Thrombophilia screening in women with recurrent first trimester miscarriage: is it time to stop testing? – a cohort study and systematic review of the literature

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STRENGTHS AND LIMITATIONS OF THIS STUDY
⇒ The examination of a large cohort of pregnancies with three or more recurrent miscarriage (RM) in specialist dedicated clinics; accurate and systematic investigation of aetiological causes of early RM including inherited and acquired thrombophilia.
⇒ Use of a standardised method of analysis of results; using the same reference range to mitigate errors introduced due to variation in laboratory reporting such as adherence to the diagnostic criteria of the revised Sapporo criteria.
⇒ Systematic review of literature using standard criteria and statistical analysis.
⇒ The retrospective nature of the study and lack of contemporaneous data from control pregnancies; however, the latter is not a part of routine clinical practice.
⇒ Studies included in this review were restricted to women with three or more first trimester miscarriages and those that a minimum of 100 women in the miscarriage arm.

INTRODUCTION
Recurrent miscarriage (RM), which affects 1% of couples trying to conceive, is defined as the loss of three or more consecutive pregnancies from the time of conception up to 24 completed weeks of gestation.1 There are differences in recommendations from professional bodies with regards to the definition of RM, with some requiring two or more clinical pregnancies with ultrasound or histological confirmation of pregnancy loss, whereas others requiring three or more losses after a positive pregnancy test with no specification.
of the need for clinical confirmation.1,2 These variations in definitions may potentially affect the prevalence of underlying causes of RM in various studies, as the etiology of biochemical pregnancy loss may be different compared with loss in the second trimester.

Many factors have been studied as possible causes of RM such as anatomical, endocrine, immunological, genetic and thrombophilia (inherited and acquired) disorders. The inherited thrombophilia includes causes such as the presence of Factor V Leiden (FVL) and prothrombin G20210A mutation (PGM), antithrombin and protein C and S deficiencies, whereas acquired thrombophilia includes the presence of lupus anticoagulant (LA), anticardiolipin (ACL) and anti-beta 2 glycoprotein antibodies. There are many studies reporting a disproportionally high prevalence of thrombophilia in women with a history of RM, which has led to this condition being frequently overdiagnosed.3–6 These studies included women at various gestations and the number of pregnancy losses as well as those identified as having acquired thrombophilia based on values that do not meet the diagnostic criteria. This has led to unnecessary investigations and empirical treatment without improvement in clinical outcomes.7–9 In contrast, there is evidence from studies suggesting that the prevalence of inherited thrombophilia in women with RM is similar to the general population and that its presence in women with RM is more likely to be an association rather than causation.4,8–11

The objective of our study was to assess the prevalence of inherited and acquired thrombophilia in a large cohort of women with a history of early RM using internationally agreed diagnostic criteria and inclusion parameters. We also undertook a systematic review of literature and meta-analysis for studies that reported data regarding inherited and acquired thrombophilia for women with RM and control population.

**METHODS**

**Cohort study**

Setting
This is a retrospective cohort study set-up for women with RM in Epsom and St Helier University Hospitals NHS Trust and the Centre for Reproductive Immunology and Pregnancy, London, and Surrey. We reviewed all the available literature related to causes of RMs. We ascertained the prevalence of thrombophilia in the study population and compared it with historical and published prevalence in the general population.

Participants
A total of 1155 women between 2012 and 2017. All couples who have a history of three or more recurrent first trimester losses are eligible for referral to these specialist RM clinics. We record parental demographic information, medical, surgical and gynaecological history and offer comprehensive investigations which include testing for inherited and acquired thrombophilia.

**Inclusion and exclusion criteria**
The study population included women with a history of three or more first trimester miscarriages and those who had available results for inherited and acquired thrombophilia. We excluded cases that had incomplete results for the thrombophilia screen.

**Patient and public involvement**
No patients were involved in the conduct or design of the study.

**Outcome measures for inherited and acquired thrombophilia**
The investigations included in this paper are for homozygous or heterozygous mutations in FVL and PGM. Comparison of prevalence of FVL and PGM was made with prevalence in women with RM and controls reported in literature. Data of other inherited thrombophilia were available for a proportion of study cohort study population but were not reported or analysed due to the rare incidence in the population.

The investigations for acquired thrombophilia included testing for LA and ACL. We ensured strict criteria for defining a positive antibody test, and therefore by implication, an acquired thrombophilia was present if the result fulfilled the revised Sapporo criteria.12 A positive LA test was defined as the presence of positive result on two occasions at least 12 weeks apart. The LA screen consisted of a dilute Russell’s viper venom time (dRVVT) and an activated partial thromboplastin test test.13 The normal result for both screening tests was a ratio of ≤1.6. If either test was >1.6, a correction was carried out. The test was considered positive if the correction test is >10%. Similarly, a positive ACL antibody test was defined as presence of IgG or IgM antibodies measured using a standardised ELISA with a level ≥40 U/mL on two occasions at least 12 weeks apart.

