Prediction of Recurrent Pregnancy Loss by a New Thrombophilia Based Genetic Risk Score

José Antonio Páramo[a], Pilar Llamas[b], José Bellver[c], Ramón Lecumberri[d], Arantxa García-Raso[e], Pilar Alamá[f], César Díaz-García[g], Ana Alfonso Pierola[h], Ana Carolina Miranda[i], Víctor Hugo Gómez[j], Sergio Cabanillas[k], Sheila Vázquez[l], Kevin Guillén[m], Sara Pich[n], Oriol Casagran[o], Israel Ortega[p], Jose Manuel Soria[q], Eduardo Salas[r]*

[a,d,h]*Clinica Universidad de Navarra, Hematology, Pamplona, 31008, Spain*

[a,d]*CIBER CV, ISCIII, Spain*

[b,e,i]*Hospital Universitario Fundacion Jimenez Dıaz, Hematology, Madrid, 28040, Spain*

[c,f,j,k]*IVI-RMA Valencia, Valencia, 46015, Spain*

[c,g]*University of Valencia, Department of Pediatric, Obstetrics and Gynecology, Valencia, 46010, Spain*

[g,l]*IVI-RMA London, London, W1G 9RQ, United Kingdom*

[g]*Instituto de Investigaciones Sanitarias La Fe, Reproductive Research Group, Valencia, 46026, Spain*

[m,n,o,p,r]*Gendiag.exe, Scientific Department, Esplugues de Llobregat, 08950, Spain*

[q]*Institut d′Investigacio Sant Pau (IIB-Sant Pau), Unitat de Genomica de Malalties Complexes, Barcelona, 31008, Spain*

[a]*Email: japaramo@unav.es ;* [b]*Email: pllamas@fjd.es ;* [c]*Email: Jose.Bellver@ivi.es*

[d]*Email: rlecumber@unav.es ;* [e]*Email: ArGarciaR@quironsalud.es ;* [f]*Email: Pilar.Alama@ivi.es*

[g]*Email: cdiaz_med@yahoo.fr ;* [h]*Email: aalfonso@unav.es ;* [i]*Email: ACMiranda@hospitalreyjuancarlos.es*

[j]*Email: VictorHugo.Gomez@ivi.es ;* [k]*Email: Sergio.Cabanillas@ivi.es ;* [l]*Email: sheilita16_04@hotmail.com*

[m]*Email: kevin.guillen@gendiag.com ;* [n]*Email: sara.pich@gendiag.com ;* [o]*Email: oriol.casagran@gendiag.com*

[p]*Email: Israel.ortega@gendiag.com ;* [q]*Email: jsoria@santpau.cat ;* [r]*Email: eduardo.salas@gendiag.com*

----

* Corresponding author.

Abstract

We examined the predictive ability of the new thrombophilia-based genetic risk score that has been developed (TiC-RPL) to acutely determine the risk of recurrent pregnancy loss (RPL) closely related to thrombophilia and to compare it with the ability of the classical genetic thrombophilia variants F5 rs6025 and F2 rs1799963. This is a case-control observational study, with retrospective data analysis. We included 180 healthy women with at least one uncomplicated pregnancy to term and no previous miscarriage and 184 cases of idiopathic recurrent pregnancy loss (RPL). The predictive ability was assessed in terms of discrimination (AUC), sensitivity, specificity, positive and negative predictive values (PPV, NPV), and positive and negative likelihood ratios (PLR and NLR). TiC-RPL has a better AUC (95 CI) than F5 rs6025+F2 rs1799963 [0.763 (0.715-0.811) vs 0.540 (0.514-0.567); $p<0.0001$], with a sensitivity of 70.65%, a specificity of 67.78%, a PPV of 69.15%, an NPV of 69.32%, a PLR of 2.19, and an NLR of 0.43. Our results show that the new score TiC-RPL is significantly better than F5 rs6025+F2 rs1799963 in identifying RPL women in whom RPL appears to be associated with thrombophilia. This identification can guide a personalized approach in the prevention of RPL.

