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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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ABSTRACT

Objective There are numerous studies reporting a disproportionally high prevalence of thrombophilia in women with a history of recurrent miscarriage (RM), which has led to overdiagnosis and treatment without an improvement in clinical outcomes. 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 and compare it to the meta-analysis results of existing literature.
Methods

Design Retrospective cohort study and systematic review of literature.

Setting This is a retrospective cohort study set-up in two dedicated tertiary centres for women with RM in Southwest 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 1155 women between 2012 and 2017. All patients had three or more first trimester miscarriages and a full thrombophilia screen.

Results The overall prevalence of thrombophilia in our study population is 9.2% (106/1155) with 8.1% (94/1155) of cases positive for inherited thrombophilia, which is similar to the general population; Factor V Leiden (4.9%; 57/1155) and prothrombin gene mutation (2.9%; 34/1155) were the most common inherited thrombophilias, while only 1% (12/1155) tested positive for acquired thrombophilia. Persistent positive lupus anticoagulant (LA) was found in 0.5% (6/1155) and persistent positive anticardiolipin (ACL) antibodies with a value ≥40 U/mL was found in 0.5% (6/1155) of patients. Tests for LA/ACL were performed a minimum of 12 weeks apart thus meeting the revised Sapporo criteria for a diagnosis of antiphospholipid syndrome.

Conclusion The findings of our study demonstrate that the prevalence of inherited thrombophilia is similar in women with RM to that in the general population. Similarly, the prevalence of acquired thrombophilia, using the revised Sapporo criteria, in the cohort of RMs is similar to that in the general population. Therefore, we do not recommend investigation or treatment of inherited or acquired thrombophilia in women with RM.

PROSPERO registration number CRD42020223554.

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 aetiology 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
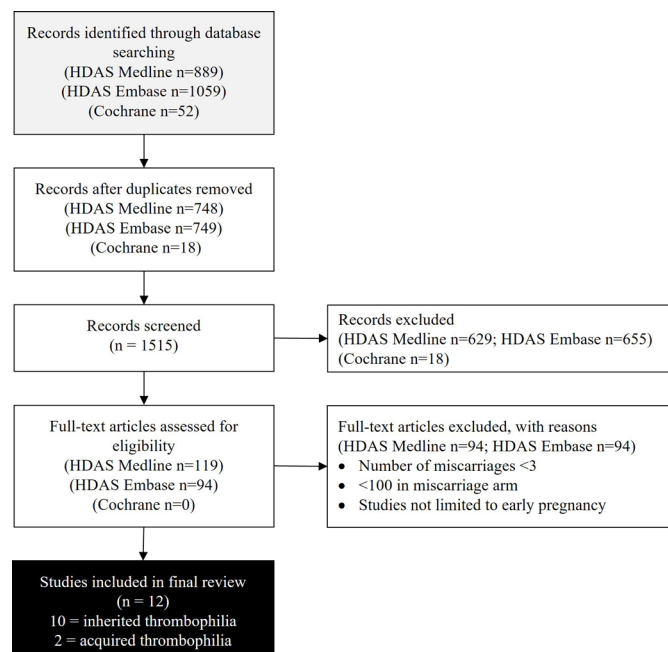
Figure 1 Flow chart of Healthcare Database Advanced Search (HDAS) demonstrating process of selection of studies included in the systematic review.

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 crossed checked the selection process between them. Our 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 which was calculated as a weighted average of the individual study estimates in the RM and control groups using both fixed and random-effects model. The fixed-effects model weighs each study by the inverse of its variance and only considers variability in results within studies and not between studies. The random-effects model allows for between-study variability in results by weighting studies using a combination of their own variance and the between-study variance. To determine whether there was a significant difference in the prevalence of thrombophilia between the RM and control groups, we estimated the difference in prevalence (95% CI) between the RM group and control group for each study using the incidence-rate

difference (IRD) meta-analysis; the individual summary statistics of difference in prevalence in each study were combined to calculate a weighted pooled estimate for FVL and PGM with IRD meta-analysis. We also estimated the effect size of the significance of difference in prevalence by calculating relative risk (RR) (95% CI) for each study and then a weighted pooled estimate from the RR for each study. Forrest plots of summary statistics for each study and final pooled estimates were constructed using data from the random-effects models.

The heterogeneity between studies was estimated using Cochrane's heterogeneity statistic Q, which was calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method (R). Inconsistency between study results was assessed using the I^2 statistic, which was calculated as $I^2 = 100\% \times (Q - df)/Q$, where Q is the Cochrane's heterogeneity statistic and df the df. The statistical software package StatsDirect V.2.7.9 (StatsDirect, Cheshire, UK) and MedCalc Statistical Software V.16.4.3 (MedCalc Software, Ostend, Belgium) were used for data analysis.

