

# Thromboelastography, whole-blood haemostasis and recurrent miscarriage

R.Rai<sup>1,3</sup>, E.Tuddenham<sup>2</sup>, M.Backos<sup>1</sup>, S.Jivraj<sup>1</sup>, S.El’Gaddal<sup>1</sup>, S.Choy<sup>1</sup>, B.Cork<sup>1</sup> and L.Regan<sup>1</sup>

<sup>1</sup>Faculty of Medicine, Department of Obstetrics and Gynaecology, Imperial College London, St Mary’s Hospital, Mint Wing, South Wharf Road, London W2 1PG and <sup>2</sup>MRC Haemostasis Unit, Hammersmith Hospital, London, UK

<sup>3</sup>To whom correspondence should be addressed. E-mail: r.rai@imperial.ac.uk

**BACKGROUND:** Some cases of recurrent miscarriage have a thrombotic basis. Thromboelastography is a rapid, reproducible test of whole-blood haemostasis. **METHODS:** Thromboelastography was performed in 494 consecutive, non-pregnant women (median age 35 years; range 21–48) with a history of miscarriages at <12 weeks gestation (median 4; range 3–12) and 55 parous women (median age 33 years; range 20–41) with no history of pregnancy loss. The prospective outcome of untreated pregnancies amongst 108 women with recurrent miscarriage was studied. **RESULTS:** The maximum clot amplitude (MA) (median 66.0 mm; range 48.0–76.0) was significantly higher and the rate of clot lysis (LY30) (median 2.5%; range 0.5–7.8) significantly lower amongst women with recurrent miscarriage compared with controls (MA 61.5 mm; range 50.0–67.0;  $P = 0.01$ ; LY30 4.9%; range 2.9–9.7;  $P = 0.01$ ). The pre-pregnancy MA was significantly higher amongst women who subsequently miscarried (median 66.0 mm; range 54.0–73.0) compared with those who had a live birth (median 61.7 mm; 48.0–71.5;  $P < 0.01$ ). A pre-pregnancy MA  $\geq 64$  mm has a sensitivity of 68% and specificity of 82% to predict miscarriage. **CONCLUSIONS:** Thromboelastography identifies a subgroup of women with recurrent miscarriage to be in a prothrombotic state outside of pregnancy. Women in such a state are at increased risk of miscarriage in future untreated pregnancies.

*Key words:* recurrent miscarriage/thromboelastography/whole-blood haemostasis

## Introduction

Pregnancy is a hypercoagulable state secondary to an increase in the levels of certain coagulation factors, a decrease in the levels of anticoagulant proteins and an increase in fibrinolysis (Stirling *et al.*, 1984; Hellgren *et al.*, 1996). The increase in coagulation that occurs during pregnancy counteracts the inherent instability associated with haemochorial placentation. However, evidence is accumulating to suggest that some cases of recurrent miscarriage are the result of an exaggerated haemostatic response during pregnancy, which results in thrombosis of the utero-placental vasculature and subsequent fetal loss (Rushton *et al.*, 1988; Preston *et al.*, 1996; Rai *et al.*, 2001).

Whilst a number of individual haemostatic abnormalities have been reported in association with recurrent miscarriage and later obstetric complications, it is clear that some women who have these abnormalities have uncomplicated pregnancies (Kupferminc *et al.*, 1999; Rai *et al.*, 2002). The major limitation of conventional haemostasis tests, such as measuring the levels of individual coagulation and anticoagulant proteins, are that they ignore the fact that *in vivo* haemostasis is a dynamic process which involves the interaction of coagulation and fibrinolytic factors with cellular elements such as platelets

and the blood vessel wall. Thromboelastography, a ‘near patient’ test of whole-blood haemostasis, addresses these deficiencies by dynamically assessing the kinetics, strength and stability of the end result of coagulation—the fibrin clot (Salooja *et al.*, 2001).

The aims of this study were: (i) to compare the thromboelastographic parameters outside of pregnancy amongst women with recurrent miscarriage and parous controls; and (ii) to determine the value of pre-pregnancy thromboelastographic assessment of whole-blood haemostasis in predicting the outcome of future untreated pregnancies amongst women with a history of recurrent early miscarriage.