Keywords: recurrent pregnancy loss; thrombophilia screening; genetic risk score; highly sensitive predictive tool.

1. Introduction

Thrombophilia is defined as a hypercoagulable state that leads to thrombotic tendency [1]. Thrombophilia can be inherited, acquired, or mixed (congenital and acquired), and the risk of venous thromboembolism (VTE) differs, based on the resulting modification of the coagulation [2]. Women with thrombophilia are at increased risk of venous thrombosis during pregnancy, placenta-mediated pregnancy complications, and recurrent pregnancy loss (RPL) [3,4]. Although 15 percent of clinically recognized pregnancies miscarry, the rate of total pregnancy losses is close to 50 percent [5]. Recurrent pregnancy loss (understood as two or more clinical pregnancy losses, per the Practice Committee of American Society for Reproductive Medicine, 2013) affects 0.4 to 2 percent of couples [6].

The most commonly tested types of inherited thrombophilia include deficiencies in antithrombin, protein C, or protein S and the gain-of-function genetic variants F5 rs6025 and F2 rs1799963. Several cohort and case control studies have noted a positive association between thrombophilia and pregnancy loss [3,7]. The risk for RPL is higher in women with inherited thrombophilia [3]; however, the value of routine inherited thrombophilia screening is under debate [8]. Most likely the doubt about the clinical utility of inherited thrombophilia screening is due to the fact that only two low frequency genetic variants are tested. Therefore, greater knowledge of the genetic basis of thrombophilia [9] could be used to identify thrombophilia-related RPL more accurately and to establish an effective thromboprophylaxis protocol with improve pregnancy outcomes.

Our study developed a new thrombophilia-based genetic risk score (TiC-RPL), based on the current knowledge on the genetic basis of thrombophilia, and compared its predictive ability with that of the classical genetic thrombophilia variants F5 rs6025 + F2 rs1799963 in identifying RPL.

2. Material and Methods

This study was registered at ClinicalTrials.gov under registry number NCT03336463. The study was conducted in compliance with the Helsinki Declaration and was approved by the corresponding institutional ethics committees. All patients signed informed consent forms before inclusion.

This multicenter, case-control, observational study, with retrospective data analysis, was performed in 4 centers throughout Spain.

Accepting an alpha risk of 0.10 and a beta risk of 0.20 in a two-sided test, 182 cases and 180 controls are needed to recognize as statistically significant an odds ratio greater than or equal to 2. The proportion of exposed subjects in the control group has been estimated to be 15.1 per cent. The GRANMO program (https://www.imim.cat/ ofertadeserveis/software-public/granmo, Version 7.12) has been used to make the calculations, which are derived from the Poisson approach for the case of sample size estimation based on odds ratios.

Women with idiopathic RPL (184) attending the Obstetrics and Gynecology or Reproductive medicine Units at the participant centers were eligible for study participation if they fulfilled the following criteria: age older than 18 years, a history of RPL (two or more clinical pregnancy losses) from spontaneous or assisted pregnancies, use of their own gametes, normal karyotype in both members of the couple, normal or corrected thyroid function, BMI <30, normal uterine anatomy (as assessed by 3D ultrasound, hysterosalpingography, or hysteroscopy), with negative antiphospholipid and anti-beta 2 glycoprotein antibodies, non-diabetic, no chronic pathologies, no hydrosalpinx, and not taking concomitant anticoagulant or anti-aggregant therapies. The couple's sperm could be analyzed in 112 of the 184 PRL cases, and the count was higher than $2x106$/ml.

Control subjects (180) attending the Obstetrics and Gynecology Units of the participant centers were eligible for study participation if they fulfilled the following criteria: age older than 18 years at first pregnancy, at least 1 pregnancy to term, no chronic pathology, no personal or family history of thrombosis, no history of obstetric complications (miscarriage or fetal death, pre-eclampsia, eclampsia, intrauterine growth restriction, placental abruption), and not taking concomitant anticoagulant or anti-aggregation therapies during pregnancy.

