. Traditionally, and often legally, the terms *spontaneous abortion* and *miscarriage* are used to categorize the loss of the conceptus before 20 weeks' gestation. These terms are used whether the loss is caused by embryonic death, fetal death, or maternal causes. After 20 weeks' gestation, a fetal death usually is classified as a stillbirth. These traditional and legal definitions ignore the concepts of developmental biology. The causes of embryonic loss and many fetal deaths (10 through 20 weeks' gestation) do not fall into two distinct and well-defined etiologic categories. There is no biologic evidence that an arbitrary cutoff of 20 weeks' gestation distinguishes the causes of fetal deaths that occur before 20 weeks' gestation (spontaneous abortions) and after 20 weeks' gestation (stillbirths). The same pathologic process (eg, severe placental insufficiency) may result in the live birth, and ultimate survival of one fetus delivered at a very early gestational age may result in fetal death or subsequent stillbirth.

Recurrent pregnancy loss, or recurrent miscarriage, may present as recurrent-anembryonic loss, recurrent-embryonic loss, recurrent fetal death, or any combination of the three. Typically, recurrent means three or more consecutive losses with the same partner. Because subsequent pregnancy success is similar in patients with two or three consecutive losses, some clinicians initiate an evaluation or treatment after the second consecutive loss.

Approximately half of pregnancy failures occur before an expected last normal menstrual period. In this circumstance, women usually are not aware of the conception—much less the loss. Of clinically recognized pregnancies, 10% to 15% result in pregnancy loss, usually before 14 weeks. Most women with a diagnosis of recurrent abortion have recurrent anembryonic or embryonic loss, and most clinical abortion occurs between 6 and 12 postmenstrual weeks' gestation. Because the embryo typically dies 1 to several weeks before the onset of clinical symptoms, most pregnancy losses at 10 to 12 weeks' gestation are embryonic, not fetal, in nature. Recurrent fetal death is relatively uncommon compared with recurrent embryonic loss.

To understand a possible link between autoimmunity and pregnancy loss, the definitions of autoimmune conditions must be understood. Well-characterized autoimmune diseases, such as SLE, are defined by an aggregate of specific signs and symptoms that are associated with the presence of clinically meaningful levels of pertinent autoantibodies. A single clinical symptom or sign rarely is used to make the diagnosis of an autoimmune disease. An isolated finding of arthralgia, thrombocytopenia, or proteinuria is too nonspecific to diagnose SLE. Diagnostic criteria require more than one clinical sign and symptom to be present before the diagnosis of SLE can be made [3].

In addition to typically having more than one clinical feature, patients with well-characterized autoimmune diseases have clinically meaningful levels of pertinent autoantibodies. In the laboratory, this finding means that autoantibodies are present in significant levels (ie, serum binding exceeds two to three standard deviations above the control population mean).

Positive results of low titer are likely to be clinically irrelevant. Antinuclear autoantibodies (ANAs), the autoantibody hallmark of SLE, may be present in low titers in patients with other conditions and in a small percentage of apparently normal individuals. In contrast, patients with true SLE tend to have higher titers of ANAs. The diagnosis of SLE is suspect, even in a patient with several symptoms of SLE, if the ANA titer is less than 1:160.

Systemic lupus erythematosus

Pregnancy loss is more common among women with SLE than among normal women. In a summary of data from 11 studies of pregnancy loss in women with SLE, the median rate of pregnancy loss was 22% [4]. In four prospective studies, the overall pregnancy loss rates were 22%, 31%, 19%, and 10.5% [5–8]. These studies did not include appropriately matched, prospectively acquired controls. Mintz followed more than 100 women in a high-risk clinic for comparison and found a statistically higher rate of pregnancy loss among patients with SLE [7].

These studies suggest that the median rate of spontaneous abortions among patients with SLE is comparable with that of the normal population (10%). In contrast, the median rate of late (second or third trimester) pregnancy loss (6%) is considerably higher than that in the normal population (approximately 1%–3%). Excess pregnancy loss among women with SLE primarily is caused by a high rate of fetal death in the second and third trimester. In one study, nearly three quarters of pregnancy losses among women with SLE were second or third trimester fetal deaths [6].

Potential benefits of therapy

Kleinman and colleagues suggest potential benefits of diagnosing SLE in women with recurrent pregnancy loss [9]. They evaluated 56 pregnancies in 21 women with SLE and compared the pregnancy outcomes that occurred before SLE was diagnosed with the pregnancy outcomes that occurred after the SLE diagnosis. Outcomes improved significantly after the diagnosis was made; there were decreased incidences of spontaneous miscarriages (from 36% to 10%) and fetal losses (18% to 3.3%) and a decreased overall loss rate (54% to 15%). The improved outcome likely resulted from careful monitoring and therapeutic interventions. Buchanan's study also suggested improved live birth rates (63 of 100) in women with APS or SLE who were treated in a tertiary center versus the live birth rates (19 of 101) in the same women who were treated outside the center [10]. In the tertiary center, heparin and aspirin were used as pharmacotherapy.