RESULTS
Study population
There was a total of 1267 women with a history of three or more consecutive early RM in our study population. We excluded cases with missing data and duplicates (n=112) and included data from 1155 women. The overall prevalence of thrombophilia in our study population was 9.2% (106/1155) of which most cases were positive for inherited thrombophilia 8.1% (94/1155) and 1.0% (12/1155) tested positive for acquired thrombophilia. The median age was 37 years (IQR 33–40) and 66.6% (769/1155) of the cohort had no previous live birth.

Inherited thrombophilia
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 FVL mutation in our study versus that reported in literature in the RM group ((4.8%) 55/1155 vs (5.8%) 92/1584, respectively; p=0.263) as well as the prevalence of FVL in RM group versus that of control population from literature ((4.8%) 55/1155 vs (4.9%) 47/966, respectively; p=0.354) (figure 2). Similarly, there was no significant difference in the prevalence of PGM 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
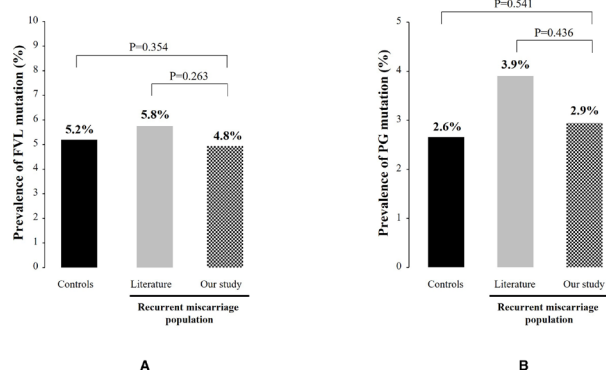
Figure 2 Prevalence of Factor V Leiden (FVL) mutation (A) and prothrombin gene (PG) mutation (B) in our study in women with recurrent miscarriages (crossed bar) compared with prevalence reported in literature in recurrent miscarriages (grey solid bar) and general population (black solid bar).

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.
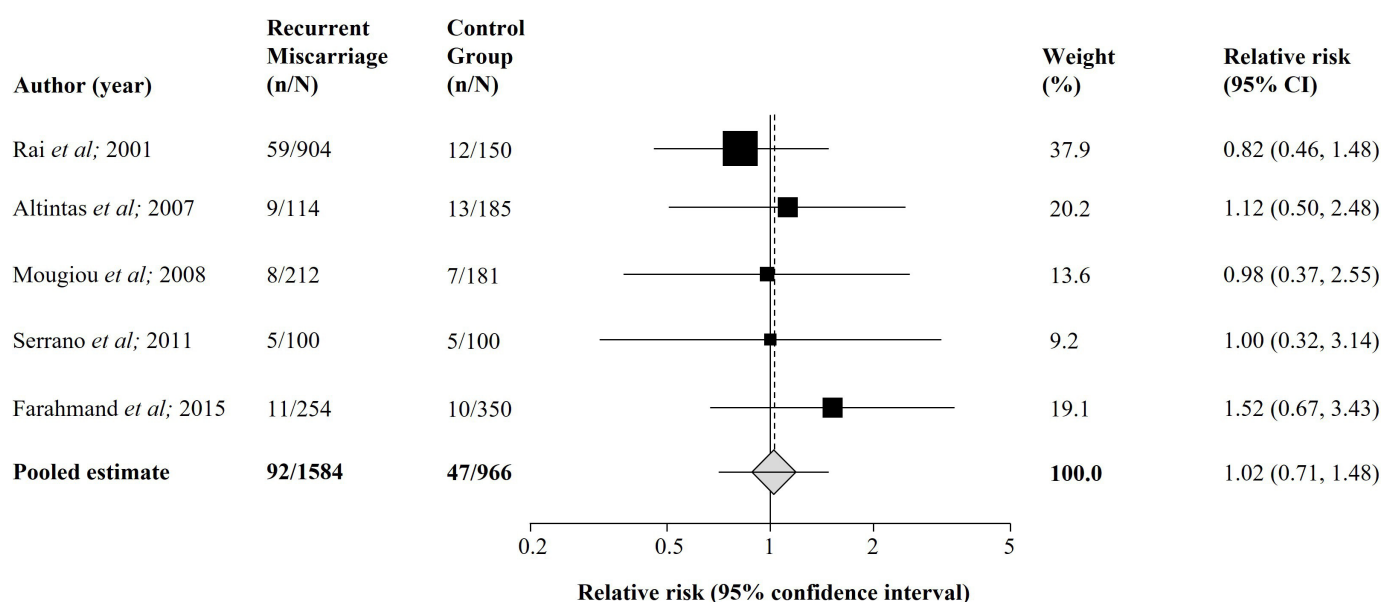
Author (year)	Recurrent Miscarriage (n/N)	Control Group (n/N)		Weight (%)	Relative risk (95% CI)
Rai *et al;* 2001	59/904	12/150		37.9	0.82 (0.46, 1.48)
Altintas *et al;* 2007	9/114	13/185		20.2	1.12 (0.50, 2.48)
Mougiou *et al;* 2008	8/212	7/181		13.6	0.98 (0.37, 2.55)
Serrano *et al;* 2011	5/100	5/100		9.2	1.00 (0.32, 3.14)
Farahmand *et al;* 2015	11/254	10/350		19.1	1.52 (0.67, 3.43)
Pooled estimate	**92/1584**	**47/966**		**100.0**	1.02 (0.71, 1.48)