The genetic analysis entailed the collection of a saliva sample (by oral mucosal smear) or blood sample, DNA extraction (by digestion and selective precipitation with ethanol), and genotyping of the prothrombotic genetic variables that were identified as (gene-rs) using the standard F5-rs6025 and F2-rsl799963 panel and Thrombo inCode (TiC, Ferrer inCode, Barcelona, Spain), being TiC based on the current knowledge on the genetic basis of thrombophilia [9]. The genetic variants included in TiC have been shown to be of clinical utility in the identification of subjects at risk of suffering a venous thromboembolic event and we want to study if all those genetic variants or a subset of them could be of clinical utility if the identification of RPL. The TiC panel included 12 genetic variables: F2 rs1799963, F5 rs6025, F5 rs118203905, F5 rs118203906, F12 rs1801020, F13 rs5985, Serpin-C1 rs121909548, Serpin-A rs2232698, AB0 rs8176719, AB0 rs7853989, AB0 rs8176743 , and AB0 rs8176750 (all 4 ABO rs forming the haplotype for identification of A1 AB0 group carriers). The genetic analysis was performed at Gendiag.exe.

The clinical variables that we considered were age, family history of VTE, and week at which the pregnancy loss occurred.

All variables were analyzed for patients with recurrent pregnancy loss and controls.

The association between genetic variables and recurrent pregnancy loss was determined, taking into account the confounding effect of age. For this purpose, a logistic regression model was fitted, including the individual genetic variable and age as the independent variables in the model.

For the development of the Thrombo inCode for repeated pregnancy loss (TiC-RPL) risk score, age and genetic variables included in TiC panel that were individually associated with recurrent miscarriage ($p<0.10$) were analyzed by multivariate logistic regression. Hosmer-Lemeshow test was used to assess the correct calibration of the models. TiC-RPL score was compared against F5 rs6025 + F2 rs1799963, a binary score that was defined as 1 in the presence of the F5 rs6025 or F2 rs17799963 risk allele and 0 otherwise.

The predictive capacity of the risk scores was evaluated using the area under the receiver operating characteristic curve (AUC; larger values indicate better discrimination). DeLong test was used to compare AUC values between the 2 scores. Standard measures of sensitivity, specificity, positive and negative predictive values (PPV, NPV), and positive and negative likelihood ratios (PLR, NLR) were calculated. These measures were compared between scores using the R package DTComPair (http://CRAN.R-project.org/package=DTComPair), which implements several methods for each of the measures. Briefly, sensitivity and specificity were compared by McNemar test, PPV and NPV were compared using a generalized score statistic and likelihood ratios were compared using a regression model approach [10].

The cutoff for high risk using the F5 rs6025 +F2 rs1799963 score was 0.5 (which is equivalent to define as high risk individuals with the presence of any risk allele), and for the TiC-RPL score, this threshold was the point on the ROC curve that corresponded to balanced sensitivity/specificity of approximately 70. The cutoff for relevant thrombophilia that could be responsible for RPL was established as the presence of any thrombophilia for which the risk was similar or higher to that for F5-rs6025. Cross validated AUC was also computed to correct for any over optimism bias, because all samples were used to fit the regression model for the TiC-RPL score. For this purpose, we used a leave-one-out cross validation (LOOCV) approach. Briefly, 1 sample was eliminated, and a new regression model was fitted with the remaining samples to estimate their coefficients. The predicted risk for the omitted sample was then computed, based on the new model. This step was repeated until every sample was left out once. The newly generated risk values were used to calculate the corrected AUC. All calculations were performed using R, version 3.1.3 (R Development Core Team, 2015).

