Association Between Antiphospholipid Antibodies and Recurrent Fetal Loss in Women Without Autoimmune Disease: A Metaanalysis

LUCIE OPATRNY, MICHÈLE DAVID, SUSAN R. KAHN, IAN SHRIER, and EVELYNE REY

ABSTRACT. ***Objective.*** To assess the strength of association between recurrent fetal loss (RFL) and presence of antiphospholipid antibodies (aPL) in women without autoimmune disease, and to examine whether magnitude of association varies according to type or titer of antibody and timing of fetal loss.

Methods. We searched Medline and Current Contents for articles published between 1975 and 2003 with terms denoting early (less than 13 weeks) and late (less than 24 weeks) RFL associated with various aPL. Published case-control, cohort, and cross-sectional studies rated moderate or strong were included in our metaanalysis. Pooled odds ratios with 95% CI were generated using the random-effects models with Cochrane Review Manager software.

Results. Our analysis included 25 studies. Lupus anticoagulant (LAC) was associated with late RFL (OR 7.79, 95% CI 2.30–26.45); the association of LAC was stronger than that of any other aPL. IgG anticardiolipin antibodies (aCL), when combining all titers, were associated with both early (OR 3.56, 95% CI 1.48–8.59) and late RFL (OR 3.57, 95% CI 2.26–5.65). Restricting analysis to include only women with moderate to high titers increased the strength of association (OR 4.68, 95% CI 2.96–7.40). It was not possible to extract data on isolated low IgG aCL positivity. IgM aCL were associated with late RFL (OR 5.61, 95% CI 1.26-25.03). There was no association found between early RFL and anti-$ß_2$-glycoprotein I antibodies (OR 2.12, 95% CI 0.69–6.53).

Conclusion. The magnitude of the association between aPL and RFL varies according to type of aPL. More data on the relationship between recurrent fetal loss and isolated IgM aCL as well as with low titer IgG aCL would be useful. The place of testing for anti-$ß_2$-glycoprotein I antibodies remains to be determined. (First Release Oct 1 2006; J Rheumatol 2006;33:2214–21)

Key Indexing Terms:
RECURRENT FETAL LOSS ANTIPHOSPHOLIPID ANTIBODIES METAANALYSIS
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Recurrent fetal loss (RFL), variably defined as the loss of either 2 or 3 pregnancies, is an incompletely understood process. While etiologic factors such as infections, karyotype abnormalities, and underlying endocrine and gynecologic conditions are identified in some patients, many cases remain unexplained.

The association between antiphospholipid antibodies (aPL) and RFL has been recognized for many years[1-3], and RFL is one of the clinical criteria included in the definition of the antiphospholipid syndrome (APS)[4]. aPL are a heterogeneous group of autoantibodies that bind to negatively charged phospholipids, phospholipid-binding proteins, or both[5]. The most commonly detected aPL are lupus anticoagulants (LAC), anticardiolipin antibodies (aCL), and anti-$ß_2$-glycoprotein I antibodies (anti-$ß_2$-GPI)[5]. LAC are detected by coagulation assays, and are either dependent on $ß_2$-GPI or prothrombin[6]. aCL and anti-$ß_2$-GPI are detected by immunoassays[5]; aCL assays detect antibodies that react with cardiolipin (a phospholipid), or more commonly, with complexes of cardiolipin and $ß_2$-GPI, the source and quantity of which vary among assays. Some antibodies can bind directly to $ß_2$-GPI in the absence of phospholipids, and specific assays have been devised to detect these antibodies[6].

The association of aPL with fetal loss has been described in women with systemic lupus erythematosus, as well as in women without evidence of autoimmune disease. Among

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published studies, the presence and the strength of association between aPL antibodies and RFL in women with autoimmune disease are consistent and strong throughout studies[7-9], while in women without autoimmune disease, the association is variable. Anti-ß$_2$-GPI is considered by some authors to be a more specific marker of APS than aCL[10]. The relationship between RFL and IgM aCL, and between RFL and low titers of IgG aCL, is uncertain[11].

