CHAPTER 9

ACQUIRED APC RESISTANCE RELATED TO ORAL CONTRACEPTIVES AND PREGNANCY AND ITS POSSIBLE IMPLICATIONS FOR CLINICAL PRACTICE

Johan R. Meinardi, Cilia M.A. Henkens, Martijn P. Heringa, and
Jan van der Meer

Blood Coagulation and Fibrinolysis 1997; 8:152-154

9.1 LETTER TO THE EDITOR

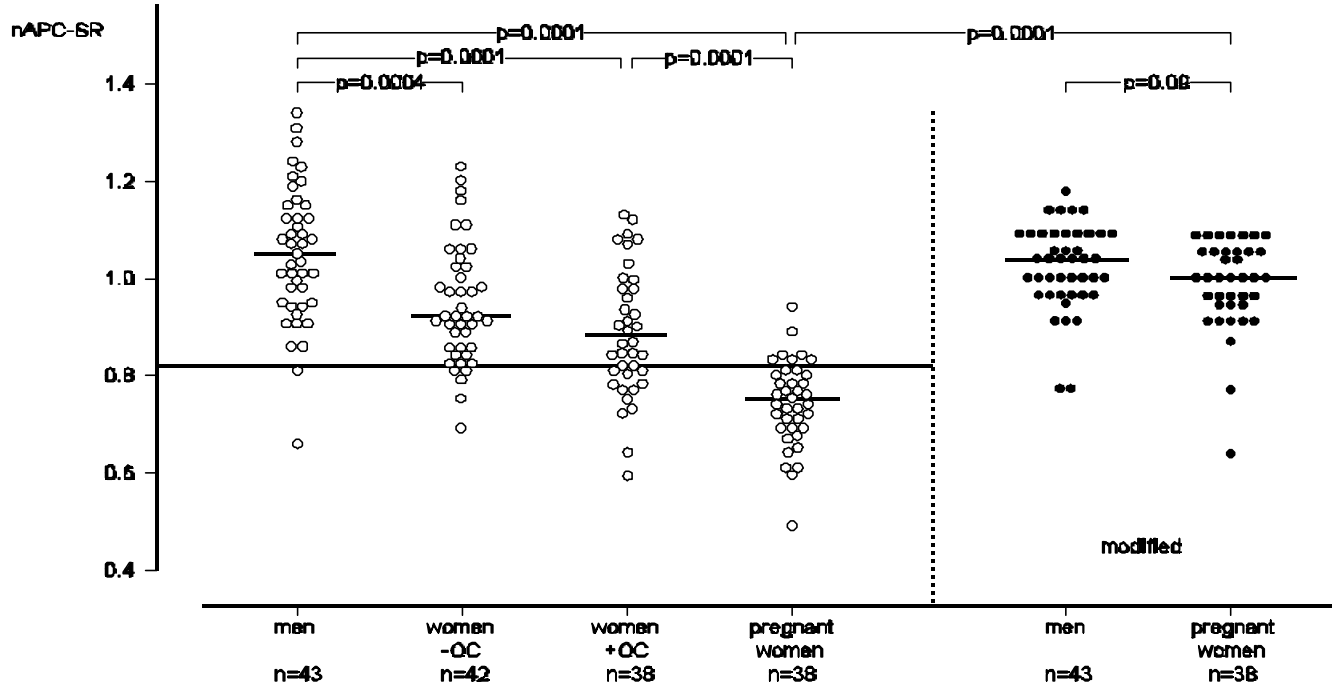
Hereditary activated protein C (APC) resistance is an established risk factor of venous thromboembolism, mostly associated with the Arg^{506}-Gln mutation of the factor V gene [1]. A decreased sensitivity to APC is widely used to identify carriers of this mutation, as an alternative to DNA-analysis. To improve the accuracy of the original (standard) APC resistance test in acquired conditions that may influence its results, a modification has been introduced [2, 3]. Using the standard test, we recently demonstrated differences in sensitivity to APC between healthy men and women, particularly those who used oral contraceptives [4]. These data suggested acquired APC resistance due to a hormonal effect, possibly caused by oestrogens. In order to obtain supportive evidence of this assumption, we extended our study to healthy pregnant women.

Citrated blood samples were collected at 36 weeks' gestation in 38 women (median age 31 years, range 19-40 years) with an event-free pregnancy and a negative personal and family history of thromboembolism. Sensitivity to APC, expressed as normalized APC sensitivity ratio (nAPC-SR) [5], was measured by the Coatest™ resistance kit (Chromogenix AB, Mölndal, Sweden). Standard nAPC-SR values in pregnant women were compared with those previously obtained in healthy men (n=43; median age 35 years, range 22-63 years), women not using oral contraceptives (n=42; median age 36 years, range 24-53 years) and women who used oral contraceptives (n=38; median age 27 years, range 19-47 years). The modified test was performed in the groups of pregnant women and men by measuring nAPC-SR in test plasma, 1:5 prediluted with factor V-deficient plasma.