## Materials and methods

### Subjects

#### Prevalence study

The study population was comprised of 494 consecutive Caucasian women with a history of recurrent early miscarriage (three or more consecutive miscarriages at <12 weeks gestation). The demographic details and outcome of previous pregnancies of these women are shown in Table I. All women were investigated according to our published protocol (Rai *et al.*, 2001). In particular, all women and their partners had a normal peripheral blood karyotype (46XX and 46XY,

**Table I.** Demographic details of the study populations

	Recurrent early miscarriages only ( <i>n</i> = 494)	Controls ( <i>n</i> = 55)	<i>P</i> -value
Age (years) [median (range)]	35 (21–48)	33 (20–41)	0.72
Number of previous miscarriages [median (range)]	4 (3–12)	0	
Number with a previous live birth (%)	205 (41)	55 (100)	

respectively) and no woman had the Antiphospholipid Syndrome (Wilson *et al.*, 1999). The study was performed over 18 calendar months between 2001 and 2002. Women were tested at random intervals during the menstrual cycle.

An age-matched control population of 55 unrelated Caucasian women (median parity 1; range 1–3) with no previous history of miscarriage or late pregnancy complication was also studied (Table I). These women were recruited from the obstetric wards.

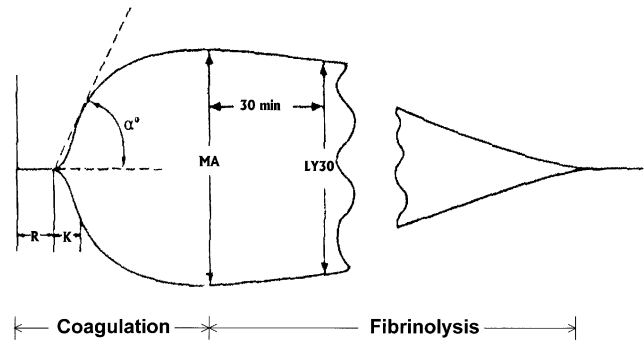
All women were investigated at least 12 weeks after their last pregnancy. In answer to direct questioning, no woman in this study said that she had a personal or family history of thromboembolic disease, smoked tobacco, or was taking any pharmacological medication, prescribed or non-prescribed, such as the combined oral contraceptive pill or aspirin, which are recognized to effect the haemostatic pathways.

#### Pregnancy study

The prospective outcome of the next untreated pregnancy amongst the first 108 consecutive women of the cohort of those with recurrent miscarriage was studied. All women were asked to contact the clinic once they had a positive urine pregnancy test, and attended a dedicated early pregnancy clinic for serial ultrasound scans between 5 and 12 weeks of amenorrhoea. No woman received any pharmacological treatment during pregnancy except for folic acid (400 µg daily) as prophylaxis against neural tube defects. Only women whose pregnancy had advanced to the fetal stage were analysed. The fetal stage was defined as being reached once a fetal pole was seen on transvaginal pelvic ultrasound. Ultrasonography was performed by trained personnel who were blinded to the results of the thromboelastographic parameters.

#### Antiphospholipid antibodies

All women were screened for lupus anticoagulant (LA) and anticardiolipin antibodies (aCL) on at least two occasions more than 6 weeks apart prior to pregnancy. Lupus anticoagulant was detected using the dilute Russell's viper venom time (dRVVT) together with a platelet neutralization procedure. Patient samples with a dRVVT ratio (test/control) of  $\geq 1.1$  were retested with a platelet neutralization procedure. A decrease of 10% or more in the ratio was considered to be positive for LA (Lupus Anticoagulant Working Party, 1991). Anticardiolipin antibodies were identified using a standardized enzyme-linked immunosorbent assay (ELISA). An IgG aCL level  $\geq 20$  GPL units and an IgM aCL level  $\geq 20$  MPL units was considered to be positive (Wilson *et al.*, 1999). Women with a positive test for LA or a positive aCL titre had a confirmatory test performed on a second sample taken at least 6 weeks after the initial sample. Only women with persistently positive tests for either LA or aCL were considered to have the Antiphospholipid Syndrome. These women were excluded from the present study.



**Figure 1.** R = reaction time; K = clot formation time;  $\alpha$  = speed of clot formation; MA = maximum clot amplitude; LY30 = % clot lysis at 30 min.