In order to clarify the magnitude of risk associated with aPL in women without autoimmune disease with RFL, we conducted a metaanalysis to determine the strength of the association between aPL and RFL according to the type of antibody (LAC, aCL, and anti-ß$_2$-GPI), as well as to the isotype and titer of antibody. We also examined whether these associations varied according to the timing of the RFL.

MATERIALS AND METHODS

Medline and Current Contents were searched independently by 3 investigators (LO, MD, ER) for English language articles published between January 1, 1975, and September 1, 2003, that contained the following terms: fetal loss, RFL, habitual abortion, recurrent abortion, abortion, spontaneous abortion, or miscarriage combined with the terms LAC, aCL antibodies, aPL antibodies, and anti-ß$_2$-GPI antibodies.

Data abstraction. Inclusion and exclusion criteria for articles were established prior to data abstraction. Case-control, cohort, and cross-sectional studies were potentially eligible to be included. Studies without controls, or control groups that included men or non-parous women were excluded. Unpublished data, case reports, abstracts, editorials, letters, reviews, meta-analyses, and studies with cases selected on the basis of autoimmune disease, thrombotic disorders, *in-vitro* fertilization, or other adverse pregnancy outcomes were also excluded from analysis, but their references were used to obtain other potentially appropriate articles.

The outcome of interest was RFL, which was defined as 2 or more losses that occurred during the gestational period under study. RFL was further categorized as early RFL, defined as loss occurring at less than 13 weeks' gestational age, and late RFL, defined as loss occurring at less than 24 weeks' gestational age.

The exposure of interest was the presence of aPL. Data were extracted for each aPL specificity and antibody isotype (IgG or IgM). Diagnosis of LAC required a prolonged phospholipid-dependent coagulation test (activated partial thromboplastin time, kaolin clotting time, dilute Russell viper venom time, tissue thromboplastin time, dilute prothrombin time), absence of correction with plasma, and correction with the addition of excess anionic phospholipids[12]. The following criteria were required to diagnose aCL of moderate to high titer: titer of more than 5 standard deviations (SD) above normal, titer greater than 99th percentile, more than 20 GPL/MPL units or 5 SBI (specific binding index) for IgG aCL[13]. Any other values set for detection of aCL were considered low-titer[13]. Based on the published criteria for APS, whereby only moderate to high titers are considered clinically significant, we tried to separate data for patients with low and those with moderate to high titers in each study. Since very few studies confirmed positive aPL antibodies with retesting, we did not consider absence of repeat positivity as an exclusion criterion. For anti-ß$_2$-GPI, we separated studies that used a phospholipid based assay with purified ß$_2$-GPI from those that used an assay with purified ß$_2$-GPI alone, although authors referred to both these types of assays as anti-ß$_2$-GPI.

Studies were reviewed and rated independently by LO, ER, and MD. Quality scores (weak, moderate, or strong) were assigned to each of the studies using a quality assessment grid previously used for observational studies[14]. A fourth investigator (SK) was used as arbitrator in case of discrepancy. The criteria used to judge study quality included adequate description of the study population, appropriate control group, appropriate laboratory method for the measurement of aPL, and provision of enough information to allow extraction of the required data. Studies graded as moderate or strong were retained for analysis.

Data from retained studies were extracted separately for each type of aPL, and were further subdivided according to timing of fetal loss (by gestational week), antibody titer (moderate to high vs other), and antibody isotype (IgG or IgM).

The analysis for late fetal loss also included women with early fetal loss. Data were also retrieved on whether women who had other conditions associated with fetal loss were excluded. These conditions included karyotype abnormalities, endocrine abnormalities, uterine anomalies, infections, and other systemic diseases. Women in some studies had had prior thrombotic events, but for the most part, whether cases or controls had suffered from a thrombotic event in the past was not explicitly mentioned or was excluded by study authors, as described in Table 1.

Statistical analysis. Two-by-two tables were constructed from the extracted data. Cochrane Review Manager software (version 4.0.3) was used to generate individual and pooled odds ratios (OR) with 95% confidence intervals. Confidence intervals not crossing the unity value were considered statistically significant. A summary statistic was only provided if the p value of the heterogeneity test was greater than 0.05. We used a random-effects model to combine the data that provides a more conservative estimate of effect compared to a fixed-effects model. In addition, to allow inclusion of older studies that used assays that did not discriminate between IgG and IgM aCL, a combined summary statistic was also performed for IgM and IgG. In more recent studies where the antibody isotype was determined, patients were included when they had IgG, IgM, or both. Therefore, the data represent women with either IgG or IgM positivity or both.