Relative risk (95% confidence interval)

Figure 3 Forest plot demonstrating summary statistics (relative risk (95% CIs)) derived from a random effects model for prevalence of Factor V Leiden in women with recurrent miscarriages compared with the control population. Study weights derived from random effect model.
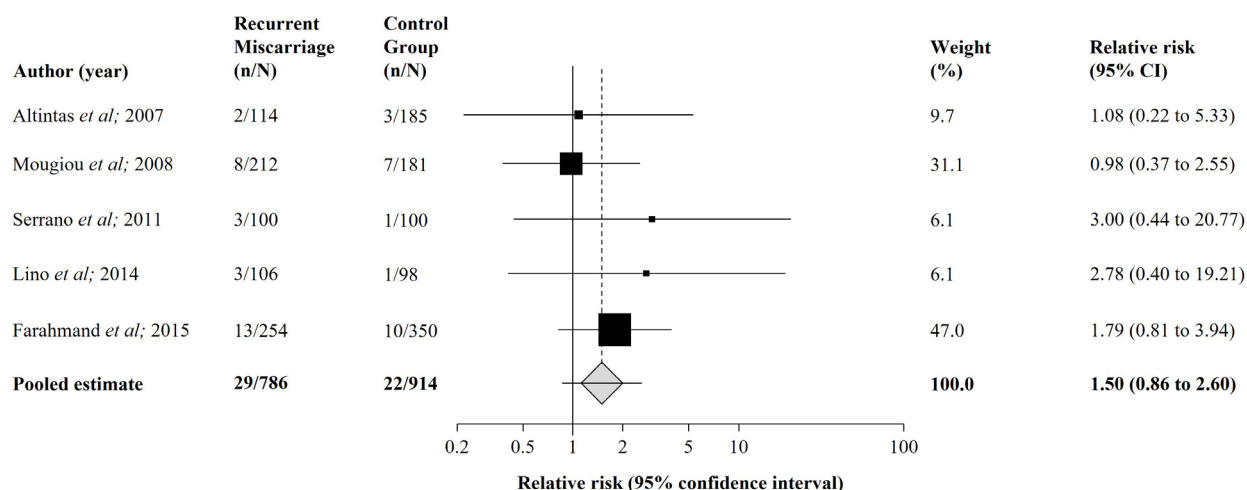
Author (year)	Recurrent Miscarriage (n/N)	Control Group (n/N)		Weight (%)	Relative risk (95% CI)
Altintas *et al*; 2007	2/114	3/185		9.7	1.08 (0.22 to 5.33)
Mougiou *et al*; 2008	8/212	7/181		31.1	0.98 (0.37 to 2.55)
Serrano *et al*; 2011	3/100	1/100		6.1	3.00 (0.44 to 20.77)
Lino *et al*; 2014	3/106	1/98		6.1	2.78 (0.40 to 19.21)
Farahmand *et al*; 2015	13/254	10/350		47.0	1.79 (0.81 to 3.94)
Pooled estimate	**29/786**	**22/914**		**100.0**	**1.50 (0.86 to 2.60)**

Relative risk (95% confidence interval)

Figure 4 Forest plot demonstrating summary statistics (relative risk (95% CIs)) derived from a random effects model for prevalence of prothrombin gene mutation in women with recurrent miscarriages compared with the control population. Study weights derived from random effect model.

