Antiphospholipid antibodies and antibodies to tissue factor pathway inhibitor in women with implantation failures or early and late pregnancy losses

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The association of antiphospholipid antibodies (aPL) with early recurrent spontaneous abortions and fetal deaths is well established [1,2]. A role for aPL as a possible cause of failure to achieve pregnancy after *in vitro* fertilization (IVF), however, is controversial. An association between the hypercoagulable state because of the presence of aPL and unsuccessful embryo implantation has been observed in some [3], but not in other studies [4–6]. Pregnancy complications constitute one of the two major clinical criteria of the antiphospholipid syndrome (APS) [7] and adverse pregnancy outcomes may result from poor placental perfusion because of localized thrombosis. Recently, we found that high titers of antibodies to tissue factor pathway inhibitor (TFPI) (anti-TFPI) in women with autoimmune aPL seem to increase the risk of aPL-related reproductive failures [8]. TFPI is a Kunitz-type protease inhibitor that tightly regulates tissue factor-mediated coagulation. During normal pregnancy, some of the hemostatic changes occurring in the placenta are characterized by increased tissue factor expression and low expression of TFPI. Both tissue factor and TFPI seem to be essential for the maintenance of hemostasis in the placenta [9]. The objective of the present study was to evaluate the presence of aPL [lupus anticoagulant (LA) and moderate or high titers of anticardiolipin antibodies (aCL)] and anti-TFPI in women with a history of pregnancy loss, as well as in women with recurrent IVF failures.

The study included patients referred to our institution in Argentina for evaluation of aPL because of a history of two or more consecutive spontaneous abortions (early pregnancy loss before 10 weeks of gestation), at least one fetal death (late pregnancy loss at or beyond 10 weeks of gestation) or recurrent (two or more) IVF failures despite good visual quality embryos. Patients were referred after excluding other common etiologies of pregnancy failures (infections and hormonal, metabolic, uterine anatomic or genetic abnormalities). There were 243 women (median age 32 years old, range 21–37) with early and/or late pregnancy losses (98 with early, 116 with late, and 29 with both), and 48 (median age 33 years old, range 23–39) with IVF failures. The median number of pregnancy losses was three (range 2–7) in the early group and two (range 1–5) in the late group, and the median number of IVF procedures was three (range 2–6) in the IVF failure group. A group of 80 normal control women (median age 35 years old, range 25–42) was also evaluated. They had had only successful pregnancies. None of the patients or control women had a history of thrombosis or systemic lupus erythematosus. In all cases, blood collection took place at least 3 months after pregnancy complications and if aPL were positive a second blood sample was taken 3 or 6 months later. LA activity was identified through at least three different screening tests, mixing studies and confirmatory procedures according to the ISTH guidelines. aCL (IgG and IgM isotypes) were measured by using a standardized in-house ELISA and titers above 20 GPL or MPL units were considered to be a positive result. The in-house ELISAs for IgG and IgM antibodies to β_2 glycoprotein I (anti-β_2GPI) and to prothrombin (anti-PT) were performed as previously reported using electron beam- (100 kGy) and γ-irradiated microtiter plates, respectively. The cut-off values (10 arbitrary units for IgG or IgM) were previously determined as the 99th percentiles of 95 normal sera [10]. Antibodies to TFPI (IgG and IgM isotypes) were detected in patients' sera as recently described using recombinant full-length TFPI coated on γ-irradiated microtiter plates [8]. Results were expressed as U mL^{-1}, referred to internal standards arbitrarily fixed at 100 U mL^{-1}. The 99th percentiles in 70 healthy controls were previously chosen as the cut-off points (18 U mL^{-1} and 15 U mL^{-1} for IgG and IgM anti-TFPI, respectively).