Antiphospholipid antibodies in women with systemic lupus erythematosus

Most women with SLE who have experienced fetal loss also have significant levels of aPLs [11–13]. Lockshin and colleagues studied 21 women with SLE

and found that aPLs were the most sensitive indicator of fetal distress or fetal death [12]. The same group found that aPLs were present in 10 of 11 patients with fetal losses. The positive predictive value of aPLs for fetal death was over 50% [14]. Other investigators determined that the presence of aPLs and a history of fetal loss predict over 85% of pregnancy losses in women with SLE [15].

Studies performed before the current understanding of the effects of aPLs suggested that three other factors may be associated with the likelihood of pregnancy loss in women with SLE: (1) underlying renal disease, (2) disease activity before conception, and (3) onset of SLE during pregnancy.

Among pregnancies in women with SLE and underlying renal disease [16–20], the median rate of spontaneous abortions is 13% and the median rate of fetal death is 7.5%. The rate of fetal death seems to be higher after the diagnosis of SLE and lupus nephropathy than before the diagnosis [21], and the severity of renal impairment may be important [18,19].

Prepregnancy lupus flares also portend a worse prognosis for successful outcome [19]. Similarly, women who are first diagnosed with lupus during pregnancy seem to have a relatively high rate of pregnancy loss. Taking the aggregate data from four different studies, 5 of the 21 pregnancies ended in fetal death, and 1 ended in spontaneous abortion; overall loss rate was 29% [19,22–24].

Based on the foregoing, the authors recommend that: Women with active SLE should be advised to delay conception until remission is established; women with moderate renal insufficiency should be warned of their higher pregnancy loss rate, and women with severe renal insufficiency should be advised against pregnancy; women with SLE also should be counseled that even among patients with successful pregnancies, there is a higher rate of preeclampsia and premature delivery than among controls [21,25–30]; and treatment aimed at preventing pregnancy loss in women with SLE and aPLs should be similar to that for APS (discussed later).

Antiphospholipid syndrome

Antiphospholipid syndrome is characterized by the production of moderate-to-high levels of aPLs and specific clinical features. In addition to pregnancy loss, the other clinical criterion for APS is venous or arterial thrombosis, including stroke [31]. APS probably occurs most commonly in patients with other underlying autoimmune diseases such as SLE, but it also is diagnosed in women with no other recognizable autoimmune disease. To be classified as having APS, the patient must have at least one clinical feature of the syndrome along with moderate-to-high levels of aPLs.

Of the clinical problems associated with aPLs, none has generated more interest and debate than pregnancy loss. Beginning in the 1950s, case reports and subsequent small series focused attention on a relationship between aPLs and pregnancy loss [32]. The initial reports were retrospective, and the patients were highly selected. Most patients had underlying conditions, such as SLE, previous

pregnancy loss, or a history of thrombosis. Until the 1980s, when standardized ELISAs became available, skeptics argued that aPLs were not associated independently with pregnancy loss but were an epiphenomenon that occurred in women with previous fetal loss or thrombosis who were destined to have another pregnancy loss for other reasons.

With better laboratory testing and publication of larger studies, the independent association between aPLs and pregnancy loss was established. The evidence largely comes from four perspectives. The first perspective is that a small, but clinically important, proportion of women with recurrent pregnancy loss have aPLs [4].

The second supportive evidence is that prospective studies show an increased rate of pregnancy loss among women positive for aPLs on early pregnancy screening. In one study of 737 consecutively tested patients, 2 tested positive for lupus anticoagulant (LA) (both patients later experienced a second trimester fetal death), and the 16 women who tested positive for immunoglobulin G (IgG) anticardiolipin antibodies (aCLs) or IgM aCLs had a significantly higher rate of fetal loss (38%) did than women who tested negative for aCLs (6%) [33]. In another study, the investigators measured LA and aCLs in over 900 consecutive pregnant women [34]. Of these women, 1.2% had LA and 1% had either IgG or IgM aCLs. Fetal loss occurred in 16% of the 19 women with aPLs but in only 2% of the 915 women without aPLs.

Third, studies have shown that even when most women who were positive for aCLs or LA were treated (glucocorticoids, heparin, low-dose aspirin, or a combination of these medications) and followed prospectively through another pregnancy, 24% to 32% of the women had pregnancy losses [35–38]. In one study of highly selected patients, 60% of patients had another fetal death [39]. Using appropriate controls (aPL-negative women with SLE or SLE-like conditions), Out and colleagues found that the fetal death rate among women with LA was significantly higher (35%) than among women without aPLs (6%) [38].

The fourth piece of evidence linking aPLs to pregnancy loss comes from animal models. Investigators have demonstrated that some aPL–IgG fractions, but not all [40,41], from women with APS [42] and some monoclonal aCLs [43] cause fetal death when injected into mice in the first half of pregnancy.

Since 1987, fetal loss has been included as one clinical criterion for the diagnosis of APS [1,2]. The criteria for APS were reworked and clarified in 1999 at the International Antiphospholipid Symposium in Sapporo, Japan [31]. Three types of pregnancy loss and complications are included in the clinical criteria: (1) one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal morphology documented by ultrasound or direct examination of the fetus; (2) one or more premature birth before the 34th week of gestation as a consequence of severe preeclampsia or placental insufficiency; or (3) three or more consecutive spontaneous abortions before the 10th week of gestation (maternal and paternal chromosomal abnormalities and other known causes of miscarriages must be excluded).