**Systematic review and meta-analysis**

**Study registration**
This study was registered with International Prospective Register of Systematic Reviews.

**Data sources and searches**
A search of MEDLINE and Embase via the NICE Healthcare Databases Advanced Search interface and a Cochrane database search was performed using Medical Subject Heading (MeSH) terms including exp THROMBOPHILIA/, *FACTOR V LEIDEN/, *ANTIBODIES, ANTICARDIOLIPIN/ and *PROTHROMBIN GENE MUTATION/. The search was very specific and to ensure as much relevant literature on the search topic was retrieved, the major search option was used for the MeSH terms to ensure that the focus of the papers was that particular term. Example terms where the major option was chosen includes *LUPUS COAGULATION INHIBITOR/, *ANTIBODIES and ANTICARDIOLIPIN/. A limit of English language, and all papers related to humans and papers dated from 1990 to 2020 were applied. Studies included in this review consisted...
of observational, case control, prospective and retrospective cohort studies that had women with three or more first trimester miscarriages and those that a minimum of 100 women in the miscarriage arm. Four independent reviewers (AA, MS, E, HJ and HS) applied the inclusion criteria to the identified articles from the search and cross checked the selection process between them. Of the search identified 2000 citations; details of search criteria are outlined in (online supplemental file 1) and process of article selection are detailed in figure 1. There was a variation in antiphospholipid antibody levels as well as the interval between tests. After applying the diagnostic criteria, the final analysis included 12 papers; 10 on inherited and 2 on acquired thrombophilia.

**Statistical analysis**

For all studies in the systematic review, the summary statistics for prevalence of FVL and PGM were derived in the RM and control groups. These individual study statistics were then combined to obtain a pooled summary estimate for FVL and PGM with IRD meta-analysis. The most common inherited thrombophilia was FVL heterozygous mutation, which was noted in 4.8% (55/1155) followed by heterozygous PGM which was identified in 2.9% (34/1155) of patients. There was no significant difference in the prevalence of PGM mutation in our study versus that reported in literature in the RM group ((4.9%) 55/1155 vs (4.8%) 92/1584, respectively; p=0.263) as well as the prevalence of FVL in RM group versus that of control population from literature ((4.9%) 55/1155 vs (5.8%) 92/1584, respectively; p=0.354) (figure 2). Similarly, there was no significant difference in the prevalence of FVL mutation in our study versus that reported in literature in the RM group ((2.9%) 34/1155 vs (3.7%) 29/786, respectively; p=0.436) as well as the prevalence of PGM in RM group in our study versus that of control population from literature ((2.9%) 34/1155 vs (2.4%) 22/914, respectively; p=0.541) (figure 2). Only one woman had compound heterozygous for FVL and PGM and one homozygous FVL result. The meta-analysis of
proportions demonstrated that the weighted pooled prevalence of FVL in the RM and control group was 5.8% (95% CI: 4.6% to 7.1%) and 5.2% (95% CI: 3.3% to 7.5%), respectively. There was no significant difference in the weighted pooled prevalence of FVL in the RM and control group (0.4 (95% CI: −1.6 to 2.3); Z-test=0.396, p=0.692) (online supplemental table 2). Similarly, there was no significantly increased risk of RM in women with FVL (RR=1.02 (95% CI: 0.71 to 1.48); p=0.906) (online supplemental table 2, figure 3). The meta-analysis of proportions demonstrated that the weighted pooled prevalence of PGM in the RM and control group was 3.9% (95% CI: 2.7% to 5.4%) and 2.6% (95% CI: 1.6% to 3.7%), respectively (online supplemental table 3). There was no significant difference in the weighted pooled prevalence of PGM in the RM and control group (1.2% (95% CI: −0.4% to 2.8%); Z-test=1.463; p=0.143) (online supplemental table 3, figure 4). Similarly, there was no significantly increased risk of RM in women with PGM (RR=1.50 (95% CI: 0.86 to 2.60); p=0.154) (figure 4).

Acquired thrombophilia

An initial positive LA test was found in 18 patients, of these, only a third remained positive on repeat testing, giving an LA prevalence of 0.5% (6/1155). A total of 23/1155 (2.0%) patients had two positive ACL tests performed a minimum of 12 weeks apart, based on a laboratory cut-off value of ≥10 U/mL. Of these, two patients had a value ≥40 U/mL on initial or repeat testing, and four had two results with a value ≥40 U/mL giving an ACL prevalence of 0.5% (6/1155). The data concluded the total prevalence of antiphospholipid syndrome is 1.0% (12/1155).

DISCUSSION

Principal findings of the study

The findings of our study demonstrate that the prevalence of inherited thrombophilia in a large cohort of women with a history of early three or more RM is similar to that of the general population, and that the prevalence of acquired thrombophilia is low and not significantly different than the prevalence reported in the general population. Women and their clinical practitioners should be aware that the contribution of inherited and acquired thrombophilia in causing early RM is extremely low, and hence empirical testing and treatment for thrombophilia, such as low dose aspirin and low molecular weight heparin should not be advised, unless there is unequivocal clinical and laboratory evidence of established disease.
Strengths and limitations

The strengths of our study are the examination of a large cohort of pregnancies with three or more RM in specialist dedicated clinics; accurate and systematic investigation of aetiological causes of early RM including inherited and acquired thrombophilia; use of a standardised method of analysis of results; using the same reference range to mitigate errors introduced due to variation in laboratory reporting; and the adherence to the diagnostic criteria of the revised Sapporo criteria. The limitations of our study are the retrospective nature of the study and lack of contemporaneous data from control pregnancies; however, the latter is not a part of routine clinical practice. Studies included in this review were restricted to women with three or more first trimester miscarriages and those with a minimum of 100 women in the miscarriage arm. The minimum number of 100 women in the study arm could be seen as a limitation.

