

Association between non-O blood type and early unexplained recurrent spontaneous abortion in women with and without inherited thrombophilia

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ABSTRACT

We retrospectively evaluated the prevalence of non-O blood type – the most frequently inherited prothrombotic factor – and inherited thrombophilia (IT) in a group of women with recurrent spontaneous abortion (RSA). All consecutive women with a history of early unexplained RSA who underwent a screening for IT between December 2008 and December 2021 were considered for enrolment. A group of healthy, age-matched women with ≥ 1 normal pregnancy and no adverse pregnancy outcomes acted as controls. Two hundred and seventeen women were enrolled. The adjusted odds ratio (aOR) of RSA in non-O vs. O blood type was 1.37 (95% CI, 1.04-2.78), and in women with vs. without IT was 1.26 (95% CI, 1.08-3.61); aOR of RSA in women with non-O blood type and IT was 2.52 (95% CI, 1.12-5.47). We observed a significant association between non-O blood group or IT and RSA. The concomitant presence of non-O blood group and IT further increases RSA risk.

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Key words: recurrent spontaneous abortion, inherited thrombophilia, blood group.

Contributions: Conceptualization, AP and LS; methodology, AP, LS, PS; validation, AP, LS, EC and PS; formal analysis, LS; investigation, AP, EB, GT, MM, EC, DT; resources, PS; data curation, AP and LS; writing and original draft preparation, AP and LS; writing, review and editing, all authors; supervision, PS. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Conference presentation: Data from this paper were included in an oral presentation at the XXVII National Congress of the Italian Society for the Study of Hemostasis and Thrombosis (SISSET) – Perugia 2-5 November 2022.

Ethical approval: Padova University Hospital's Ethics Committee was notified, as required for retrospective studies.

Received for publication: 24 July 2022.

Revision received: 13 October 2022.

Accepted for publication: 25 October 2022.

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Bleeding, Thrombosis and Vascular Biology 2022; 1:47

doi:10.4081/btvb.2022.47

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INTRODUCTION

Spontaneous abortion is considered one of the most frequent complications during pregnancy.¹ A great body of evidence, published in the literature so far, points to placental damages due to microvascular thrombosis as one of the main pathogenetic mechanisms involved in the gestational vascular pathology and in particular, recurrent spontaneous abortion (RSA).² Several studies have investigated the possible association between hypercoagulability due to inherited thrombophilia (IT) and RSA without reaching definitive conclusions.^{3,4} Some more solid data has been published in relation to prothrombin G20210A mutation (PM) and Factor V Leiden (FVL). A meta-analysis by Gao & Tao reported a significant association between PM and RSA (OR 1.81; 95% CI, 1.26-2.60).⁵ Furthermore, two meta-analyses by Sergi *et al.* and Hamed *et al.* conducted on Middle Eastern women, found a significant association between FVL and RSA (OR 1.68; 95% CI, 1.16-2.44 and OR 2.37; 95% CI, 1.50-3.75), respectively.^{6,7} Two small studies – in 2011 by Klai *et al.*⁸ and in 2013 by Akdemir *et al.*⁹ – previously found no significant association between RSA and non-O blood type, the most frequently inherited prothrombotic factor.¹⁰ Therefore, we aimed to retrospectively evaluate the prevalence of non-O blood and IT in a large cohort of Italian women with early unexplained RSA. Moreover, we evaluated whether the concomitance of non-O blood type and IT carries a higher risk of RSA as compared to each condition separately.

MATERIALS AND METHODS

All consecutive women with a history of early (<12 weeks of gestation) unexplained recurrent (*i.e.* three or more consecutive) spontaneous abortion tested for IT at our laboratory of Thrombotic and Hemorrhagic Diseases Unit between December 2008 and December 2021 were considered for enrolment. Exclusion criteria were: age <18 yrs, a history of venous and/or arterial thrombosis, severe renal or liver insufficiency, ongoing anticoagulant/ antiplatelet treatment or oral contraceptives, and a diagnosis of antibody antiphospholipid syndrome. The women enrolled as controls in this study were selected among a group of healthy women, as previously reported: age-matched (± 3 yrs) with cases, with at least one normal pregnancy and no history of adverse pregnancy outcomes.¹¹ Age, body mass index (BMI) and comorbidities were recorded at the time of the previous pregnancy that resulted in pregnancy loss in cases, and at the time of blood sample collection in controls. Obesity was defined as a BMI >30 and hypertension as a confirmed systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg, in accordance with the World Health Organization. Diabetes was defined according to the criteria of the American Diabetes Association.¹² The whole study population was screened for antithrombin (AT), protein C (PC) and protein S (PS) deficiencies and for the following polymorphisms: prothrombin G20210A, FVL G169A, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, as described elsewhere.¹³ In particular, antithrombin activity was detected using a thrombin-based chromogenic substrate assay (Roche Diagnostics GmbH, Mannheim, Germany); protein C activity was measured using a commercial kit (Protein C Reagent, Siemens Healthcare Diagnostics, Milan, Italy). The above tests were performed on a BCS XP coagulation analyzer (Siemens Healthcare Diagnostics, Milan, Italy). Protein S activity was assessed using the ProS kit (Instrumentation Laboratory, Milan, Italy) on an ACL TOP 300 CTS coagulation analyzer (Instrumentation Laboratory, Milan, Italy). Finally, genetic polymorphisms were determined using ABI Prism 3100 Genetic Analyzer (Applied Biosystem, USA) according to manufacturer's instructions. Each enrolled woman underwent ABO genotyping. All participants provided a written informed consent in compliance with the principles of the Declaration of Helsinki, and the Padova University Hospital's Ethics Committee was notified as required for retrospective studies. The data supporting our findings in this study are available from the corresponding author upon reasonable request.