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.[7 14–16] 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.[14]

Our literature search identified five suitable publications for FVL prevalence[14 17–20] and five for PGM prevalence.[17–21] The prevalence of FVL in the miscarriage control group is 5.2% compared with 7.7% in the general population,[14 17–20] 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,[17–21] 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.[22–24] There is large variation however in the prevalence of FVL and PGM in the general population.[25] 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[26] and the American Society for Reproductive Medicine guidelines[2] 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.[1]

Similar to inherited thrombophilia, there is controversial evidence about the association between acquired thrombophilia and RM, with some studies reporting a strong association,[27 28] whereas others question this association.[29 30] The prevalence of antiphospholipid antibodies in the healthy general population is 1%–5%.[31] 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%[32] and 1.4% of LA and ACL,[33] respectively. A frequently cited study by Rai *et al* reported a prevalence of antiphospholipid antibodies in women with RM as high as 15%.[34] 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,[30] while another study reported 0.2% prevalence of LA.[29]

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.[35]

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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Supplementary Table 1. Search strategy used in the systematic review

Medline database – Search strategy			
#	Database	Search term	Results
1	Medline	exp "ABORTION, HABITUAL"/	8085
2	Medline	("recurrent miscarriage*").ti,ab	2070
3	Medline	(1 OR 2)	8848
4	Medline	exp THROMBOPHILIA/	24961
5	Medline	("acquired thrombophilia").ti,ab	317
6	Medline	*"PROTEIN C DEFICIENCY"/	979
7	Medline	*"PROTEIN S DEFICIENCY"/	750
8	Medline	*"ANTIPHOSPHOLIPID SYNDROME"/	6568
9	Medline	*"LUPUS COAGULATION INHIBITOR"/	1040
10	Medline	*"ANTIBODIES, ANTICARDIOLIPIN"/	1461
11	Medline	*"BETA 2-GLYCOPROTEIN I"/	568
12	Medline	("factor V Leiden").ti,ab	3998
13	Medline	("prothrombin gene mutation").ti,ab	300
14	Medline	(4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13)	35905
15	Medline	(3 AND 14)	889
16	Medline	15 [DT 1990-2020] [Languages English]	760
Embase database – Search strategy			
#	Database	Search term	Results
1.	EMBASE	exp "ABORTION, HABITUAL"/	6141
2.	EMBASE	("recurrent miscarriage*").ti,ab	3620
3.	EMBASE	(21 OR 22)	8255
4.	EMBASE	exp THROMBOPHILIA/	13382
5.	EMBASE	("acquired thrombophilia").ti,ab	623
6.	EMBASE	*"PROTEIN C DEFICIENCY"/	954
7.	EMBASE	*"PROTEIN S DEFICIENCY"/	781
8.	EMBASE	*"ANTIPHOSPHOLIPID SYNDROME"/	9102
9.	EMBASE	*"LUPUS COAGULATION INHIBITOR"/	2122
10.	EMBASE	*"ANTIBODIES, ANTICARDIOLIPIN"/	2548
11.	EMBASE	*"BETA 2-GLYCOPROTEIN I"/	1237
12.	EMBASE	("factor V Leiden").ti,ab	6368
13.	EMBASE	("prothrombin gene mutation").ti,ab	578
14.	EMBASE	(24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33)	31404
15.	EMBASE	(23 AND 34)	1327

16.	EMBASE	35 [DT 1990-2020] [English language] [Humans]	1059

Cochrane database – Search strategy

#	Database	Search term	Results
1.	Cochrane	MeSH descriptor: [Abortion, Habitual] 1 tree(s) exploded	
2.	Cochrane	"recurrent miscarriage*"	
3.	Cochrane	#1 OR #2	
4.	Cochrane	MeSH descriptor: [Thrombophilia] explode all trees	
5.	Cochrane	"acquired thrombophilia"	
6.	Cochrane	MeSH descriptor: [Protein C Deficiency] this term only	
7.	Cochrane	MeSH descriptor: [Protein S Deficiency] this term only	
8.	Cochrane	MeSH descriptor: [Antiphospholipid Syndrome] this term only	
9.	Cochrane	MeSH descriptor: [Lupus Coagulation Inhibitor] explode all trees	
10.	Cochrane	MeSH descriptor: [Antibodies, Anticardiolipin] this term only	
11.	Cochrane	MeSH descriptor: [Antibodies, Anticardiolipin] explode all trees	
12.	Cochrane	"factor V Leiden"	
13.	Cochrane	"prothrombin gene mutation*"	
14.	Cochrane	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	
15.	Cochrane	#3 AND #14	

Supplementary Table 2. Meta-analysis demonstrating the summary statistics for prevalence of heterozygous Factor V Leiden (FVL) in the recurrent miscarriage and control groups with the prevalence difference (95% confidence intervals (CI) and effect size of the prevalence difference, expressed as relative risk (RR) with 95% CI.