3. Results

Among clinical variables, age differed ($p<0.001$) between the healthy control and RPL groups (median 31 vs 35 years, respectively). In the 184 subjects with RPL, 407 pregnancy losses were registered, 401 (98.52%) of which occurred before week 20 of pregnancy.Among genetic variables, F12 rs1801020 ($p=0.019$), F13 rs5985 ($p=0.062$), F2 rs1799963 ($p=0.037$), and AB0 haplotype ($p=0.030$) were individually associated with RPL (Table I).

Table I: prevalence of genetic variants in patient with recurrent miscarriage

	Control (n=180)	Recurrent miscarriage (n=184)	P value*	P value**
F12-rs1801020			0.202	0.019
0***	121 (67.2)	107 (58.2)		
1	48 (26.7)	63 (34.2)		
2	11 (6.1)	14 (7.6)		
Serpin A10-rs2232698			0.751	0.533
0***	176 (97.8)	178 (96.7)		
1	4 (2.2)	6 (3.3)		
Serpin C1-rs121909548			0.244	0.981
0***	178 (98.9)	184 (100)		
1	2 (1.1)	0 (0)		
F5-6025			0.565	0.633
0***	176 (97.8)	177 (96.2)		
1	4 (2.2)	7 (3.8)		
F5-rs11820390				
0***	180 (100)	184 (100)		
F5-rs118203905				
0***	180 (100)	184 (100)		
F13-rs5985			0.394	0.062
0***	109 (60.5)	99 (53.8)		
1	61 (33.9)	71 (38.6)		
2	10 (5.6)	14 (7.6)		
F2-rs1799963			0.006	0.037
0***	178 (98.9)	170 (92.4)		
1	2 (1.1)	14 (7.6)		
AB0			0.163	0.03
0***	106 (58.9)	126 (68.5)		
1	62 (34.4)	49 (26.6)		
2	12 (6.7)	9 (4.9)		

* p value for standard chi-square test
** p value adjusted for the confounding effect of age
*** 0-2, number of minor alleles
Data are expressed as n (%)

3.1. Development of the TiC-RPL risk model

Table II shows the genetic and clinical variables that were included in the TiC-RPL risk score. F5 rs6025 was incorporated, based on a meta-analysis[11,12,13]. The weights that were assigned to each variable were defined from a meta-analysis for F5 rs6025 and F2 rs1799963 [13] and by the multivariate logistic regression for the rest of the variables.

Table II: association (odd ratio) between age and genetic variants with recurrent miscarriage

Variable	OR (95% CI)	P value
Age	1.24 (1.17-1.32)	<0.0001
F12	1.67 (1.14-2.47)	0.0097
F13	1.49 (1.02-2.20)	0.0401
AB0	1.89 (1.17-3.09)	0.0103
F2	2.32*	
F5	2.01*	

F12, F12-rs1801020; F13, F13-rs5985; F2, F2-rs1799963; A1, AB0-rs8176719-rs7

rs8176743-rs8176750; F5, F5-rs6025

* OR extracted from meta-analysis

Data are expressed as OR (95% CI)

For the TiC-RPL score to identify patients who are at risk, a cutoff that yielded a balanced sensitivity of 70.65% and specificity of 67.78% was selected. By comparison, for the F5 rs6025 + F2 rs1799963 to identify such patients at risk, the presence of any risk allele in F5-rs6025 and F2-rs1799963 was selected as cutoff that yielded a sensitivity of 11.4% and specificity of 96.7%.

3.2. Accuracy and validation of the risk model

The TiC-RPL score had an area under the ROC curve of 0.763 (0.715-0.811), a sensitivity of 70.65%, and a specificity of 67.78%. It had a PPV of 69.15%, an NPV of 69.32%, a PLR of 2.19, an NLR of 0.43 (Table III), and a cross validated AUC value of 0.742 (0.682-0.784). The F5 rs6025 + F2 rs1799963 score did not distinguish between patients who did and did not experience an RPL as well (0.763 vs 0.540; p<0.0001, Fig. I).