Antiphospholipid antibodies testing

There are two aPLs for which well-established assays are available: aCL and LA. In both assays, aPLs bind moieties on negatively charged phospholipids or moieties formed by the interaction of negatively charged phospholipids with other lipids, phospholipids, or proteins. In 1990, independent investigators discovered that some aCLs require the presence of the plasma phospholipid-binding protein β_2 -glycoprotein I (β_2 -GP-I) to bind to cardiolipin [44,45]. β_2 -GP-I is required for aCL binding in patients with SLE or APS, but not in patients with syphilis or other infectious diseases [44–47]. The latter antibodies react directly with cardiolipin, independent of β_2 -GP-I [46,47]. After it was discovered that autoimmune aCLs are directed against a phospholipid-binding protein, rather than the phospholipid itself, attention shifted from phospholipids to phospholipid-binding proteins [48] and led to the discovery that some autoantibodies bind directly to β_2 -GP-I in the absence of phospholipid [47–50].

Anticardiolipin testing

The assay for aCL is standardized and uses sera that can be obtained from the Antiphospholipid Standardization Laboratory in Atlanta, Georgia. Results calibrated against these standards are measured as GPL (IgG aCL), MPL (IgM aCL), or APL (IgA aCL) units. Results should be interpreted in semiquantitative terms as negative, low positive, medium positive, or high positive [51].

There is considerable controversy regarding the clinical significance of varying titers and isotypes of aCL. To diagnose APS, the International Consensus Criteria require that patients meet clinical criteria and have positive LA or more than 20 units of aCLs on at least two occasions at least 6 to 8 weeks apart [31]. There is clear agreement that patients with medium- and high-positive titers of IgG aCLs suffer pregnancy complications. Whether low-positive results of IgG or of isolated IgM or IgA aCLs (low or high titer) are clinically significant is subject to ongoing debate.

Low-positive IgM aCLs occur in 3% to 5% of otherwise normal individuals. Silver et al compared the pregnancy outcomes of 93 women with low-positive IgG (fewer than 20 IgG-binding units) and 97 women with IgM only (more than 9 IgM-binding units) with those of 131 women with medium-to-high positive IgG (LA or more than 19 IgG-binding units of aCLs) and 153 women who tested negative. Low-positive IgG and isolated IgM were not associated with adverse pregnancy outcome [52]. Although some investigators have included patients with isolated IgM aCLs in diagnostic or therapeutic studies, these studies do not allow a separate analysis of this subgroup [36,53,54]. Despite these limitations, the International Consensus Criteria allow that patients with more than 20 MPL units of IgM can be identified as having APS. As with IgG, however, at least two positive tests that are at least 6 to 8 weeks apart are required for the definite diagnosis [31]. One large study (n = 500) of consecutive patients with recurrent abortion (≥ 3) showed that only 36% women who tested positive for IgM or IgG

aCLs were positive for aCLs, and only 12% of women were persistently positive for the same antibody on repeat testing [55]. Overall, the prevalence of persistently positive tests was 9.6%, 3.3%, and 2.2% for LA, IgG aCLs, and IgM aCLs, respectively. There were no patients who were initially negative for aCLs who subsequently tested positive. Only one patient who was initially negative for LA subsequently tested positive. The authors concluded that repeat testing was necessary for patients with initial positive tests and not necessary for patients with initial negative tests.

Substantial levels of aPLs may be found among otherwise normal women. Two studies that each included over 1000 unselected obstetric patients found aPLs in 1% to 4% of participants. In Harris' study [56], over 80% of the positive results were in the low-positive range, with only 0.2% of IgG results and 0.7% of IgM results being in the medium- or high-positive range. In the other report [57], 3.5% and 0.94% of women had IgG and IgM aCLs, respectively. Most women had low-positive results.

Whether or not IgM and IgA aCLs are included, pregnancy loss uncommonly is caused by aPLs. This finding is not surprising given the infrequency of APS. One author found only one patient with medium-to-high positive IgG aCLs among 309 patients with sporadic second or third trimester fetal death (all deaths occurred after 14 weeks' gestation) [58]. In another study of 331 women with their first pregnancy loss, investigators found no relationship between aPLs and abortion when results were compared with those in 993 controls [59]. These two studies suggest that APS is not the cause of pregnancy loss in a large proportion of unselected cases. Similarly, parental karyotype abnormalities or uterine malformations uncommonly cause pregnancy loss in unselected cases. Although APS is infrequent, testing for APS in selected patients is warranted, because APS is a potentially treatable cause of pregnancy loss and has other long-term implications [35,37,53,59].

β_2 -GP-I antibody testing

The anticardiolipin cofactor β_2 -GP-I was identified independently in 1990 by three different groups [44,45,60]. This glycoprotein has several anticoagulant properties in vitro. It inhibits contact activation of the coagulation cascade, ADP-induced platelet aggregation, activated protein C, and the conversion of prothrombin to thrombin. The thrombotic tendency in patients with aCLs may be related to the interference of aCLs in the biologic function of β_2 -GP-I. Evidence suggests that conformational changes occur following the interaction of β_2 -GP-I with phospholipid and render the protein antigenic to aCLs [61].