RESULTS

We initially retrieved 128 published studies, of which 103 were excluded: 47 used unsuitable controls, 40 used unsuitable definitions of cases (including studies that examined non-recurrent fetal loss), 4 used an inadequate definition of exposure, 7 used an inadequate definition of outcome, 4 were studies that retested initially negative patients, and one was a systematic review. Therefore, 25 studies are included in this metaanalysis. All are case-control studies[15-39]. Among retained studies, there is variability as to definitions of RFL, including the number and timing of losses, and some studies did not exclude women with other conditions associated with RFL (Table 1-3). In 13/25 studies, women were tested for more than one type of aPL antibody or antibody isotype[16-18,22-25,28,30,31,34-36]. Subanalysis of women having more than 3 fetal losses was only possible using very few studies for isolated outcomes, and are therefore not further presented.

Lupus anticoagulant. Pooled data for LAC are available from 9 studies (n = 2195)[16-24]. There were no data available to pool for RFL occurring prior to 13 weeks' gestation. RFL occurring prior to 24 weeks' gestation showed a strong, consistent, and significant association with LAC (OR 7.79, 95% CI 2.30–26.45)[16-19,22-24]. All studies except one[24] excluded women with other potential causes of RFL. When reanalyzed without this study, the association between LAC and RFL increased (OR 13.35, 95% CI 4.49–39.70; Figure 1). Pooled analysis of all studies of RFL regardless of timing of fetal loss showed a very similar result to the preceding analysis (OR 9.59, 95% CI 3.30–27.88). When analysis was restricted to

Table 1. Characteristics of studies examining lupus anticoagulant.

Author	Case Definition	Control Definition
Balasch[17]	≥ 2 consecutive losses < 20 wks. Other pathologies excluded; presence or absence of thrombosis unknown	Healthy parous women
Barbui[18]	≥ 2 consecutive losses < 20 wks. Other pathologies excluded; presence or absence of thrombosis unknown	Ill parous women
Das[19]	≥ 3 non-consecutive losses < 24 wks. Other pathologies excluded; presence or absence of thrombosis unknown	Pregnant parous women
Edelman[20]	≥ 2 non-consecutive losses at any fetal age. Other pathology excluded; thrombosis in 2% of patients	Parous women; some with a previous fetal loss
Gris[16]	≥ 3 consecutive losses < 16 wks. Other pathologies excluded; thrombosis in 0.4% of patients	Healthy parous women
Howard[21]	> 3 non-consecutive, fetal loss at any age. Other pathologies excluded; thrombotic disease absent	Healthy women
Maier[22]	≥ 3 non-consecutive losses < 20 wks. Other pathologies excluded. Presence or absence of thrombosis unknown	Healthy parous women
Parazzini[23]	≥ 2 consecutive losses < 20 wks. Other pathologies excluded. Presence or absence of thrombosis unknown	Ill parous women
Parke[24]	≥ 3 non-consecutive losses < 20 wks. Other pathologies not excluded	Healthy parous women

studies in which all other conditions were excluded (8 studies, n = 2026), the strength of the association was increased (OR 15.42, 95% CI 5.90–40.38)[16-23].

Anticardiolipin antibodies

IgG anticardiolipin antibody. Only 2 studies (n = 907) examined RFL that occurred at less than 13 weeks' gestational age and included all titers (low and moderate to high). These demonstrated a significant association between IgG aCL and RFL (OR 3.56, 95% CI 1.48–8.59; Figure 2)[28,35].