#### Thromboelastography

Thromboelastography was performed, according to the manufacturer's guidelines, using a Computerized Thromboelastograph Coagulation Analyser (TEG™) Model 3000C (Haemoscope Corporation, Skokie, IL, USA). In brief, 0.36 ml of celite-activated, citrated whole blood was placed into a cup pre-heated to 37°C. The cup oscillates through an angle of  $\sim 5^\circ$  in either direction every 10 s. A metal pin, attached to a torsion wire, is suspended into the cup of blood and the movement of the pin is converted by a mechanical–electrical transducer to an electrical signal, which is monitored by a computer. Initially, when no clot exists, the motion of the cup does not affect the pin and a straight-line trace is recorded (Figure 1). As the blood in the cup clots, the motion of the rotating cup is transmitted to the pin and a TEG trace generated (Figure 1). We studied five parameters of the TEG trace. The 'R time' (reaction time) is the latency between placing the blood in the cup until the first fibrin strands form; the 'K time' is a measure of the kinetics of clot formation; ' $\alpha$ ' (angle) is closely related to the 'K time' and also assesses the kinetics of clot formation; 'MA' is the maximum amplitude and a measure of the maximum strength of the blood clot; and 'LY30' is a measure of the percentage of clot lysis 30 min after MA is reached.

During the study period, two TEG analysers were used and  $>90\%$  of the tests were performed by the same trained technician. Women were tested randomly during the menstrual cycle. Samples were tested in duplicate. The intra-assay coefficient of variation (CV) was 4.7% and the inter-assay CV 7.3%.

#### Statistical analysis

Statistical analysis was performed using SPSS version 10 (SPSS Inc., Chicago, IL, USA). Discrete variables were analysed using the  $\chi^2$ -test and continuous variables analysed using the Mann–Whitney *U*-test. *P*-values of  $<0.05$  were taken as statistically significant. The area under the receiver-operating characteristic (ROC) curve was calculated to assess the value of pre-pregnancy thromboelastography in predicting the outcome—miscarriage or live birth—of future untreated pregnancies amongst women with recurrent early miscarriage.

#### Results

The MA was significantly higher and the LY30 significantly lower amongst women with recurrent early miscarriages compared with parous controls (Table II). There was no significant difference in the remaining TEG parameters between the two groups of women (Table II).

**Table II.** Thromboelastographic parameters amongst women with a history of miscarriage and controls

	Recurrent early miscarriages only (n = 494)	Controls (n = 55)	P-value
R (mm)	8 (3.5–12.0)	8.8 (4.5–11.5)	0.08
K (mm)	2.5 (1.0–4.5)	3.0 (1.6–3.5)	0.17
$\alpha$ (°)	73 (57.0–82.0)	72.8 (68–78.5)	0.73
MA (mm)	66.0 (48.0–76.0)	61.5 (50.0–67.0)	0.01
LY30 (%)	2.5 (0.5–7.8)	4.9 (2.9–9.7)	0.01

Values are medians (range).

**Table III.** Sensitivity and specificity of pre-pregnancy MA for prediction of miscarriage in future untreated pregnancy

MA (mm)	Sensitivity	Specificity
60	0.76	0.39
61	0.73	0.47
62	0.70	0.60
63	0.70	0.74
64	0.68	0.82
65	0.57	0.86
66	0.46	0.88
67	0.40	0.88
68	0.27	0.90

In the prospective pregnancy study, 57 of the 108 women had a live birth (53%); 37 miscarried a pregnancy that had reached the fetal stage (34%), 10 an embryonic pregnancy (9%); two a tubal ectopic pregnancy (2%) and two a biochemical pregnancy (2%). The pre-pregnancy MA was significantly higher amongst women who miscarried a fetal pregnancy (median 66.0 mm; range 54.0–73.0) compared with those whose pregnancy resulted in a live birth (median 61.7 mm; range 48.0–71.5;  $P < 0.01$ ). Women who had live birth were of similar age (median 34 years; range 20–41) and had a similar number of previous miscarriages (median 3; range 3–7) compared with those who miscarried a fetal pregnancy (median age 35 years; range 24–44;  $P = 0.14$ ; median number of previous miscarriages 3; range 3–9;  $P = 0.71$ ). There was no significant difference in the remaining thromboelastograph parameters between those women who subsequently miscarried a pregnancy that had reached the fetal stage compared with those who subsequently had a live birth.

For the MA, the area under the ROC curve was 0.7 (95% confidence interval 0.58–0.82). The sensitivity and specificity of pre-pregnancy MA for predicting miscarriage after the fetal stage is shown in Table III. At a cut-off value for the MA of 64.0 mm the sensitivity of pre-pregnancy MA for predicting miscarriage after the fetal stage is 68%, and the specificity 82%. The positive predictive value for miscarriage using an MA of >64 mm is 71% and the negative predictive value (livebirth) is 81%.

