Activated protein C resistance and pregnancy loss

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Activated protein C resistance is a thrombophilia with an established role in producing thrombosis which more recently has been implicated in the pathogenesis of pregnancy loss. This review will analyse recent literature to evaluate this association and address the gestation and type of pregnancy loss.

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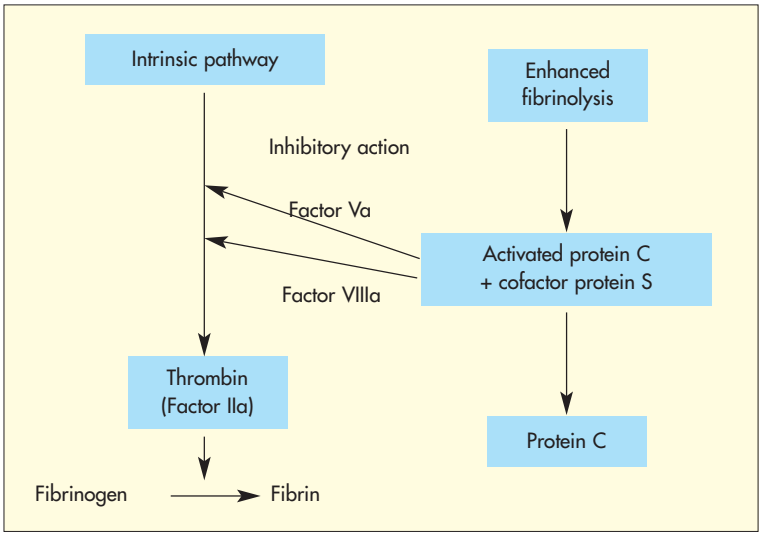
Recently, considerable interest has been generated regarding the association between thrombophilia and recurrent pregnancy loss. Thrombophilia can be acquired such as antiphospholipid syndrome (Roubey and Hoffman, 1997) or inherited such as factor V Leiden and activated protein C resistance (APCR) (Dahlback et al, 1993; Sheppard, 2000).

Hypercoagulation may encourage placental thrombosis, inflicting a fatal interruption in the uteroplacental blood flow, resulting in pregnancy loss, both early (<12 weeks) and late (12–24 weeks). This has led researchers to postulate that the presence of APCR may lead to pregnancy loss. The authors have reviewed the published literature attempting to elucidate this hypothesis.

Figure 1. The role of protein C and activated protein C resistance in haemostasis.



WHAT IS APCR?

The first description of APCR was derived from a familial study of thrombosis in Leiden in 1993 (Dahlback et al, 1993). APCR causes prolongation of the activated partial thromboplastin time (PTT) by interfering with the protein C pathway (*Figure 1*).

Protein C and its cofactor substrate, protein S, are integral components of the anticoagulation pathway. Protein C is a natural anticoagulant and limits the conversion of fibrinogen to fibrin through the degradation of factors Va and VIIIa (Van Cott et al, 2002). By degrading activated clotting factors Va and VIIIa, activated protein C functions as one of the major inhibitors of the coagulation system. When factor Va is resistant to degradation by activated protein C, the anticoagulation pathway defaults, increasing the risk of thrombosis.

CONGENITAL AND ACQUIRED APCR

Congenital APCR is most commonly the result of a point mutation causing the replacement of an amino acid (Arg 506→Gln) at a predominant cleavage site within the factor V gene. This renders the activated form of factor V, factor Va, less susceptible to proteolysis by activated protein C. This mutation has been designated the factor V Leiden mutation.

In-vitro resistance to activated protein C (causing APCR) may occur in the absence of factor V Leiden. The term used to describe this phenomenon is acquired APCR. Known associations of acquired APCR include pregnancy, the second and third generation combined contraceptive pills, age, hormone replacement therapy, smoking, and antiphospholipid antibodies (Clark and Walker, 2001). The exact mechanism of acquired APCR, however, remains an enigma.

MEASURING ACTIVATED PROTEIN C RESISTANCE

APCR may be detected by an impaired response to activated protein C in functional clotting tests, such as the activated PTT. Sensitivity to activated protein C is expressed as a ratio of the activated PTT obtained in the presence and the absence of activated protein C. A ratio of <2.0–2.5 generally represents APCR; however, there seems to be a considerable overlap between normal subjects and heterozygosity for the factor V Leiden mutation in the 2.0–3.0 range (Van Cott et al, 2002). Therefore, in order to prevent non-detection of positive patients and, because of differences in usage of commercially available testing kits and equipment calibration, each laboratory needs to determine its own cut-off value. The sensitivity and specificity of the test approaches 100%, when patient plasma is first diluted 1:5 with factor V-deficient plasma, as it offsets any PTT-related factor deficiencies and minimizes the effect of a shortened PTT.