Comparison with other studies

Our findings are consistent with results of other studies, including large systematic reviews and meta-analyses, which report no significant difference in the prevalence of inherited thrombophilia in women with a history of RM compared with controls. In one large case–control study of 1111 women examining the prevalence of FVL mutation in women with early and late RM compared with controls, the authors reported no significant difference in the prevalence of FVL mutation in early or late RM and controls, which was 3.3%, 3.9% and 4.0%, respectively.

Our literature search identified five suitable publications for FVL prevalence and five for PGM prevalence. The prevalence of FVL in the miscarriage control group is 5.2% compared with 7.7% in the general population, while the prevalence of FVL in our miscarriage population is 4.8%. Similarly, the prevalence of PGM in the miscarriage control group is 2.6% compared with 3.7% in the general population, with our miscarriage population at 2.9%. Therefore, this suggests that there is no significant association of heterozygous FVL or PGM with RM.

In contrast, older studies have reported a high prevalence of inherited thrombophilia in women with RM and therefore advocate routine testing and treatment. There is large variation however in the prevalence of FVL and PGM in the general population. This is most likely due to genetic variations with more prevalence seen in the Levant region and Europe compared with the USA and the Far East. Despite the widespread practice of screening and treatment, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine guidelines do not recommend screening for inherited thrombophilia. At present the Royal College of Obstetricians and Gynaecologists recommends screening only if women present with pregnancy loss in the second trimester despite little evidence to support this. Similar to inherited thrombophilia, there is controversial evidence about the association between acquired thrombophilia and RM, with some studies reporting a strong association, whereas others question this association. The prevalence of antiphospholipid antibodies in the healthy general population is 1%–5%. The prevalence of acquired thrombophilia in women with RM in our study population (1%) is similar or lower when compared with healthy pregnant controls in other studies with a prevalence of ACL IgM at 3%, ACL IgG antibodies at 1% and 1.4% of LA and ACL, respectively. A frequently cited study by Rai et al reported a prevalence of antiphospholipid antibodies in women with RM as high as 15%. However, this predated the original Sapporo criteria published in 1999 and hence included levels of antiphospholipid antibodies that were even lower than 10 U/mL, which is the laboratory threshold for diagnosis, as well as less than a 12-week interval between the
tests. Applying the revised Sapporo criteria (2006) with the higher laboratory level (40 U/mL) to this study, the number of patients with raised ACL IgG antibodies will reduce from 15 to 4, and the number of raised IgM will reduce from 9 to 3 out of a total of 500 patients giving a prevalence of raised ACL antibodies of 1.4% (7/500) compared with the 5.5% (24/500) quoted in the original paper. With regards to LA, the new revised criteria states that levels of DRVVT ratio should be ≥1.2. If this is applied, the number of patients with positive LA will reduce from 46 to 9 which would give a prevalence of 1.8% (9/500) compared with 9.6% (46/500) quoted in the original paper. Hence the revised total prevalence for the Rai et al paper would boast at best a prevalence of 3.2% which most likely would reduce further if the 12-week interval is applied, and not the widely quoted 15%. A more recent study examined the association of antiphospholipid syndrome and RM using the revised Sapporo criteria for diagnosis and reported that this is present in only 2% of the population of RM, while another study reported 0.2% prevalence of LA.

Our results are consistent with these findings and reiterate that the prevalence of acquired thrombophilia in women with RM is extremely low and is similar to the general population. This was highlighted in the recent recommendations from the 14th International Congress on Antiphospholipid Antibodies Task Force report, which stated that studies reporting the association between antiphospholipid antibodies and RM are heterogeneous with considerable variation in the definition of RM and in the cut-offs used for defining what constitutes positive antibody levels. They concluded that the association between antiphospholipid antibodies and RM remains inconclusive and further research is required to establish recommendations.

CONCLUSION
The prevalence of inherited and acquired thrombophilia in women with history of RM in the first trimester of pregnancy is similar to that of the general population. The prevalence of acquired thrombophilia is only 1% in our study and between 0.2% and 2% in other good quality publications that adhered to the appropriate diagnostic criteria, which implies that the prevalence of not just inherited but also acquired thrombophilia in women with a history of RM is similar to that of the general population.

Therefore, we do not recommend routine investigation or treatment of thrombophilia in women with history of RM, and specifically do not recommend empirical thromboprophylaxis with low molecular weight heparin based on obstetrical history in the absence of any clinical evidence of thrombophilia. Further research should be carried out into other aetiologies of RM such as immunological factors and other treatment options.

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