Author	Recurrent miscarriage group		Control group		Prevalence difference	Relative risk
	Total	N (%,95% CI)	Total	N (%; 95% CI)	% (95% CI)	RR (95% CI)
Rai *et al.,* 2001 [14]	904	59 (6.5; 5.0-8.3)	150	12 (8.0; 4.2-13.6)	-1.5 (-6.0-3.0)	0.82 (0.46-1.48)
Altintas *et al.,* 2007 [31]	114	9 (7.9; 3.7-14.5)	185	13 (7.0; 3.8-11.7)	0.9 (-5.5-7.2)	1.12 (0.50-2.48)
Mougiou *et al.,* 2008 [32]	212	8 (3.8; 1.6-7.3)	181	7 (3.9; 1.6-7.8)	-0.1 (-4.0-3.8)	0.98 (0.37-2.55)
Serrano *et al.,* 2011 [33]	100	5 (5.0; 1.6-11.3)	100	5 (5.0; 1.6-11.3)	-	1.00 (0.32-3.14)
Farahmand *et al.,* 2015 [34]	254	11 (4.3; 2.2-7.6)	350	10 (2.9; 1.4-5.2)	1.5 (-1.5-4.5)	1.52 (0.67-3.43)
Summary statistics						
Fixed effects model	1584	92 (5.9; 4.8-7.1)	966	47 (4.9; 3.6-6.3)	0.4 (-1.6-2.3)	1.03 (0.72-1.49)
Random effects model	1584	92 (5.8; 4.6-7.1)	966	47 (5.2; 3.3-7.5)	0.4 (-1.6-2.3)	1.02 (0.71-1.48)
Cochrane's Q (p value)		4.401 (0.354)		8.414 (0.078)	1.135 (0.889)	1.456 (0.835)
I^2 statistic (%; 95%CI)		9.1 (0 to 67.3)		52.5 (0 to 80.6)	0 (0 to 64.1)	0 (0 to 64.1)
Bias (p value)		-0.886 (0.524)		3.969 (0.256)	-1.016 (0.542)	1.003 (0.465)

CI: confidence interval; RR: relative risk

Supplementary Table 3. Meta-analysis demonstrating the summary statistics for prevalence of heterozygous Prothrombin gene mutation (PGM) in the recurrent miscarriage and control groups with the prevalence difference (95% confidence intervals (CI) and effect size of the prevalence difference, expressed as relative risk (RR) with 95% CI.

Author	Recurrent miscarriage group		Control group		Prevalence difference	Relative risk
	Total	n (%,95% CI)	Total	n (%; 95% CI)	% (95% CI)	RR (95% CI)
Altintas *et al.,* 2007 [31]	114	2 (1.8; 0.2-6.2)	185	3 (1.6; 0.3-4.7)	0.1 (-2.9- 3.2)	1.08 (0.22-5.33)
Mougiou *et al.,* 2008 [32]	212	8 (3.8; 1.6-7.3)	181	7 (3.9; 1.6-7.8)	-0.1 (-4.0-3.8)	0.98 (0.37-2.55)
Serrano *et al.,* 2011 [33]	100	3 (3.0; 0.6-8.5)	100	1 (1.0; 0.0-5.4)	2.0 (-1.9-5.9)	3.00 (0.44-20.77)
Lino *et al.,* 2014 [35]	106	3 (2.8; 0.6-8.0)	98	1 (1.0; 0.0-5.6)	1.8 (-2.0-5.7)	2.78 (0.40-19.21)
Farahmand *et al.,* 2015 [34]	254	13 (5.1; 2.8-8.6)	350	10 (2.9; 1.4-5.2)	2.3 (-0.9-5.4)	1.79 (0.81-3.94)
Summary statistics						
Fixed effects model	786	29 (3.9; 2.7-5.4)	914	22 (2.6; 1.6-3.7)	1.2 (-0.4-2.8)	1.52 (0.88-2.62)
Random effects model	786	29 (3.9; 2.7-5.4)	914	22 (2.6; 1.6-3.7)	1.2 (-0.4-2.8)	1.50 (0.86-2.60)
Cochrane's Q (p value)		2.548 (0.636)		3.293 (0.510)	1.556 (0.817)	1.689 (0.793)
I^2 statistic (%; 95%CI)		0 (0 to 64.1)		0 (0 to 64.1)	0 (0 to 64.1)	0 (0 to 64.1)
Bias (p value)		-1.718 (0.613)		-0.560 (0.841)	0.683 (0.823)	0.473 (0.592)

CI: confidence interval; RR: relative risk