Statistical analysis

Continuous variables were expressed as mean \pm standard deviation, categorical variables were expressed as percentages. The chi-square and the student's t-test were used to calculate the differences between categorical and parametric variables, respectively. ORs and 95% CIs were calculated as a likelihood estimate of recurrent pregnancy loss. To adjust for possible confounders, a logistic regression model was used taking into account age, comorbidities, thrombophilia and non-O blood type. $P < 0.05$ was considered statistically significant. Analyses were performed using the SPSS statistical (package for Windows 17.0 software: Chicago, Illinois, USA).

RESULTS

Among n. 248 eligible patients, n. 31 (12.5%) were excluded: n. 14 for missing laboratory data; n. 7 for a previous thrombotic event; n. 6 for a diagnosis of antibody antiphospholipid syndrome; n. 2 refused to give their informed consent; n. 2 for liver or renal insufficiency. Overall, n. 217 (mean age 32 yrs, range 25-42 yrs) women were enrolled. The main characteristics of the study population are reported in Table 1. The prevalence of obesity was significantly higher in cases than controls ($p = 0.02$). The remaining comorbidities were distributed similarly between cases and controls. The prevalence of IT was significantly higher in cases n. 63 (29.0%) than in controls n. 42 (19.6%, $p = 0.02$). The most frequent polymorphisms in both cases and controls were MTHFR (4 homozygous in cases and 2 in controls) and FVL (1 homozygous in cases) followed by PM (0 homozygous). Non-O blood type was significantly more frequent in cases than in controls (n. 129, 59.4% vs. n. 107, 49.3%; $p = 0.03$). The distribution of blood groups observed in the control group mirrored that expected in the general Italian population.¹⁴ The prevalence of IT and blood types are reported in Table 2. The adjusted OR of RSA in women with vs. without IT was 1.37 (95% CI, 1.04-2.78). The adjusted OR of RSA in non-O vs. O blood type women was 1.26 (95% CI, 1.08-3.61). We found concomitant IT and non-O group in n. 37 (17.0%) cases and n. 20 (9.2%) controls with a resulting adjusted OR 2.52 (95% CI, 1.12-5.47).

DISCUSSION

Several studies published in the literature so far have investigated the possible association between hypercoagulability and adverse pregnancy outcomes, particularly pregnancy loss, yielding inconclusive results. Our large case-control study revealed that women with non-O blood

type – the most commonly inherited prothrombotic factor¹⁰ – carry a higher risk to develop early unexplained RSA *vs.* women with O blood type. It bears noting that two small previous studies by Klai *et al.* and by Akdemir *et al.* found no association between non-O blood type and RSA.^{8,9} The discrepancy may be ascribed to our bigger sample size and the fact that we enrolled a cohort of Caucasian women versus Tunisian and Turkish women in the previous two studies; different inclusion and exclusion criteria may have also played a role.

We also found that women with IT have a two-fold increased risk to develop early unexplained RSA *vs.* healthy women. Our findings are in line with the data of three meta-analyses recently published in the literature that

identified a significant association between PM or FVL and RSA.⁵⁻⁷ The main mechanisms that may predispose to placenta-mediated pregnancy complications such as RSA, are thrombosis of placental vessels and abnormal placental development.¹⁵ Nevertheless, there is no definitive consensus on the causal nexus between IT and RSA. Among IT conditions, we also considered MTHFR testing, though only for research purposes. There are conflicting reports in the literature. A meta-analysis by Wu *et al.* found that homozygous MTHFR C677T genotype is associated with increased risk of recurrent pregnancy loss in Asian population but not in Caucasians.¹⁶ A recent Focus by Deloughery *et al.* citing guidelines from multiple medical societies, concluded that MTHFR polymor-

Table 1. Patients' characteristics.