Table III: TiC panel performance metrics and comparison with standard F5+F2 panel in patients with recurrent miscarriage

Variable	TiC	F5+F2	*P* value
AUC (95% CI) (*p*-value)	0.763 (0.715-0.811) (<0.0001)	0.540 (0.514-0.567) (0.003)	<0.0001
Sensitivity	70.65%	11.4%	<0.0001
Specificity	67.78%	96.7%	<0.0001
PPV	69.15%	77.8%	0. 2772
NPV	69.32%	51.6%	<0.0001
PLR	2.19	3.42	0.3218
NLR	0.43	0.92	<0.0001
Calibration (p)	0.616	>0.999	
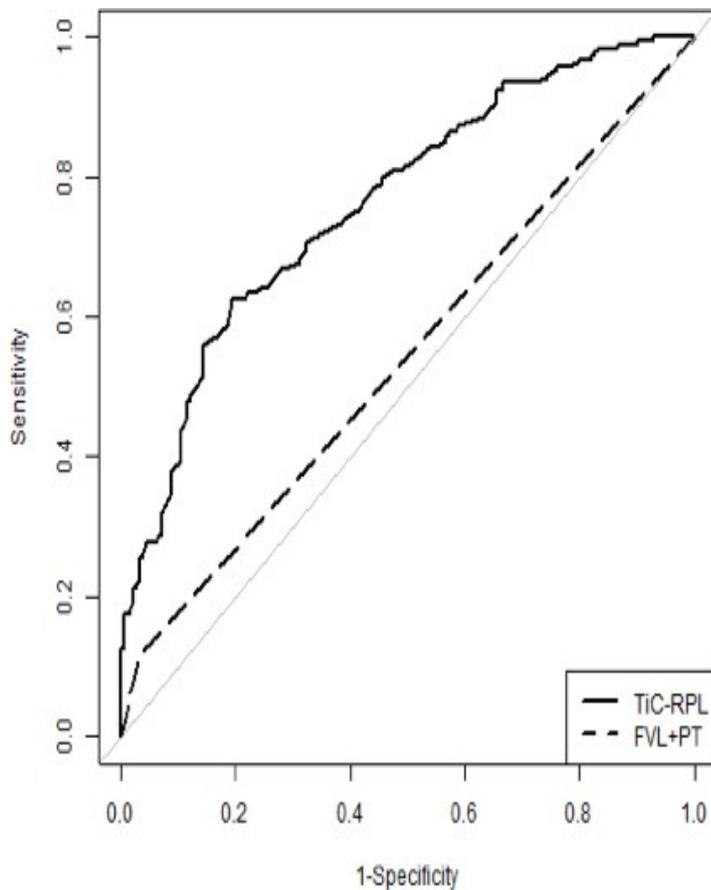
Figure I: Area under the ROC curves: F5+F2 and TiC-RPL

The sensitivity of the TiC-RPL score was significantly higher than that of the F5 rs6025 +F2 rs1799963 (70.65% vs. 11.4%; p<0.0001), whereas its specificity was lower (67.78% vs. 96.7%; p<0.0001). The NPV of the TiC-RPL score exceeded that of the F5 rs6025 + F2 rs1799963 score (69.32% vs. 51.63%; p<0.0001), but its PPV scores were similar (69.15% vs. 77.8;% p=0.2772). The NLR of the TiC-RPL score was also significantly better versus the F5 rs6025 + F2 rs1799963, but their PLRs were similar (Table III).

The proportion of RPL patients who were classified as high- or low-risk according to the F5 rs6025 + F2 rs1799963 or TiC-RPL score was also compared. Most patients who suffered an RPL (88.59%) were identified by the F5 rs6025 +F2 rs1799963 score as low-risk. Notably, among these patients, 68.1% was reclassified as high risk by the TiC-RPL score. TiC-RPL considered 70.65%t of patients who suffered an RPL to be at high risk of developing RPL.