For RFL occurring at less than 24 weeks' gestation, 10 studies (n = 3631) were pooled, providing an odds ratio similar to that obtained for RFL occurring at less than 13 weeks' gestation (OR 3.57, 95% CI 2.26–5.65; Figure 2)[18,23,24,28,30,31,33-36]. When the analysis was restricted to studies that included only women with moderate to high IgG aCL titers (6 studies, n = 2724), a slight increase in the strength of association resulted (OR 4.68, 95% CI 2.96–7.40)[18,23,24,28,33,36]. It was not possible to generate a summary statistic of women with RFL and low aCL IgG.

IgM anticardiolipin. No study examined the association between RFL before 13 weeks' gestation and IgM aCL. Four studies (n = 1822) examined the association between RFL occurring at less than 24 weeks and IgM aCL[23,24,30,36]. Patients with other conditions associated with RFL were excluded in some but not all studies. When all antibody titers were examined, the strength of association was similar to that of IgG (OR 5.61, 95% CI 1.26–25.03; Figure 3). Restricting the analysis to those studies that included only women with moderate to high titers (3 studies, n = 1579) provided a similar point estimate for the odds ratio, which, however, was no longer significant (OR 4.03, 95% CI 0.84–19.34)[23,24,36]. It was not possible to extract data for women with isolated positivity for IgM aCL. Women included in the analysis were not all positive exclusively for IgM aCL.

IgG and IgM anticardiolipin antibodies combined. Older studies used aCL assays that did not distinguish between IgG and IgM antibodies. We attempted to combine studies that specified aCL isotype with those that did not. Using the investigator's definition of a positive assay, 15 studies (n = 4567) that examined RFL occurring at less than 24 weeks' gestation were identified, but these were found to be too heterogeneous (p = 0.031) to be combined statistically. When the analysis was restricted to studies using our *a priori* definition for moderate to high antibody titers, 10 statistically homogeneous (p = 0.9) studies (n = 3534), when combined, generated a pooled odds ratio similar to that of either antibody separately (OR 5.39, 95% CI 3.72–7.82)[17,18,22-24,26,28,29,33,36]. In both the preceding analyses, other conditions associated with RFL were not fully excluded in all studies.

Anti-$ß_2$-glycoprotein I. There were 5 studies looking at anti-$ß_2$-GPI that met criteria for inclusion in this metaanalysis[16,28,35,37,38]. Four used an assay with cardiolopin and purified $ß_2$-GPI (n = 1585), and one used an assay with purified $ß_2$-GPI without phospholipids (n = 203). All looked at women with losses prior to 13 weeks' gestation. The relationship between anti-$ß_2$-GPI antibodies and RFL was not statistically significant, irrespective of whether the first (OR 2.12, 95% CI 0.69–6.53; Figure 4) or second type of assay (OR 1.10, 95% CI 0.34–3.53) was used.

Two studies examining the relationship between RFL and IgM anti-$ß_2$-GPI met our inclusion criteria. However, the 2 studies used different assay methods, and therefore could not be pooled. One of these, by Gris, *et al*, used an assay with cardiolipin and purified $ß_2$-GPI and found no association with RFL (OR 2.02, 95% CI 0.26–15.96)[16]. The other study had no

Table 2. Characteristics of studies examining anticardiolipin antibodies.