	Cases n. 217	Controls n. 217	p value
Age, yrs	32±5	33±6	0.40
Body Mass Index, kg/m ²	27±4	25±6	<0.01
Co-morbidities, n (%)			
Obesity	27 (12.4)	13 (6.0)	0.02
Hypertension	15 (6.9)	12 (5.5)	0.55
Active smoking	6 (2.8)	7 (3.2)	0.78
Diabetes	5 (2.3)	4 (1.8)	0.74
Thrombophilia, n (%)			
Methylene tetrahydrofolate reductase	22 (10.1)	15 (6.9)	0.23
Factor V Leiden	16 (7.3)	10 (4.6)	0.22
Prothrombin mutation	10 (4.6)	8 (3.7)	0.63
Protein S deficiency	6 (2.8)	2 (0.9)	0.15
Protein C deficiency	5 (2.3)	4 (1.8)	0.74
Antithrombin deficiency	1 (0.5)	1 (0.5)	1.00
Combinations	3 (1.4)	2 (0.9)	0.65
Blood group, n (%)			
O	88 (40.5)	110 (50.7)	0.03
A	79 (36.4)	65 (30.0)	0.30
B	31 (14.3)	27 (12.4)	0.57
AB	19 (8.8)	15 (6.9)	0.47

Numbers in brackets indicate percentages. Age and Body Mass Index are expressed as mean ± Standard Deviation. Values in italics are statistically significant.

Table 2. Thrombophilias and blood groups in study subjects.

	Cases n. 217	Controls n. 217	Crude OR (95% CI)	Adjusted OR (95% CI)
Non-thrombophilia	154	175	1	1
Thrombophilia	63	42	1.70 (1.09-2.66)	1.37 (1.04-2.78)
O blood group	88	110	1	1
Non-O blood group	129	107	1.51 (1.03-2.20)	1.26 (1.08-3.61)
O blood group without thrombophilia	63	89	1	1
O blood group with thrombophilia	25	21	1.68 (0.87-3.27)	1.54 (0.71-3.62)
Non-O blood group without thrombophilia	91	86	1.49 (0.97-2.31)	1.44 (0.85-2.28)
Non-O blood group with thrombophilia	38	21	2.56 (1.37-4.77)	2.52 (1.12-5.47)

OR, Odds Ratio; CI, Confidence Interval. ORs were adjusted for age, comorbidities, thrombophilia and non-O blood type.

phism should not be included in any routine thrombophilia workup.¹⁷

Finally, the combined presence of non-O blood type and IT further increased the risk of early unexplained RSA vs. O blood type and no IT. This may be attributable to the hypercoagulable state stemming from the association between IT and increased levels of factor VIII:C and von Willebrand factor (VWF) observed in non-O blood vs. O blood type.¹⁸ In fact, Albanez *et al.* reported a significant age-related increase in plasma levels of VWF and FVIII in subjects with non-O blood type.¹⁹ There have been reports in the literature of significantly increased FVIII levels in women with a history of spontaneous abortion.²⁰

Our findings suggest that the risk of developing early unexplained RSA increases if a woman has simultaneously more than one procoagulant factor (*e.g.* non-O blood group and IT). Considering these results, it would be interesting to reconsider the data obtained in those studies that evaluated the efficacy of low molecular weight heparin (LMWH) prophylaxis in preventing early unexplained RSA in thrombophilic women. In particular, a recent meta-analysis by Intzes *et al.* found that LMWH had no beneficial effect on live birth rates in women with thrombophilia.²¹ We hypothesize that the subgroup of women with concomitant non-O blood group and IT enrolled in these studies, with their greater hypercoagulable state than women with (only) IT may benefit from early LMWH prophylaxis, thus preventing early unexplained RSA.

CONCLUSIONS

Our findings suggest that blood typing may provide additional information to stratify patients at increased risk of early unexplained RSA. This may help clinicians identify women at high risk of pregnancy loss, suitable for counselling, further testing, closer monitoring or thromboprophylaxis changes/implementation.

Some of the limitations of our study include the heterogeneity of the controls and the difficulty to ascertain the cause of RSA. In some cases, we were not able to discern genetic abnormalities of the fetus vs. placental phenomena, the exact stage of the pregnancy when the SA occurred, the mother's health conditions at the time of the SA. Unlike previous studies, we did not investigate the role of ABO incompatibility between mother and fetus as a possible risk factor for SA.²²

In conclusion, our findings suggest that either IT or non-O blood type may be risk factors for early unexplained RSA. The combined presence of non-O blood type and IT increases said risk. Larger studies are needed to confirm the strength of our results and evaluate their clinical relevance.

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