AUC, area under the curve (measure of discrimination capability); PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; F5+F2, (F5-rs6025+F2-rs1799963)As the algorithm in TiC-RPL is formed by two elements (age and the thrombophilia genetic variants) some patients could be identified as high risk for RPL by a combination of relative high age together with a relative low number of thrombophilia genetic variants. In that particular case and depending of other clinical circunstances, the use of thromboprophylaxis could not be adequate given the relative low number of thrombophilia genetic variants. To solve this issue and therefore to identify patients with a level of thrombophilia who could be considered candidates for thromboprophylaxis among subjects who were at high risk of RPL according to TiC-RPL score, we established a more conservative approach using a second criterion: the presence of thrombophilia (as a single genetic variant or a combination of them, based on the proposed multivariate model) to a similar or greater degree than that of a single thrombophilia variant in F5-rs6025 (OR 2.01, the level of association that is used by most guidelines as the degree of thrombophilia that requires intervention). According to this approach, TiC-RPL identified 130 (70.65%) of the 184 RPL women as being at high risk for RPL. Applying the second criterion of the 130 women who were at high risk for RPL, 91 (70%) were considered to be patients in whom thrombophilia was relevant and for whom thromboprophylaxis could be suggested.

4. Discussion

The TiC-RPL score that has been developed in this study identifies women in whom RPL is associated with significant thrombophilia. This identification can guide personalized approached to prevent the development of RPL.

By multivariate analysis, we established a model with 8 genetic variants and age to define the algorithm for the TiC-RPL, which initially allowed the patients to be classified as being at high or low risk of RPL. Among patients in the TiC-RPL-based high-risk group, 69.15% eventually suffered an RPL, whereas 30.68% of the low-risk group did so (Table III). By comparison, 77.78% of high-risk patients according to F5 rs6025 + F2 rs1799963 score

experienced an RPL. Similarly, 48.36% of patients in the low-risk group, based on F5 rs6025 +F2 rs1799963 score, did so. Nevertheless, we must consider that TiC-RPL detects more women with RPL as being high-risk (130 vs 21 who were identified by F5+F2); yet, TiC-RPL classifies fewer women with RPL as low-risk (54 vs 163 as classified by F5 rs6025 + F2 rs1799963).

The contribution of thrombophilia to pregnancy loss and other adverse outcomes in pregnancy remains debated [14]. Thus, the guidelines of the American College of Chest Physicians recommend against screening for inherited thrombophilia in women with a history of pregnancy complications [15]. These conflicting results on thrombophilia are most likely attributed to the use of a single-marker marginal analysis approach using F5 rs6025 alone or in combination with F2 rs1799963. This standard approach might suffer from low power and poor reproducibility. One useful strategy for solving these problems is marker set analysis, in which a set of genetic markers is assembled, based on previous knowledge. We have obtained good results using this approach in the current work and in previous research on VTE [9].

As a result, we have developed a new algorithm from clinical and genetic markers. That the variables that we have included in our algorithm have also been individually associated with RPL by other authors validates our strategy and algorithm. In our study, age was significantly associated with RPL—women with RPL were older than those with noncomplicated pregnancies (35 versus 31 years, $p<0.001$); this association has been observed previously. George and his colleagues reported that the risk of repeated miscarriage higher for women aged 35 or more years (adjusted OR 2.9) [16]. In another study of more than 600,000 women, a similar conclusion was reached, wherein the risk of repeated miscarriage rose significantly from age 35 years [17].

Because our study focused on idiopathic RPL, we excluded patients with certain clinical factors, such as obesity, that have been linked to RPL and that clinicians should consider in evaluating patients with RPL [18].

The genetic variants in our algorithm have been also individually linked to RPL by other authors. The association of F2 rs1799963 and F5 rs6025 with RPL has been studied extensively [8,10,11] although the clinical sensitivity has not been reported [19]; in our case, the clinical sensitivity was low. F13 rs5985(V34L) is associated with an elevated overall risk for RPL in women, likely due to low normal levels of fibrinogen—at such levels, F13 rs5985 might alter the structure of fibrin and increase its resistance to fibrinolysis [20,21]. The F12 rs1801020 variant is associated with low plasma levels of F12 and low F12 activity, which have been confirmed to be risk factors for RPL [22,23]. Most of the studies that have associated ABO groups with RPL have been based on ABO incompatibility between the mother and fetus. However, ABO groups could contribute to the presentation of repeated abortions by influencing von Willebrand factor and factor VIII levels [24]. Among women with early RPL, a high percentage has persistently elevated levels of factor VIII [25,26].