Of the 80 control women, positive aCL was found in only one subject (1.2%). Similarly, only two of 48 (4.2%) patients with IVF failures had aCL. None of them had either LA or anti-β_2GPI and/or anti-PT. On the other hand, 43 of the 243 (17.7%) women with pregnancy loss had persistent classical aPL. LA was positive in 32; aCL in 37 and 34 women had anti-β_2GPI and/or anti-PT. All women with positive anti-β_2GPI and/or anti-PT had LA and/or aCL. The prevalence of aPL

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was statistically different between women with pregnancy loss and normal controls ($P < 0.0001$) as well as with those with IVF failures ($P < 0.02$). No significant differences were observed in the frequencies of LA, aCL, anti-β_2GPI and anti-PT when cases were divided into three groups: early, late and both early and late pregnancy losses. Anti-TFPI was evaluated in a subgroup of 84 women belonging to the aPL negative group of patients with pregnancy loss ($n = 200$). They were matched by age, type of obstetric complication, and number of pregnancies with the aPL positive group ($n = 43$). In this aPL negative group with pregnancy loss, only one patient had IgG anti-TFPI. Similarly, only one of the 48 women with IVF failures and one of the 80 normal controls had positive IgG anti-TFPI (Fig. 1A). Figure 1B shows the distribution of TPFI in the 43 aPL positive women with pregnancy loss and in the control group of 80 women with only uncomplicated pregnancies. Within the former group, there were 10 women with IgG, five with IgM, and one with both IgG and IgM anti-TFPI. Anti-TFPI was significantly more prevalent in aPL positive women with pregnancy loss (37.2%) when compared with normal control women (1.3%) and aPL negative women with pregnancy loss (1.2%) ($P < 0.0001$). The frequency of high titers of anti-TFPI (defined previously as above 50 U mL^{-1}) [8] was also significantly higher in cases with history of pregnancy loss than in normal control women (13.9% vs. 0%, $P < 0.002$). No significant differences were observed in the frequencies of anti-TFPI when cases were divided into three groups: early, late and both early and late pregnancy losses. The occurrence of the major inherited prothrombotic risk factors among normal control, early pregnancy loss, late pregnancy loss and IVF failure women were 1.2%, 5.4%, 2.9%, and 2.1% for factor V Leiden, and 1.2%, 2.2%, 1.6%, and 0% for prothrombin G20210A, respectively.

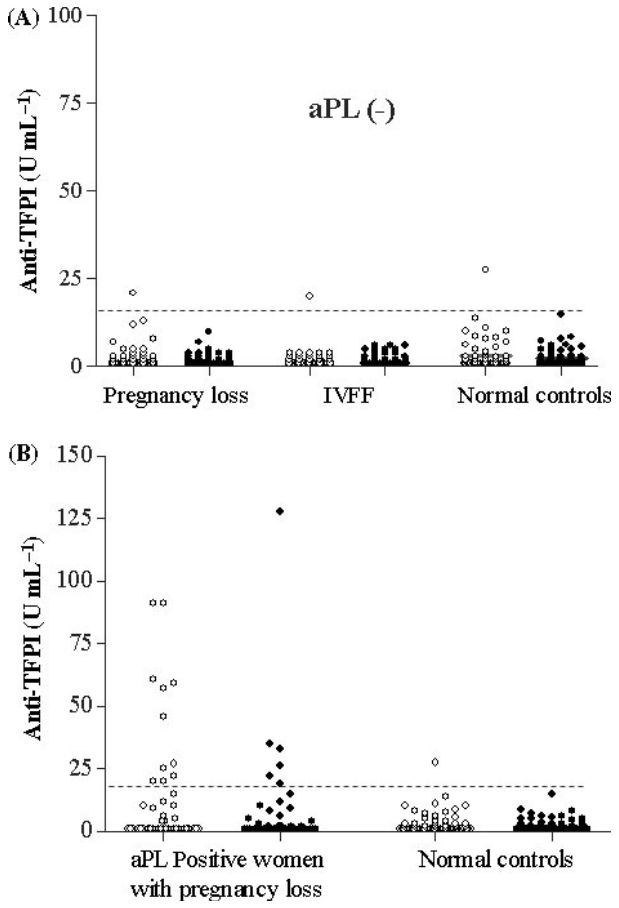

Fig. 1. Distribution of IgG (o) and IgM (•) antibodies to TFPI in (A) aPL negative women with early or late pregnancy loss, IVF failure and normal controls; and (B) aPL positive women with early or late pregnancy loss. The broken lines indicate the cut-off points for IgG and IgM.