Study	Antibody Type and Titer (low or moderate-high)	Case Definition	Control Definition
Bahar[25]	IgG, IgM. Low cut-off; positive test not repeated	≥ 3 non-consecutive losses < 25 wks; Other pathologies excluded. Thrombotic disease absent	Healthy parous women
Balasch[17]	IgG, IgM. High cut-off; positive tests repeated	≥ 2 consecutive losses < 20 wks; Other pathologies excluded; Presence or absence of thrombotic disease unknown	Healthy parous women
Barbui[18]	IgG. High cut-off; Positive test not repeated	≥ 2 consecutive losses < 20 wks; Other pathologies excluded. Presence or absence of thrombotic disease unknown	Hospital control parous women
Costa[39]	IgG, IgM. Low cut-off; Positive test not repeated	≥ 3 consecutive fetal losses < 22 wks. Other pathologies partially excluded; Presence or absence of thrombosis unknown	Healthy parous women
Decarolis[26]	IgG, IgM. High cut-off; Positive test repeated	> 2 consecutive losses < 20 wks or > 20 wks. Other pathology excluded. Presence or absence of thrombosis unknown	Healthy parous women
Deleze[27]	IgG, IgM. Low cut-off; Positive test not repeated	> 2 fetal losses at any age. Other pathologies partially excluded. Thrombosis in 3% of patients	Healthy women, many pregnant
Gris[16]	IgG, IgM. High cut-off; positive test repeated	≥ 1 fetal loss > 22 wks; many had previous losses. Other pathologies excluded; Presence or absence of thrombosis unknown	Healthy parous women
Higashino[28]	IgG. High cut-off; positive test not repeated	≥ 2 or more fetal loss < 13 wks. Other pathologies not excluded. Presence or absence of thrombosis unknown	Healthy parous women
Kutteh[29]	IgG, IgM. High cut-off; positive test not repeated	≥ 3 consecutive losses < 20 wks. Other pathological exclusion unclear. Thrombotic disease excluded	Healthy parous women
Kwak[30]	IgG, IgM. Low=cutoff; positive test sometimes repeated	≥ 3 consecutive loss < 24 wks. Other pathologies excluded. Presence or absence of thrombotic disease unknown	Healthy parous women
Maier[22]	IgG, IgM. High cut-off; positive test not repeated	≥ 3 non-consecutive losses < 20 wks. Other pathologies excluded. Thrombosis in 5% of patients	Parous women
Melk[31]	IgG, IgM. Low cut-off; positive test not repeated	> 3 consecutive losses < 17 wks. Other pathologies excluded; Presence or absence of thrombosis unknown	Healthy parous women
Out[32]	IgG, IgM. Low cut-off; positive test not repeated	≥ 3 non-consecutive loss < 12 wks. Other pathologies excluded. Thrombotic disease absent	Pregnant healthy women
Panton[33]	IgG, Low & high cut-offs; positive test repeated	≥ 3 non-consecutive losses < 20 wks. Other pathologies excluded; Presence or absence of thrombosis unknown	Healthy parous women
Parazzini[23]	IgG, IgM. High cut-off; positive test not repeated	≥ 2 consecutive losses < 20 wks. Other pathologies excluded. Presence or absence of thrombosis unknown	Ill parous women
Parke[24]	IgG, IgM. High cut-off; positive tests repeated	≥ 3 non-consecutive losses < 20 wks. Other pathologies not excluded. Presence or absence of thrombosis unknown	Healthy parous women
Ruiz[34]	IgG. High cut-off; positive test not repeated	≥ 3 consecutive losses < 20 wks. Other pathologies excluded. Presence or absence of thrombosis unknown	Healthy parous women
Stern[35]	IgG, IgM. Low cut-off; positive test not repeated	≥ 3 consecutive losses < 12 wks. Other pathologies excluded; Presence or absence of thrombosis unknown	Healthy parous women
Yetman[36]	IgG, IgM. High cut-off; positive test not repeated	≥ 2 consecutive losses < 20 wks. Other pathologies not excluded.Presence or absence of thrombosis unknown	Healthy non pregnant women

events of RFL in the control group, making risk estimates difficult to calculate[16,35].

DISCUSSION

Our findings indicate that the relationship between RFL and aPL differs according to the type and isotype of aPL studied. This varies from a very strong risk of RFL with LAC and IgG aCL antibodies to no apparent association of RFL with anti-$ß_2$-GPI.

We observed a strong, consistent, and significant association between LAC and the risk of RFL in women without autoimmune disorders. The magnitude of the association was considerably higher for LAC than for any other aPL antibodies including IgG aCL. This finding is consistent with a recent metaanalysis by Galli and coworkers on the association of aPL antibodies and the risk of thrombosis, which found a stronger association with LAC than with aCL[40]. There is, however, a lack of data for isolated first trimester RFL in association with LAC, despite this being a diagnostic criterion for the APS[4].

We demonstrated a significant association between IgG aCL and early and late RFL. The association was of similar magnitude whether or not analysis was restricted to studies that included only women with high-titer antibodies.

Table 3. Characteristics of studies examining ß2-dependent anticardiolipin antibodies, anti-$ß_2$-glycoprotein I antibodies, and fetal loss.