The exact mechanism by which inherited thrombophilia causes RPL is unknown. It has been suggested that inherited thrombophilia impairs placental function by causing arterial or venous thrombosis at the maternal-fetal interface. Also, thrombophilia has been proposed to effect syncytiotrophoblast invasion of the maternal blood vessels, leading to the formation of microthrombosis at the site of implantation and thus resulting in RPL [27].

There are limitations and strengths of our study. The main limitations are: a) the small number of subjects, b) the initial exclusion of F5 rs6025 from the algorithm and the weight of the rare genetic variants (F5 rs6025 and F2 rs1799963) could be a vias due also to the small number of subjects studies, c) lack of meaning for PPV and NPV in a case/control study, and d) lack of replication. However, we performed several steps to overcome those limitations: a) in the case of small number of subjects, subjects were recruited from 4 hospitals in 3 areas in Spain; b) to avoid this bias, we included F5 rs6025, based on the literature, and obtained the weights for F5 rs6025 and F2 rs1799963 from a meta-analysis of 3753 women [13]; c) with regard the lack of meaning of PPV and NPV in an artificially composed 50/50 group of cases and controls as those predictive values are vulnerable to disease prevalence [28]. However, the likelihood ratios which are stable in the face of shifting disease prevalence [28] are in line with the information provided by PPV and NPV. Moreover, the ability of a test has to be analyzed not only considering the predictive value but also its sensitivity, specificity and discriminative capacity, as we have done and showed that TiC-RPL is significantly better. Finally, d) it is true that the results should be replicated. However, we have used subjects from different hospitals and we have performed an internal validation.

The main strength of our study is the development of an algorithm through marker set analysis, in which a set of genetic markers is assembled. This combination generates better results than F5 rs6025 and F2 rs1799963, 2 low-frequency genetic variants. Another strength is the selection of the variables and the analysis that was performed to characterize the goodness of the 2 algorithms: TiC-RPL and F5 rs6025 +F2 rs179996329 [30]. Most studies limit this analysis to the association between the marker and RPL. F5 rs6025 might have a strong association with RPL (OR: 2.01)[13] with a good PPV (in our case, 77.78%, combined with F2 rs1799963), but these variants are uncommon in RPL women, limiting their clinical value (in our case, the sensitivity was 11.41%). The use of only 2 variants with low sensitivity, such as F5 rs6025 and F2 rs1799963, might explain the lack of reproducible results with these variants in identifying people at risk and selecting patients for thromboprophylaxis (the AUC for F5+F2 was also low, AUC=0.540).

We have developed an algorithm that identifies women who are at risk of developing RPL. This algorithm could be used after the first pregnancy loss to identify such women. It can also be applied to women with confirmed RPL to identify those who are at high risk of RPL in whom thromboprophylaxis might be indicated. A more conservative approach could be to recommend thromboprophylaxis only to women who are at high risk for RPL according to our algorithm and in whom the presence of thrombophilia (as a single genetic variant or a combination of them, according to the proposed multivariate model) is similar or greater than that of a single thrombophilia variant in F5-rs6025 (OR 2.01)—the threshold that is used by most guidelines as the level of thrombophilia that requires intervention. Applying this criterion, of 130 high-risk women in the RPL group, 91 (70 per cent) could be considered patients in whom thrombophilia is relevant and thromboprophylaxis can be suggested. Using this criterion in an ongoing pilot study, of 80 women who were at high risk for RPL, 37 had thrombophilia (as a single genetic variant or a combination, according to the proposed multivariate model) to a similar or greater degree than that of a single thrombophilia variant in F5-rs6025. All 37 were treated with prophylactic doses of LMWH, and 33 of them (89.2 per cent) experienced a pregnancy to term (personal communication by Dr. Agius). These preliminary results should be confirmed.