Several studies have suggested that acquired or inherited prothrombotic factors may play a major role in the occurrence of obstetric morbidity. Nevertheless, there is no clear evidence that adverse pregnancy outcomes are associated with all thrombophilias [11]. The data presented here indicate that there is an association between classical aPL and an increased risk of early and late pregnancy losses. On the other hand, this study shows that failure to achieve pregnancy after IVF is not linked to the presence of aPL. Autoimmune aPL are antibodies that exhibit a broad range of target specificities and affinities [12]. Our present series of women with pregnancy losses had aPL mainly associated with anti-β_2GPI and anti-PT. In this case–control study of women with reproductive failure, we also found an association with anti-TFPI. However, the presence of anti-TFPI was mainly linked to the presence of classical aPL. The uteroplacental circulation resembles venous circulation in terms of its low pressure and low velocity, and may be particularly susceptible to thrombotic complications in thrombophilic women. Isolated IgGs from patients with anti-TFPI have been shown to impair the inhibitory effect of TFPI on factor Xa activity [8] and to be associated with increased thrombin generation [13]. Thus, it is reasonable to speculate that antibodies directed against TFPI can interfere *in vivo* with its antithrombotic properties and contribute at least in part to obstetric morbidity. To date, however, no experimental models or human data have shown such a direct correlation. In conclusion, anti-TFPI is associated with adverse obstetric outcomes in women with well-characterized aPL but not in aPL negative patients with either early or late recurrent pregnancy losses or IVF failures. More studies are needed in order to draw definite conclusions about the clinical relevance of antibodies against TFPI in pregnancy.

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Rituximab-induced long-term remission in patients with refractory acquired hemophilia

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In acquired hemophilia, a reduced level of factor VIII (FVIII) is caused by the development of autoantibodies against this coagulation factor, which are believed to be IgG in nature. The incidence is approximately 1 per million per year [1,2]. Mortality is high and ranging between 12.5% and 22%, usually because of fatal hemorrhage [3–6]. Therefore, it is important to treat to induce remission. Prednisolone alone or combined with azathioprine or cyclophosphamide is commonly used [7]. Recently, evidence has emerged suggesting that rituximab may be effective in many of these patients [1,8,9]. Rituximab is a chimeric monoclonal antibody against the CD20 antigen that blocks proliferation of normal B cells, hence interfering with antibody production of the IgG type. In many of the cases in which remission after rituximab has been described that the duration of disease was relatively recent (e.g. 1 day to 3 months)[1]. In this report, we discuss two cases with acquired FVIII inhibitors that at 12 and 30 months after initial presentation were resistant to immunosuppressive therapy and other lines of treatment. However, after rituximab therapy, both have shown a significant response.

Two female Caucasian patients (aged 47 and 80 years) presented with a normal INR and an APTT prolonged to 70 and 120 s, respectively (normal 24–37 s). In both, the FVIII level was greatly reduced to < 1 and < 5 IU dL^{-1}, respectively, (normal 45–160 IU dL^{-1}) with other coagulation factors and von Willebrand factor normal. Both were previously healthy with no significant drug history or family history of hemophilia or bleeding. In the first patient, the initial FVIII inhibitor level was 760 BU mL^{-1}. Oral prednisolone (1 mg kg^{-1} bw daily) and azathioprine (100 mg daily) were commenced and after 11 months the inhibitor had decreased to 115 BU mL^{-1}. One month later, she relapsed with life-threatening bleeding and inhibitor level of 148 BU mL^{-1}. Bleeding ceased shortly after

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