Study	Antibody Type and Titer	Case Definition	Control Definition
Gris[16]	IgG, IgM. Positive test repeated	≥ 3 consecutive losses < 16 wks; Other pathologies excluded. Thrombosis in 0.4% of patients	Healthy parous women
Higashino[28]	IgG. Positive test not repeated	≥ 2 fetal loss < 13 wks. Other pathologies not excluded. Presence or absence of thrombosis unknwon	Healthy parous women
Maejima[37]	IgG, IgM. Positive test not repeated	≥ 2 consecutive losses < 13 wks. Other pathologies excluded. Presence or absence of thrombosis unknown	Healthy parous women
Matsubayashi[38]	IgG, IgM. Positive test repeated	≥ 3 non-consecutive losses < 10 wks	Healthy women, parity unknown
Stern[35]	IgG, IgM. Positive test not repeated	≥ 3 consecutive losses < 13 wks	Healthy parous women

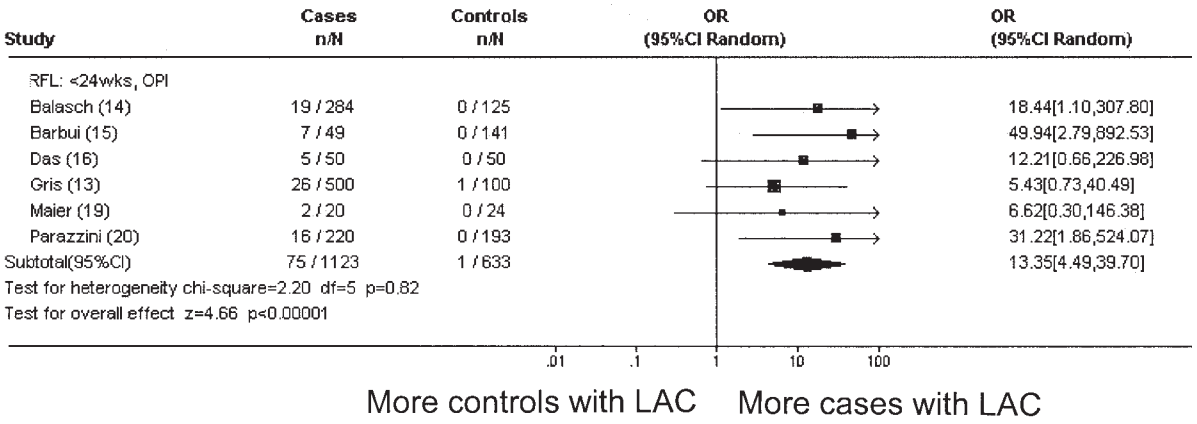

Figure 1. Lupus anticoagulant and late recurrent fetal loss.