In a study by Aoki and colleagues, β_2 -GP-I-independent aCLs and aCLs in a standard assay were found in 3.1% and 7.7%, respectively, of 130 women with recurrent abortions. Overall, β_2 -GP-I-dependent aCLs were present in 5.4% of the same group. Only the β_2 -GP-I-dependent aCLs proportion was statistically different than controls [62]. The ability to clinically apply the results of this study

is limited because the study subjects were not well characterized regarding other known causes of pregnancy loss or other autoimmune features. Other investigators have evaluated the significance of β_2 -GP-I–dependent and β_2 -GP-I–independent aCLs and have found that the latter are not associated with APS [63], whereas the former are associated with pregnancy complications [64,65].

Additional predictive value of anti- β_2 -GP-I antibodies

In 1999, Balasch and co-workers reported that the presence of anti- β_2 -GP-I antibodies in patients without aCLs did not independently influence reproductive success in 175 patients (100 patients with spontaneous recurrent abortion and 75 patients with repeated failure of embryo transfer) compared with 210 (positive and negative) controls [66].

Lee et al compared the frequency of IgM and IgG aCL and anti- β_2 -GP-I antibodies in sera from four groups of women: (1) 152 healthy, fertile controls, (2) 141 subjects with unexplained recurrent spontaneous abortions, (3) 58 subjects with unexplained fetal deaths, and (4) 73 subjects with well-characterized APS [67]. Although strong correlations were found between the presence of IgG and IgM anti- β_2 -GP-I antibodies and aCLs, measuring IgG or IgM anti- β_2 -GP-I antibodies did not improve the ability to diagnose or predict adverse pregnancy outcome.

Neither of these two studies evaluated the significance of IgA anti- β_2 -GP-I antibodies. Prompted by reports of positive findings in nonpregnant patients with rheumatologic disorders [68–71], Lee and colleagues addressed the IgA issue in a separate study [72]. They compared 133 women with unexplained recurrent abortion, 48 with unexplained fetal death, 67 with well-characterized APS, and 145 healthy, fertile controls. Isolated IgA anti- β_2 -GP-I antibodies were significantly more common in women with unexplained recurrent spontaneous abortion and unexplained fetal death than in healthy controls. This finding needs to be confirmed by other studies.

Greco and colleagues also studied the independent diagnostic value of anti- β_2 -GP-I antibodies and evaluated the IgM, IgG, and IgA isotypes. They concluded that IgA anti- β_2 -GP-I antibodies were the most important isotype in increasing the sensitivity of positive testing in women ($n = 73$) with recurrent miscarriage who previously had tested negative for aCLs. Likewise, the addition of anti- β_2 -GP-I antibody testing (particularly for the IgA isotype) increased the sensitivity of a positive test in women with previously diagnosed APS who were undergoing repeat antibody testing.

Given the results of these studies, the authors conclude that antibodies to β_2 -GP-I or β_2 -GP-I–dependent aCLs are related to autoimmune causes of pregnancy loss. Although controversy remains as to whether these assays are required to detect additional patients with APS compared with only measuring LA and aCLs, these antibodies are highly associated with thrombosis and other features of APS [64,65]. Even though anti- β_2 -GP-I antibodies are not included in the criteria for APS, the authors recommend testing for anti- β_2 -GP-I antibodies in patients who strongly are suspected to have APS and who have

negative results for aCL and LA. Although testing for IgA anti- β_2 -GP-I antibodies may prove important for increasing testing accuracy, the benefits of routine testing needs to be evaluated further.

Type of pregnancy loss associated with antiphospholipid syndrome

Although there is some controversy about the type of pregnancy loss that most commonly is associated with APS, fetal death is a dominant outcome. Branch et al summarized a group of case reports and small series and found that 30% of antiphospholipid-related pregnancy losses were second-trimester or early third-trimester fetal deaths [32]. Follow-up on retrospective analysis of women with well-characterized APS showed that 79 of 195 (41%) previous pregnancy losses were fetal deaths [35]. In 1996, Oshiro and co-workers analyzed the obstetric histories of 366 women with two or more consecutive pregnancy losses who were tested for LA and aCLs [73]. Seventy-six patients in this highly selected referral population had LA or medium-to-high positive IgG aCLs. Half of the previous pregnancies losses in this group were fetal deaths, whereas only 10% of the losses in the 290 women without significant levels of aPLs were fetal deaths. Eighty percent of the women with aPLs (LA or medium-to-high positive IgG aCLs) had experienced at least one fetal death, compared with 24% in women without aPLs. Although a history of one or more fetal deaths had a specificity of 76% for significant aPL levels, a history of two or more early first-trimester losses without fetal death had a specificity of 6% for significant aPL levels. The high rate of fetal death among patients with APS contrasts the low rate of fetal death in most series of patients with recurrent pregnancy loss.

Clinically, the death of the fetus typically is preceded by ultrasonographic evidence of fetal growth impairment, oligohydramnios, and abnormalities in the fetal heart rate that indicate hypoxemia. These conditions are attributed to spiral arteriolar vasculopathy [74,75], with subsequent inadequate blood flow to the intervillous space and resultant utero-placental insufficiency. In severe cases, decreased intervillous blood flow leads to large areas of placental infarction.