ASSESSING FOR FACTOR V LEIDEN

An APCR ratio of <2.5 mandates genetics testing for the factor V Leiden mutation to determine whether or not the mutation is present. The gold standard assay for detection of the factor V Leiden mutation is the polymerase chain reaction. DNA is isolated from whole blood and then the mutation site at nucleotide position 1691 of exon 10 of the factor V gene is amplified by polymerase chain reaction. The amplified product is digested with a digestive enzyme, *Mnl* I (New England Biolabs, Hitchin, Herts). The presence of the factor V Leiden mutation is indicated by the absence of an *Mnl* I site at position 1691. Polymerase chain reaction testing allows the identification of heterozygotes and homozygotes.

APCR AND PREGNANCY LOSS

A successful pregnancy is dependent on the development of an adequate fetomaternal circulation, relying on adequate placental circulation. In normal pregnancy, a state of procoagulation occurs with an increase in von Willebrand factors, factor V, VII, and factor X, a reduction in concentrations of anticoagulants, and a decrease in fibrinolysis. Since APCR promotes a prothrombotic state, it has been hypothesized that congenital and acquired APCR could potentially cause placental vascular insufficiency resulting in fetal loss. Another consideration is to establish whether or not APCR confers a risk factor for embryo or fetal loss. This has prompted several studies seeking to test these theories. There appears to be a degree of polarization in the findings. Thus far no consensus has been reached, as the published studies yield variable results, with some studies nullifying an association while others exhibit a convincing link.

CONGENITAL APCR AND FETAL LOSS

No link

In a case control study limited to first trimester losses only, Balasch et al (1997) could not demonstrate any clear association with congenital APCR. This finding was echoed by Dizon-Townson et al (1997) who did not find congenital APCR in any of the participating women with idiopathic recurrent miscarriage. Preston et al (1996), in a retrospective study, could not find a link between congenital APCR and first- and second-trimester losses either. In a larger study, Rai et al (2001) found that the prevalence of factor V Leiden was similar in patients with first- and second-trimester losses and a control group of parous women (*Table 1*).

Definite link

By contrast, several other studies have elicited a definite association between congenital APCR and pregnancy loss. Grandone et al (1997) report a 31.2% (5/16) prevalence of factor V Leiden in women with second trimester fetal losses compared with 4.2% (4/118) in matched controls. These findings are further supported by Younis et al (2000) who describe a significantly higher incidence of factor V Leiden in women with first- and second-trimester losses compared with a control group – 16% (6/37), 22% (9/41) and 6% (8/139) respectively. Reznikoff-Etievant et al (2001) also found a higher incidence of factor V Leiden – 10.38% (27/260) – compared with a control group – 4.7% (11/240) – although the study group was limited to patients with embryo losses only. In a more recent study, Sarig et al (2002) found an incidence of factor V Leiden of 25% (36/145) in women with fetal losses compared with 7.6% (11/145) in controls (*Table 1*).

Few studies have stratified the relationship between APCR and types of pregnancy loss. Brenner et al (1997) demonstrated that the incidence of the factor V Leiden mutation was 66% in women with first trimester losses, but that there was a higher incidence of the mutation among women with third trimester losses compared with a control group. Tal et al (1999) found a higher incidence of embryo losses in women with the factor V Leiden mutation compared with their controls (*Table 2*).

ACQUIRED APCR AND FETAL LOSS

The association between acquired APCR and fetal loss has been studied in a limited number of published papers. Younis et al (2000) were intrigued by their finding of a higher prevalence of acquired as opposed to congenital APCR in the second trimester. Rai et al (2001) also reported a significantly higher incidence of acquired APCR in women with recurrent first- and second-trimester losses – 8.8% (80/904) and 8.7% (18/207) respectively – compared with a control group of parous women 3.3% (5/150). Sarig et al (2002) report a complete absence of acquired APCR in women in their control group, but an incidence of 9% (13/145) in women with fetal losses (*Table 3*).

DISCUSSION

The incongruity of the composite results regarding congenital APCR is not surprising, as there is a wide variation in patient numbers, inherent differences in study design, acquisition bias (in selected populations), and lack of uniformity regarding pregnancy classification. The latter is fundamental in trying to establish a temporal relationship, as different aetiological processes occur at different stages in the developing conceptus. Most reported studies have adopted the terms early and late miscarriage, which lack specificity.