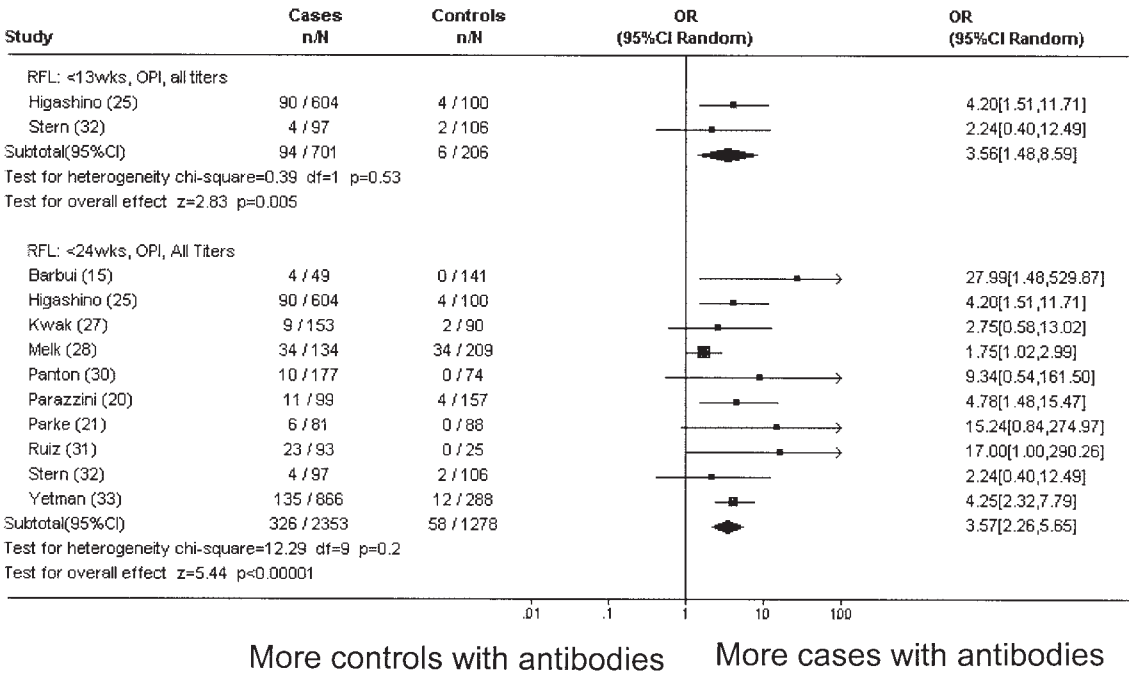

Figure 2. IgG anticardiolipin and recurrent fetal loss.

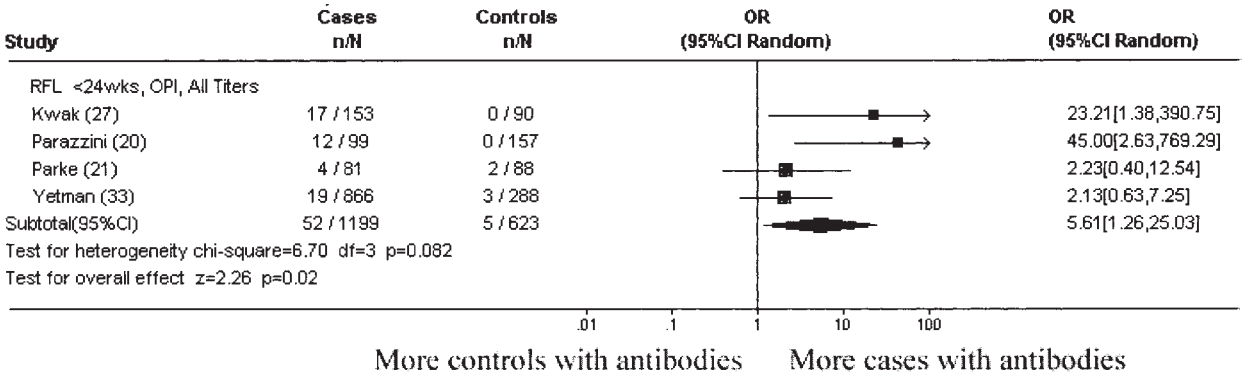

Figure 3. IgM anticardiolipin and late recurrent fetal loss.

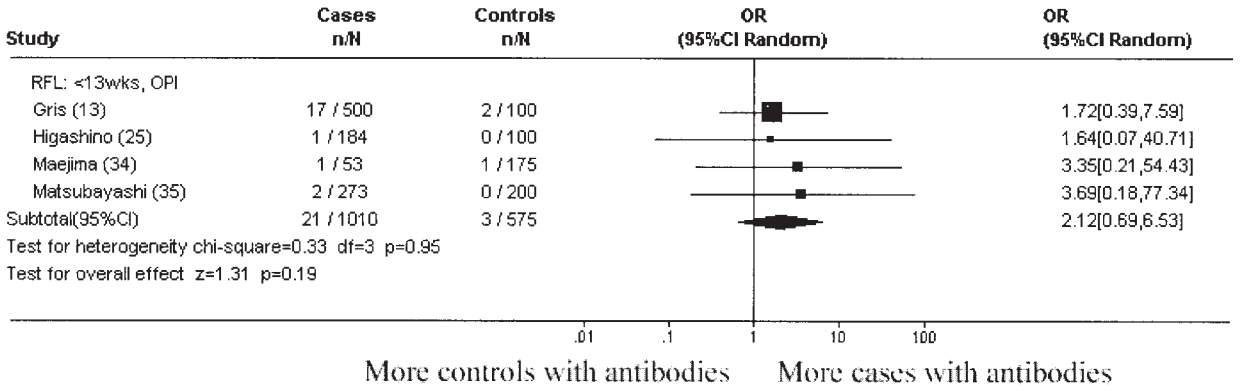

Figure 4. IgG ß$_2$-glycoprotein 1 and late recurrent fetal loss.