Therapy for antiphospholipid syndrome

Prednisone, aspirin, and heparin

Because of the apparent risk for fetal loss among women with aPLs, many investigators have treated these women with antiplatelet, anticoagulant, or immunosuppressive agents to improve pregnancy outcome. Though none included appropriately selected controls, reports that used prednisone and low-dose aspirin therapy appeared in the early 1980s [63,76], and other studies followed. Although most investigators concluded that prednisone and low-dose

aspirin therapy was beneficial, one group found that prednisone did not improve pregnancy outcome [39].

Heparin was introduced as a treatment during pregnancy in the early-to-mid 1980s — again with the lack of appropriately selected controls. Mean dosages ranged from 17,500 to 24,700 U of heparin daily, and investigators reported successful pregnancies in 75% to 93% of patients [35,36,77]. In an attempt to use patients as their own controls, one study compared the outcome of a patient's first untreated pregnancy following a fetal death with that of her first treated pregnancy [35]. The rates of surviving infants were 6% and 53%, respectively, indicating a statistically significant benefit of treatment. Heparin gained further acceptance when a small, prospective, randomized trial showed that the combination of heparin and low-dose aspirin was as effective as prednisone and low-dose aspirin in achieving successful pregnancy outcomes [36]. Two prospective trials showed that therapy with heparin and low-dose aspirin is more effective than aspirin alone in achieving live births among women with aPLs and predominantly pre-embryonic and embryonic pregnancy loss [78,79].

Although somewhat contradictory to these results, two studies suggest that patients may not require aggressive therapy. A prospective, randomized trial in which low-dose aspirin was compared with prednisone plus low-dose aspirin in 34 women with aPLs. No difference between pregnancy outcomes was found. [41]. Another trial showed that aPL-positive women with repeated pregnancy loss but without history of thrombosis or SLE achieved similar live births rates (~80%) using low-dose aspirin or placebo [80].

Despite the limitations of these studies, most experts recognize APS as a treatable cause of recurrent pregnancy loss [81]. The authors recommend maternally administered heparin, after ultrasonographic demonstration of a live embryo, as the treatment of choice. The appropriate dose is debatable and may vary with the clinical situation. Many experts agree that women with recurrent pre-embryonic and embryonic pregnancy loss and no history of thromboembolism may be treated with as little as 5000 U of heparin twice daily [78]. Women with previous thromboembolism probably should be treated with anticoagulation doses.

The optimal treatment for women with fetal loss but without history of thromboembolism is controversial. Because of the potential increased risk for maternal thromboembolism in this group, some investigators favor generous thromboprophylaxis (15,000–21,500 U/d of heparin) [35], but there are no well-designed studies to use for guidance.

Safety of heparin

When using heparin therapy, the possibility of heparin-induced thrombocytopenia (HIT) with thrombosis should be considered. HIT occurs in approximately 5% of nonpregnant patients who are treated with heparin. Thirty percent of affected patients experience thromboembolic-associated major morbidity or death. The incidence of HIT in pregnant population is not known; the authors

have compared the incidence of HIT in pregnant ($n = 244$) and nonpregnant ($n = 244$) populations [82]. There were no cases of immune-mediated HIT in the pregnant group, but there were 10 (4%) cases in the control group ($P = 0.0014$). The authors concluded that HIT is much less common in treated pregnant versus nonpregnant patients. Prednisone and heparin promote osteoporosis and can result in fractures. Patients treated with these medications may benefit from daily weight-bearing exercise and calcium supplementation.

Intravenous immunoglobulin

The use of high-dose intravenous immunoglobulin (IVIg) has generated interest because of anecdotal reports of successful pregnancy outcomes with fewer adverse effects than with prednisone or heparin [83–85]. In initial studies, APS pregnancies that were treated with IVIg also were treated with prednisone, heparin, or low-dose aspirin; the studies involved refractory cases in which other therapies had failed [84]. In 1995, Spinnato and colleagues administered high-dose IVIg to five consecutive women with well-defined APS as a part of their treatment during pregnancy [86]. All women also received low-dose aspirin; four women were treated with heparin (10,000–15,000 U/d); and all women delivered live born infants. None of these patients had preeclampsia, and none of the infants were small for gestational age. Three (60%) infants were delivered at term, one was delivered after preterm premature rupture of the membranes at 36 weeks' gestation, and one was delivered at 33 weeks' gestation. These outcomes contrast the findings of the authors' study (conducted before 1992), in which there was a 50% risk for preeclampsia and fetal distress, 30% incidence of fetal growth impairment, and 37% preterm delivery rate in patients with APS who were treated with various combinations of low-dose aspirin, prednisone, or heparin [35].

Spinnato's study prompted a pilot study to determine the feasibility of a multicenter IVIg trial and to assess the impact of the addition of IVIg to heparin and low-dose aspirin therapy on obstetric and neonatal outcomes in women with APS. This multicenter, randomized, double-blind study included 16 patients and compared treatment with heparin, low-dose aspirin, and IVIg to treatment with heparin, low-dose aspirin, and placebo [87]. All patients had LA, medium-to-high positive IgG aCLs, or both. The overall rates of antepartum complications were similar for each group. Compared with the placebo group, the IVIg group had fewer cases of fetal growth impairment (0% versus 33%, respectively) and newborn intensive care unit (NICU) admissions (20% versus 44%); however, the differences did not reach statistical significance. Although IVIg did not improve obstetric or neonatal outcomes beyond that achieved with heparin and low-dose aspirin therapy alone, the findings of fewer cases of fetal growth impairment and NICU admissions in the IVIg-treated pregnancies warrant expanding the study.