The results are summarized in Figure 9.1. A progressive decline of the standard nAPC-SR was demonstrated comparing women not using oral contraceptives, women who used oral contraceptives, and pregnant women, respectively, with men. Values below the lower limit of the normal range (0.82-1.22) were found in 10% of women not using oral contraceptives, 32% of women using oral contraceptives, and 82% of pregnant women compared with 5% of men. The normal range (95% confidence interval) of the modified nAPC-SR in men was 0.84-1.20. There was no significant difference in modified nAPC-SR between pregnant women and men. Lowered values were found in 5% of each of these groups, corresponding with the expected frequency of carriers of the factor V mutation in the general population. DNA analysis was not performed.

Our findings agree with previous reports on pregnant women [6-8]. The observed differences in standard nAPC-SR between the groups and their correction as demonstrated in pregnant women by predilution with factor V-deficient plasma, provide supportive evidence of acquired APC resistance, probably due to hormonal differences. Increased levels of fibrinogen, factor II, VIII, IX and X, as reported during pregnancy and oral contra-

Figure 9.1 Distribution of standard (▵) and modified (●) nAPC-SR in men, women not using oral contraceptives (women -OC), women using oral contraceptives (women +OC) and pregnant women. Median values are indicated per group. The horizontal line denotes the lower limit of normal range.



ceptives [9], might explain a lowered APC ratio by shortening the partial thromboplastin time. Factor VIII is of special interest, because an elevated level of this substrate of APC, might cause APC resistance, due to a relative deficiency of APC. An inverse correlation between factor VIII and APC ratio has been demonstrated during pregnancy [7], use of oral contraceptives [10], as well as in *in vitro* experiments [10]. A lowered level of protein S, also found during pregnancy and oral contraception [9, 10], is a less likely explanation of APC resistance, as its influence is limited to levels below 20% [5].

From the presented data it will be clear that the standard APC resistance test cannot reliably identify carriers of the factor V mutation. We found this test to be falsely positive in approximately 25% of women who used oral contraceptives and 75% of pregnant women. In contrast, the modified test seems more appropriate in these conditions. Nevertheless, the standard test remains worthwhile for the detection of acquired APC resistance as a potential risk factor of thrombosis. Clinical studies are warranted to assess the contribution of acquired APC resistance to the development of thrombosis in women on oral contraceptives or during pregnancy, either as an independent or concomitant risk factor.

9.2 REFERENCES

1. Bertina, R.M., B.P.C. Koeleman, T. Koster, F.R. Rosendaal, R.J. Dirven, H. de Ronde, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; 369:64-67.
2. Jorquera, J.I., J.M. Montoro, M.A. Fernández, J.A. Aznar, J. Aznar. Modified test for activated protein C resistance. *Lancet* 1994; 344:1162-1163.
3. Trossaërt, M., J. Conard, M.H. Horellou, I. Elalamy, M.M. Samama. The modified APC resistance test in the presence of factor V deficient plasma can be used in patients without oral anticoagulant. *Thrombosis and Haemostasis* 1996; 75:521-522.
4. Henkens, C.M.A., V.J.J. Bom, A.J. Seinen, J. van der Meer. Sensitivity to activated protein C; influence of oral contraceptives and sex. *Thrombosis and Haemostasis* 1995; 73: 402-404.
5. De Ronde, H., R.M. Bertina. Laboratory diagnosis of APC-resistance: a critical evaluation of the test and the development of diagnostic criteria. *Thrombosis and Haemostasis* 1994; 72:880-886.
6. Cumming, A.M., R.C. Tait, S. Fildes, A. Yoong, S. Keeney, C.R.M. Hay. Development of resistance to activated protein C during pregnancy. *British Journal of Haematology* 1995; 90:725-727.
7. Mathonnet, F., Ph. de Mazancourt, B. Bastenaire, M. Morot, N. Benattar, S. Beufé, et al. Activated protein C sensitivity ratio in pregnant women at delivery. *British Journal of Haematology* 1996; 92:244-246.
8. Cumming, A.M., R.C. Tait, S. Fildes, C.R.M. Hay. Diagnosis of APC resistance during pregnancy. *British Journal of Haematology* 1996; 92:1026-1029.
9. Beller, F.K., C. Ebert. Effects of oral contraceptives on blood coagulation. A review. *Obstetrics and Gynecological Survey* 1985; 40:425-436.
10. Henkens, C.M.A., V.J.J. Bom, J. van der Meer. Lowered APC-sensitivity ratio related to increased factor VIII clotting activity. *Thrombosis and Haemostasis* 1995; 74:1198-1199.