## Discussion

Whilst a number of traditionally held beliefs as to the aetiology and treatment of recurrent miscarriage have not withstood

critical scrutiny (Rai *et al.*, 1996), attention is currently focused on the hypothesis that many cases of recurrent miscarriage are due to an exaggerated haemostatic response during pregnancy leading to thrombosis of the utero-placental vasculature and subsequent fetal loss. In this study, we have demonstrated that thromboelastography identifies that a proportion of women with a history of recurrent early miscarriage are in a pro-thrombotic state outside of pregnancy, and that women with a raised pre-pregnancy MA are at risk for future miscarriage in untreated pregnancies.

Thromboelastography is a 'near patient' test of whole-blood haemostasis. It is sensitive to all interacting plasma and cellular components in blood that affect clot formation and its breakdown. An important advantage of thromboelastography over conventional haemostasis testing is that it is a dynamic test which yields information relating to the cumulative effect of the many components of coagulation, including platelet function. In a study of 25 patients evaluated for a hypercoagulable state, it has been reported that whilst no abnormality on conventional haemostasis testing was detected amongst those with a normal thromboelastograph trace, in contrast, the majority of those traces indicative of hypercoagulability also had an abnormality in their conventional screen (Handa *et al.*, 1997). Thromboelastography may therefore be detecting haemostatic abnormalities that lead to a pro-thrombotic state that are not identified on conventional testing. Thromboelastography also has cost benefits over conventional haemostasis testing. In our hospital, a contemporary haemostasis screen costs €550; in contrast, the cost of a thromboelastographic assessment of whole-blood haemostasis is €32, which includes the cost of consumables, technician time and hardware depreciation.

The MA assesses the maximum strength of the blood clot and is the thromboelastograph parameter that indicates a hypercoagulable state. Clot strength is a result of two components: the relatively modest contribution of fibrin and the much more significant contribution of the platelets. The finding of a raised MA outside of pregnancy complements our previous study reporting an increased level of thrombin-antithrombin complexes (a marker of thrombin generation) amongst non-pregnant women with recurrent miscarriage compared with controls (Vincent *et al.*, 1998).

Recurrent miscarriage is a heterogeneous condition and consequently no single test will detect all causes of miscarriage. However, the prospective pregnancy study reports that the pre-pregnancy MA is a useful test for predicting future pregnancy outcome. The ROC curve is a plot of the sensitivity of a test versus the false-positive rate. The area under the curve ranges from 0.5, which indicates that a test has no discriminatory property, to 1.0, a perfect test. In this study, the area under the curve was 0.7. This, of course, is likely to be a reflection not only of the heterogeneous aetiologies of miscarriage, but also of the fact that the most common cause for any single miscarriage is a fetal karyotype abnormality. We have attempted to minimize the effect of this latter variable by including in the analysis only women whose pregnancy had advanced to the fetal stage, excluding those with biochemical, anembryonic or ectopic pregnancies. It must be noted,

however, that karyotype analysis of the products of conception of those pregnancies that miscarried is not available, and it is of course possible that a pregnancy with an abnormal chromosome complement can advance to the fetal stage of development

Three key questions raised in this study are: (i) whether increases in MA during pregnancy precede pregnancy loss and later pregnancy complications, such as pre-eclampsia and intra-uterine growth restriction, which are thought in some cases to have a thrombotic basis; (ii) whether it is possible to lower the elevated MA seen amongst women with recurrent miscarriage; and (iii) whether lowering the MA leads to an improved live birth rate. As the MA primarily reflects platelet function and activity, we are exploring the effect of aspirin, a recognized anti-platelet agent, on the MA (Orlikowski *et al.*, 1996; Oshita *et al.*, 1999). Our preliminary results suggest that whilst 75 mg/day of aspirin has no effect, a dose of 150 mg/day does significantly reduce the MA. In a prospective, randomized, placebo-controlled study, we are assessing the efficacy of this latter dose of aspirin in improving the live birth rate amongst women with a history of recurrent first trimester miscarriage with an elevated pre-pregnancy MA.

The mechanism of pregnancy loss associated with thrombophilic defects has traditionally been ascribed to thrombosis of the utero-placental vasculature. Whilst this is certainly important, *in vitro* evidence suggests an alternative mechanism. It has recently been reported that thrombin, in its role as a cell signalling agent, increases trophoblast apoptosis and impairs trophoblast invasion (Isermann *et al.*, 2003). This report lends further evidence to support the contention that components of the haemostatic pathways play a cardinal role in embryonic implantation and prompts the further exploration of a field of research that can be termed 'reproductive haemostasis'. This is a field we are actively exploring.