Vaquero and associates compared the outcomes of 53 patients with APS who were treated with IVIg in one clinic with the outcomes of 29 patients with APS

who were treated with low-dose aspirin and prednisone in another clinic — both clinics were from the University of Rome [88]. Most patients had medium-to-high levels of aCLs, and the values were comparable between the groups. Compared with the patients taking IVIg therapy, the patients taking low-dose aspirin and prednisone therapy had smaller babies, more gestational diabetes, and more pregnancy-induced hypertension; however, there were no differences in the incidence of intrauterine growth restriction, preterm delivery, or live birth rate between the groups.

Optimal therapy

A direct comparison of the outcomes in the previously noted studies is impossible because of differences in patient selection. These differences may explain most of the variation in maternal and fetal outcomes noted by different investigators. Using a history of previous fetal death as an example, three of the larger treatment series included patients with only 6% [41], 20% [89], and 50% [36] rates of previous fetal death. Three other studies included populations in which over 70% of patients had a history of fetal death [35,39,90]. The antiphospholipid status of the patients differs among series. Although nearly all patients in one series had LA [35], less than 40% of patients in three other series had LA [36,41,77], and another study excluded women with LA [54]. Over 85% of patients in two series had IgG anticardiolipin [35,39], compared with 50% or less in two other series [77,91].

Because existing data do not allow valid comparisons between treatment trials, the authors urge investigators to use the International Consensus Criteria to define patients and to carefully categorize patients with regard to the type of pregnancy loss. Patients also should be defined carefully in terms of the levels and isotypes of antibodies that are used as laboratory inclusion criteria. Until larger, randomized, direct comparisons of varied therapies are performed, the reader is cautioned not to draw final conclusions regarding optimal therapy.

Other autoantibodies

Antiphospholipid antibodies other than lupus anticoagulant and anticardiolipin antibodies

A significant proportion of women with unexplained recurrent pregnancy loss have autoantibodies to other phospholipid antigens, such as phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidic acid [92–95].

The clinical implications of phospholipid-binding antibodies other than LA, aCLs, and anti- β_2 -GP-I antibodies are questionable for several reasons: (1) Women with APS often, if not always, have antiphosphatidylserine antibodies

[32,96,97]; however, binding to phosphatidylserine is highly correlated to cardiolipin binding and may represent autoantibody cross-reactivity [96,98]. (2) Other than aCL assays, assays for other phospholipid-binding antibodies are not standardized, and none have been as extensively studied as assays for LA and aCLs. The clinical relevance of other phospholipid-binding antibodies must be proven independent of LA and aCLs. (3) Many apparently healthy women have low levels of detectable aPLs [57,99,100]. (4) Investigations designed to establish whether aPLs other than LA, aCLs and anti- β_2 -GP-I antibodies are linked to recurrent pregnancy loss are limited. The same group performed most of the studies with positive findings [92,93,95]. They reported that up to 40% of women with recurrent pregnancy loss had IgG or IgM antibodies to one of six phospholipid antigens (including cardiolipin), compared with up to 17% of controls. There is some concern regarding the technical aspects of the assays and the selection of controls in this group of studies [4]. (5) A different group of investigators evaluated six aPLs in 334 patients with recurrent pregnancy loss and without known autoimmune disease and found a more credible rate of aPL positivity [101]. They defined a positive result as any value exceeding the 99th percentile of controls and normalized the results from each ELISA plate using a reference serum. Overall, 14% of patients with recurrent pregnancy loss were positive for IgG or IgM aPLs. In another larger retrospective study, Yetmen and Kutteh [102] evaluated 866 women with a history of recurrent pregnancy loss and 288 parous women without a history of reproductive problems. Using standardized testing, they measured aCLs and antiphosphatidylinositol, antiphosphatidylglycerol, antiphosphatidylserine, and antiphosphatidylethanolamine antibodies. The sera from 17.3% of the study-group patients were positive for aCLs, compared with 4% of controls. An additional 10.1% of study-group patients were negative for aCLs but had positive levels of another aPL.

Other studies refute the concept that aPLs other than LA and aCLs are related to recurrent pregnancy loss [103,104]. In a blinded study conducted in Utah, there was no difference in outcomes between patients with recurrent pregnancy loss who tested negative for LA and aCLs and patients who had antibodies binding to phospholipid antigens other than cardiolipin [104]. The few women (patients or controls) whose sera tested positive (>99th percentile) had very low levels of assay reactivity compared with the levels in women with well-characterized APS. Such low levels of aPLs may not be indicative of an autoimmune diathesis. Silver et al found that women with low levels of IgG aCLs (<20 GPL units) are at no greater risk for antiphospholipid-related medical complications than are women who test negative for aCLs [52]. In contrast, women with LA or medium-to-high positive IgG aCLs (>20 GPL units) are at substantial risk for antiphospholipid-related medical problems.

The authors agree with other investigators that given the limited and controversial data, only LA and aCL assays are appropriately standardized for routine testing. Testing for other aPLs and treatment based on such findings should remain investigational [105].