However, there were insufficient data to permit analysis of the association between RFL and low-titer IgG aCL, which is a frequent clinical scenario.

The relationship between IgM aCL and RFL has been less certain, as many disease processes other than autoimmune illnesses can lead to IgM aCL positivity. Further, although isolated IgM positivity is not an uncommon clinical scenario, studies have not distinguished between women having isolated IgM aCL and women having additional aPL antibodies. More studies would be beneficial to establish the magnitude of association between RFL and IgM aCL.

We did not detect a significant association between anti-ß$_2$-GPI and RFL. However, the wide confidence intervals suggest a power problem. The only study meeting our inclusion criteria that used an assay with ß$_2$-GPI without phospholipid was negative[35]. The role of testing for anti-ß$_2$-GPI antibodies remains to be established. Based on our results it would be premature to include anti-ß$_2$-GPI assay in the standard investigation of a woman with RFL.

Data regarding RFL after 24 weeks' gestation are not presented: few papers have been published in this area because RFL after 24 weeks is a rare clinical situation.

The poor standardization of assays testing for aPL antibodies among studies poses a problem for study comparison. Previous studies have demonstrated significant interlaboratory and interassay variability in reporting levels of a standard aliquot of aPL[41,42], and standardization of these assays remains an active objective of collaborative efforts. Further, the majority of studies included in this analysis did not confirm positive results with retesting 6 weeks later, which is recommended for diagnosis of APS[4].

Although we attempted to keep studies homogeneous, the minimum number of consecutive losses required for RFL was 2 in some studies and 3 in others. Selection of controls also differed to some extent between studies. For example, although all control subjects were parous women, 3/25 studies matched for parity, while in 22/25 studies the number of gestations differed between cases and controls. Also, although most control women were healthy, some were disease controls. Inclusion of disease controls would tend to minimize the association seen, as aPL can occasionally be found in the context of other, unrelated illnesses.

In some studies, patients were reported to have thrombotic events, while in other studies thrombotic events were not

mentioned. If thrombosis were more frequent in RFL subjects, these women would be non-healthy, and would tend to exaggerate the association seen with RFL. However, this is unlikely to have significantly altered our results, as only a small minority of women had thrombosis in the studies where it is mentioned, as noted in Tables 1–3.

The timing of fetal death reported in the studies may not be exact, as fetal death may precede clinical detection by some weeks.

Lastly, it would have been interesting to further analyze very early fetal losses (less than 6 wks) separately from fetal losses later in the first trimester, as the pathophysiology may well be distinct. This was not possible with the given data.

The pathophysiology of aPL and their role in fetal loss remains incompletely understood. It could be explained by different mechanisms, including thrombosis of placental vessels and placental infarction leading to uteroplacental insufficiency. Placental pathology in some women with aPL has shown decidual vasculopathy and placental infarction[43-45]. IgG aCL have been hypothesized to act on the fetal side of the placenta as they are able to cross the placental barrier[27,46]. While the formation of aPL has been hypothesized by some authors to be an epiphenomenon occurring as a result of either fetal loss or pregnancy, rather than the cause of the fetal loss itself, experimental animal data support a cause-and-effect model. Prospective human data on the aPL antibody/RFL relationship in otherwise healthy women are lacking[47-49].

Our metaanalysis suggests that in women without autoimmune disease, the risk of RFL varies with the aPL antibody type, and the presence of LAC represents by far the strongest risk for RFL. Future research should aim to clarify the association between low-titer IgG aCL and IgM aCL antibodies and fetal loss, as well as the significance of anti-ß$_2$-GPI antibodies. This will allow for more conclusive studies on the role of antithrombotic agents in the prevention of fetal loss in these women.

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