In conclusion, this large prospective study has demonstrated that whole-blood haemostasis testing using thromboelastography identifies that a proportion of women with recurrent early miscarriages are in a pro-thrombotic state outside of pregnancy, and that women in such a state are at increased risk of future miscarriage compared with those with a lower thromboelastograph index of coagulability. Based on animal studies we postulate that this pro-thrombotic state is associated with increased trophoblast apoptosis and impaired trophoblast invasion. At later gestational ages this hypercoagulability is amplified, leading to utero-placental vascular insufficiency and subsequent fetal loss. In addition, the findings of an increase in

clot strength amongst women with recurrent miscarriage may have important implications for the health of these women both during and beyond their reproductive years.

## References

- Handa, A.C.D.J., Pasi, K.J. and Perry, D.J.H.G. (1997) Thromboelastography: An effective screening test for prothrombotic states. *Phlebology*, **12**, 159–160.
- Hellgren, M. (1996) Hemostasis during pregnancy and puerperium. *Haemostasis*, **26** (Suppl. 4), 244–247.
- Isermann, B., Sood, R., Pawlinski, R., Zogg, M., Kalloway, S., Degen, J.L., Mackman, N. and Weiler, H. (2003) The thrombomodulin–protein C system is essential for the maintenance of pregnancy. *Nat. Med.*, **9**, 331–337.
- Kupferminc, M.J., Eldor, A., Steinman, N., Many, A., Bar-Am, A., Jaffa, A., Fait, G. and Lessing, J.B. (1999) Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N. Engl. J. Med.*, **340**, 9–13.
- Lupus Anticoagulant Working Party on behalf of the BCSH Haemostasis and Thrombosis Taskforce (1991) Guidelines on testing for the lupus anticoagulant. *J. Clin. Pathol.*, **44**, 885–889.
- Orlikowski, C.E., Roche, D.A., Murray, W.B., Gouws, E., Moodley, J., Kenoyer, D.G. and Byrne, S. (1996) Thromboelastography changes in pre-eclampsia and eclampsia. *Br. J. Anaesth.*, **77**, 157–161.
- Oshita, K., Azma, T., Osawa, Y. and Yuge, O. (1999) Quantitative measurement of thromboelastography as a function of platelet count. *Anesth. Analg.*, **89**, 296–299.
- Preston, F.E., Rosendaal, F.R., Walker, I.D., Briet, E. and Berntorp, E. (1996) Increased fetal loss in women with heritable thrombophilia. *Lancet*, **348**, 913–916.
- Rai, R. (2000) Obstetric management of antiphospholipid syndrome. *J. Autoimmun.*, **15**, 203–207.
- Rai, R., Clifford, K. and Regan, L. (1996) The modern preventative treatment of recurrent miscarriage. *Br. J. Obstet. Gynecol.*, **103**, 106–110.
- Rai, R., Shlebak, A., Cohen, H., Backos, M., Holmes, Z., Marriott, K. and Regan, L. (2001) Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. *Hum. Reprod.*, **16**, 961–965.
- Rai, R., Backos, M., Elgaddal, S., Shlebak, A. and Regan, L. (2002) Factor V Leiden and recurrent miscarriage—prospective outcome of untreated pregnancies. *Hum. Reprod.*, **17**, 442–445.
- Rushton, D.I. (1988) Placental pathology in spontaneous miscarriage. In Beard, R.W., Sharp, F. (eds) *Early Pregnancy Loss: Mechanisms and Treatment*. RCOG, London, UK, pp. 149–158.
- Salooja, N. and Perry, D.J. (2001) Thromboelastography. *Blood Coagul. Fibrinolysis*, **12**, 327–337.
- Stirling, Y., Woolf, L., North, W.R., Seghatchian, M.J. and Meade, T.W. (1984) Haemostasis in normal pregnancy. *Thromb. Haemost.*, **52**, 176–182.
- Vincent, T., Rai, R., Regan, L. and Cohen, H. (1998) Increased thrombin generation in women with recurrent miscarriage. *Lancet*, **352**, 116.
- Wilson, W.A., Gharavi, A.E., Koike, T., Lockshin, M.D., Branch, D.W., Piette, J.C., Brey, R., Derksen, R., Harris, E.N., Hughes, G.R. *et al.* (1999) International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum.*, **42**, 1309–1311.

Submitted on February 11, 2003; accepted on September 1, 2003