Antinuclear autoantibodies

Some women with recurrent pregnancy loss have detectable levels of ANAs. One group of investigators found ANAs in 5% of 130 women with recurrent pregnancy loss and in 2% of 50 controls [106]. All positive results were relatively low titer in nature ($\leq 1:200$). An additional patient was positive for anti-DNA (native) antibodies. Cowchock and colleagues investigated 61 women with unexplained recurrent pregnancy loss and 21 women with known causes of recurrent pregnancy loss [91]. They found ANAs in 29% of women with unexplained recurrent loss and 14% of women with explained recurrent loss — not statistically significant. Ten women in the unexplained-loss group and two in the explained-loss group had positive ANA titers of 1:500 or greater, and three women with unexplained losses had anti-DNA antibodies — also not statistically significant. Similarly, Maier and Parke found that 20% of 20 women with unexplained recurrent pregnancy loss and 14% of 14 women with explained recurrent pregnancy loss had low titer ANAs ($\leq 1:256$) [107]. Although none of 24 controls had positive tests, there was no statistical difference between any of the groups. Bahar et al [92] and Kwak and associates [93] reported similar findings.

In a larger study, Harger et al found that 16% of women with recurrent pregnancy loss had ANAs [108]; however, they found a significantly higher proportion of women with recurrent pregnancy loss who had ANAs at $\geq 1:80$ compared with controls. The next pregnancy outcomes (untreated) of patients with recurrent pregnancy loss and with positive ANA at $\geq 1:80$ did not differ significantly from the outcomes of ANA-negative patients with recurrent pregnancy loss (live births, 52% versus 67%, respectively). Ogasawara and colleagues also found that 17% of women with recurrent pregnancy loss had ANAs. Similarly, the next pregnancy outcomes of these women were comparable with the outcomes of women who had recurrent pregnancy loss and tested negative for ANAs [109]. These studies suggest ANAs simply may be a serum marker of previous pregnancy loss.

Whether ANAs are associated with pregnancy loss independent of aCLs is questionable. Three studies that have addressed this issue reported dramatically differing results—17% to 100% of patients with recurrent pregnancy loss who were positive for ANAs also had aPLs [91,106,107].

Mavragani and colleagues investigated the association of anti-Ro/SSA antibody with pregnancy loss and adverse pregnancy outcome in 154 women with known autoimmune diseases (78 had SLE, and 76 had non-SLE disease), who were compared with a control group of 142 anti-Ro/SSA-negative women (71 had SLE, and 71 had non-SLE disease) [110]. The patients and controls were matched for disease diagnosis and age at the time of anti-Ro/SSA diagnosis. The rate of pregnancy loss and adverse pregnancy outcome did not differ significantly between anti-Ro/SSA-positive women, anti-Ro/SSA-negative women, and healthy controls. The investigators found that anti-Ro/SSA-positive women with non-SLE disease reported a significantly higher rate (23.7%) of recurrent

pregnancy loss than did anti-Ro/SSA-negative women (7.04%; $P = 0.0063$) and healthy controls (6.4%; $P = 0.0004$). The authors concluded that anti-Ro/SSA antibody does not adversely affect pregnancy outcomes in patients with SLE but that this antibody was associated with recurrent pregnancy loss in patients with non-SLE disease. In a follow-up study, the authors reported that, when controlled for other autoimmune antibodies, each of three Ro-antigen–related activities were associated independently with fetal wastage [111]. This finding has not been confirmed by other studies.

To improve outcome in pregnant women with autoantibodies (present pre-pregnancy), including ANAs, Laskin et al randomly assigned women with a history of recurrent pregnancy loss to receive placebo or prednisone and low-dose aspirin therapy [112]. Compared with controls, patients in the treatment arm did not have improved outcomes and had a higher rate of preterm delivery. The authors concluded that prednisone and low-dose aspirin therapy was not effective in increasing the rate of live birth.

Many investigators find that women with recurrent pregnancy loss are no more likely than controls to be positive for ANAs. In one study in which a difference was noted, the next pregnancy outcomes without treatment did not differ from the outcomes in ANA-negative patients. The authors believe that studies performed to date do not support routine testing of ANA status in otherwise healthy women with recurrent pregnancy loss.

Antithyroid antibodies

Over the last decade, investigators have given increased attention to the possible role of antithyroid antibodies in pregnancy loss. Studies have evaluated the association of thyroid autoantibodies in several categories of women: the general obstetric population, pregnant and nonpregnant patients with a history of recurrent pregnancy loss, and infertile women undergoing assisted reproductive technology (ART).

Unselected pregnant women

In 1990, Stegnaro-Green and colleagues reported on the importance of antithyroid antibodies in an unselected population of pregnant patients. The investigators tested for antithyroid autoantibodies in the sera (obtained at the first prenatal visit) of 552 women [113]. Twenty percent of patients tested positive for antithyroglobulin or antimicrosomal antibodies. Subsequent pregnancy loss occurred in a higher proportion of women with these antibodies than in women without the antibodies (17% versus 8.4%). This association was confirmed in another large ($n = 730$), prospective study [114].