A further compounding factor is the remarkable variation (1.8–31.2%) in the prevalence of congenital APCR (factor V Leiden) in different population groups, determined by ethnicity

TABLE 1.
Prevalence of congenital activated protein C resistance (factor V Leiden) and relation to type of pregnancy loss

Author	Pregnancy loss classification	Cases	Controls	Odds ratio (95% CI)
Balasch et al (1997)	T1 only	1/55 (1.8)	1/50 (2)	NS
Dizon-Townson et al (1997)	NS	0/22 (0)	1/50 (2)	NS
Grandone et al (1997)	T1	2/27 (7.4)	4/118 (4.2)	NS
	T2	5/16 (31.2)		
Ridker et al (1998)	NS	9/113 (8)	16/437 (3.7)	NS
Kutteh et al (1998)	NS	1/42 (2.4)	2/50 (4)	NS
Younis et al (2000)	Fetal losses only T1	6/137 (16)	8/139 (6)	3.1 (1–10)
	Fetal losses only T2+/- T1	9/41 (22)		
Foka et al (2000)		9/61 (14.7)	2/100 (2)	8.4 (2–41)
Wramsby et al (2000)	NS	13/84 (15.4)	2/69 (2.89)	NS
Reznikoff-Etievant et al (2001)	T1 < 10/40	27/260 (10.38)	11/240 (4.7)	2.4 (1–5)
Rai et al (2001)	T1	60/1808 (3.3)	12/300 (4)	NS
	T2	16/414 (3.9)		
Raziel et al (2001)	NS	6/36 (16)	2/40 (5)	NS
Sarig et al (2002)	Fetal losses only	36/145 (25)	11/145 (7.6)	NS

T1 = First trimester pregnancy loss; T2 = Second trimester pregnancy loss; NS = not specified; 95% CI = 95% confidence intervals; Numbers in parentheses represent percentages

TABLE 2.
Percentage of different types of pregnancy loss in women with congenital activated protein C resistance (factor V Leiden)

		Percentage of pregnancy losses		
Authors	Type of pregnancy loss	Factor V Leiden carriers	Controls	OR (95% CI)
Brenner et al (1997)	T1	68/103 (66) (*n*=19)	28/36 (77.7) (*n*=11)	NS
	T2	19/103 (18.4)	7/36 (19.4)	
	T3	15/103 (14.5)	0/36 (0)	
Tal et al (1999)	Embryo	17/48 (35) (*n*=18)	25/214 (12) (*n*=100)	NS
	Fetal (T1+T2)	31/48 (65)	191/214 (89)	

CI = confidence interval; OR = odds ratio; T1 = first trimester; T2 = second trimester; T3 = third trimester; numbers in parenthesis represents percentages.

(high incidence in Jews, low incidence in Africans) which may skew results.

The majority of documented studies do not explore the entity of acquired APCR. However, in those studies that do, none of them dispute the definite association between acquired APCR and pregnancy loss. The reported prevalence of acquired APCR, from studies so far, ranges from 9% to 26.8% in women with first-, second- and third-trimester losses. It would be interesting to ascertain the converse relationship with greater emphasis on the type of pregnancy loss in women with acquired APCR. Theoretically, the pathological mechanism is ascribed to placental thrombosis, therefore it would be reasonable to assume that there should be a preponderance of fetal losses.

MANAGEMENT

Despite the discrepant results regarding a positive association between APCR and pregnancy loss, there is reasonable evidence to support a policy of screening women with recurrent miscarriage for this thrombophilia. Ostensibly, it appears that the entity of acquired APCR in the recurrent miscarriage setting cannot be ignored and is indeed gaining importance. Testing for congenital and acquired APCR should constitute part of the investigative protocol in recurrent pregnancy loss.

As far as treatment options are concerned, there is a paucity of documented evidence. Preliminary reports advocate the use of antithrombotic therapy in pregnant women with recurrent pregnancy loss and APCR (Tal et al, 1999). However, no randomized controlled trials have been reported thus far, but they are certainly warranted to evaluate the potential application of antithrombotic modalities. HM

Conflict of interest: none.

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TABLE 3.
Association between acquired activated protein C resistance and type of pregnancy loss

Author	Type of pregnancy loss	Cases	Controls
Younis et al (2000)	T1	4/37 (10.8)	3/139 (2.1)
	T2	11/41 (26.8)	
Rai et al (2001)	T1	80/904 (8.8)	5/150 (3.3)
	T2	18/ 207 (8.7)	
Sarig et al (2002)	Fetal losses only (T1, T2 +T3)	13/145 (9)	0/145 (0)

T1 = First trimester pregnancy loss; T2 = Second trimester pregnancy loss; T3 = Third trimester pregnancy loss; Numbers in parenthesis represent percentages

KEY POINTS

- Activated protein C resistance (APCR) is a relatively new thrombophilia.
- Congenital APCR is most commonly caused by the factor V Leiden mutation.
- Acquired APCR occurs in the absence of the factor V Leiden mutation.
- Acquired APCR appears to be a significant risk factor for recurrent pregnancy loss.
- Classification of pregnancy loss in recurrent miscarriage is of vital importance.
- Testing for APCR in recurrent pregnancy loss is mandatory.
- There is as yet, no documented evidence to support the use of anticoagulants in preventing pregnancy loss.