Women with recurrent pregnancy loss

Wilson and colleagues serially measured antithyroid antibody titer and antibody avidity in 15 pregnant patients with a history of recurrent miscarriage who were known to be positive for the thyroid peroxidase antibody. Seven

patients subsequently miscarried in the first trimester, and eight patients carried to term. Thyroid peroxidase antibody titer and avidity was significantly higher in women who miscarried than in women who carried to term. In women with term pregnancies, titer and avidity declined as the pregnancy progressed [115].

Esplin and co-workers tested for antithyroglobulin and antithyroid peroxidase antibodies in 74 nonpregnant (≥ 6 months after a pregnancy) women with a history of recurrent pregnancy loss and in 75 healthy, fertile controls of similar gravidity [116]. Twenty-two women with a history of recurrent pregnancy loss (29.3%) and 28 controls (37%) had positive results for one or both of the thyroid autoantibodies ($P > 0.05$). Mean levels of thyroid-stimulating hormone and the number of women with abnormal levels of thyroid-stimulating hormone did not differ between the groups. The authors found that women with a history of recurrent pregnancy loss were no more likely than were fertile controls to have circulating thyroid autoantibodies [116]. In a follow-up study, the authors published the findings of the next pregnancy outcomes in these women and found no difference in the outcomes between those who did and did not have measurable thyroid autoantibodies [117].

Although the presence and perhaps the titer of thyroid autoantibodies during a pregnancy may predict pregnancy outcome, antithyroid antibodies do not seem to pose an increased risk for future pregnancy loss in women with a history of recurrent miscarriage. A larger, prospective study that uses control patients who are negative for antithyroid antibodies and who have a history of unexplained recurrent miscarriage may resolve this question.

Women undergoing assisted reproductive technology

The results from a study by Geva et al suggest that antithyroid autoantibodies may be important in predicting the pregnancy success of women undergoing ART [118]. The investigators prospectively evaluated 78 patients with unexplained or mechanical infertility for the presence of antithyroid and antiovarian antibodies. The pregnancy rate per cycle of in vitro fertilization was statistically lower in the 23 patients who tested positive for antibodies compared with the rate in the other 55 patients.

Kutteh and colleagues retrospectively compared the prevalence of antibodies to thyroglobulin and thyroid peroxidase (or microsomal) in 700 women with a history of two or more consecutive pregnancy losses with the prevalence in 688 women with a history of infertility who were undergoing ART and with the prevalence in 200 healthy, reproductive-aged controls [119]. Antithyroid antibodies were identified in 29 of 200 (14.5%) controls, in 158 of 700 (22.5%) women with recurrent pregnancy loss, and in 132 of 688 (19.2%) women undergoing ART. The difference between the controls and the women with recurrent pregnancy loss was statistically different, whereas the difference between the women undergoing ART and the women with recurrent pregnancy loss was not statistically different. The authors concluded that thyroid autoantibodies might be markers of autoimmune activation and were associated with an increased risk for pregnancy loss.

Although the number of patients in this study is impressive, the study is subject to the limitations of retrospective evaluations. Some women in the study groups were pregnant at the time of laboratory analysis, and the frequency or distribution of concurrent pregnancy among the groups was not clarified.

There seems to be a relationship between the presence of thyroid autoantibodies during pregnancy and the risk for subsequent pregnancy loss. Likewise, the presence of thyroid autoantibodies may be associated with failed pregnancy in women undergoing ART. From a practical standpoint, the clinical usefulness of such knowledge is minimal, unless a causal relationship between antithyroid antibodies and recurrent pregnancy becomes established more firmly and efficacious therapies become available. Until such time, routine evaluation of antithyroid antibodies in women with recurrent pregnancy loss or women undergoing ART cannot be recommended.

Summary

Certain autoimmune diseases, such as SLE or APS, are associated with an increased risk for fetal wastage. A small number of women with recurrent embryonic loss and a larger number of women who have experienced fetal loss, especially when recurrent, will be diagnosed with APS. Patients with APS usually are treated with heparin and low-dose aspirin therapy to improve next pregnancy outcome. High-dose immunoglobulin treatment may decrease the severity or delay the onset of pregnancy complications, such as intrauterine fetal growth restriction (IUGR) or preeclampsia, when added to heparin and low-dose aspirin and may be superior to prednisone and low-dose aspirin therapy; optimal therapy remains to be clearly established.

Consensus criteria require that repeat testing be positive to confirm the diagnosis but allows that patients with certain clinical findings and isolated medium-to-high positive IgG or IgM aCLs be diagnosed with APS. The independent significance of low-titer (<20 GPL units) IgG aCLs and isolated IgM or IgA has not been demonstrated adequately.

The procoagulant pathology associated with aCLs seems to involve the inhibition of the proper function of β_2 -GP-I. Testing specifically for anti- β_2 -GP-I antibodies may be considered in women who test negative for LA and aCLs but who are highly suspect to have APS. The specific isotype of anti- β_2 -GP-I antibodies (eg, IgA anti- β_2 -GP-I antibodies) may be important.

The independent relationship between other aPLs, such as phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidic acid, and recurrent pregnancy loss has not been established adequately. Outside of experimental protocols, testing for the presence of these antibodies is not warranted. Likewise, although ANAs seem to be related to previous pregnancy loss and although the presence of antithyroid antibodies adds some predictability to the success of a concomitant pregnancy, there is no clinical

benefit from testing for the presence of these antibodies in women with recurrent pregnancy loss.

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