5. Recomendations

The use of clinic-genetic risk scores, such as ours, might be useful in solving the contradictory results regarding inherited thrombophilia in RPL. Patients who are identified as being at high risk by the TiC-RPL risk score and with significant thrombophilia are likely to benefit from thromboprophylaxis. A highly sensitive predictive tool, such as the TiC-RPL score, should be available to prevent the infertility that is associated with thrombophilia, considering the low risk of the thromboprophylaxis measures.

6. Conclusion

This paper reports a clinical-genetic risk score that is significantly better than F5 rs6025 + F2 rs1799963, as demonstrated by its greater AUC value, sensitivity, negative likelihood ratios, and sensitivity (70.7) in identifying RPL women. The recommendation of thromboprophylaxis might be appropriate for those with significant thrombophilia—similar to or stronger than F5. A replication of the results obtained in this work with a higher number of subjects is needed.

Acknowledgements

References

[1]. Martinelli I, Bucciarelli P, Mannucci PM. (2010) Thrombotic risk factors: basic pathophysiology. Crit Care Med 38(2 Suppl):S3-9.

[2]. Mannucci PM, Franchini M. (2014) The real value of thrombophilia markers in identifying patients at high risk of venous thromboembolism. Expert Rev Hematol 7(6):757-765.

[3]. Cao Y, Zhang Z, Xu J, et al. (2013) The association of idiopathic recurrent pregnancy loss with polymorphisms in hemostasis- related genes. Gene 530(2):248-252.

[4]. Ziakas PD, Poulou LS, Pavlou M, Zintzaras E. (2015) Thrombophilia and venous thromboembolism in pregnancy: A meta- analysis of genetic risk. Eur J Obstet Gynecol Reprod Biol 191:106-111.

[5]. Rai R, Regan L. (2006) Recurrent miscarriage. Lancet 368:601-611.

[6]. Cohn DM, Goddijn M, Middeldorp S, Korevaar JC, Dawood F, Farquharson RG. (2010) Recurrent miscarriage and antiphospho- lipid antibodies: Prognosis of subsequent pregnancy. J Thromb Haemost 8(10):2208-2213.

[7]. Middeldorp S. (2014) Anticoagulation in pregnancy complications. Hematology (1):393-399.

[8]. Simcox LE, Ormesher L, Tower C, Greer IA. (2015) Thrombophilia and Pregnancy Complications. Int J Mol Sci 16(12):28418-28

[9]. Soria JM, Morange P-E, Vila J, et al. (2014) Multilocus genetic risk scores for venous thromboembolism risk assessment. J Am Heart Assoc 3(5):e001060.doi:10.1161/JAHA.114.001060.

[10]. Gu W, Pepe MS. (2009) Estimating the capacity for improvement in risk prediction with a marker. Biostatistics. 10(1):172- 186.

[11]. Skeith L, Carrier M, Kaaja R, et al. (2016) A meta-analysis of low-molecular-weight heparin to prevent pregnancy loss in women with inherited thrombophilia. Blood 127(13):1650-1655.

[12]. Sergi C, Al Jishi T, Walker M. (2014) Factor V Leiden mutation in women with early recurrent pregnancy loss: a meta-analysis and systematic review of the causal association. Arch Gynecol Obstet 291(3):671-679.

[13]. Rey E, Kahn SR, David M, Shrier I. (2003) Thrombophilic disorders and fetal loss: a meta-analysis. Lancet (London, England) 361(9361):901-908.

[14]. Battinelli EM, Marshall A, Connors JM. (2013) The Role of Thrombophilia in Pregnancy.Thrombosis 516420. doi:10.1155/2013/516420.

[15]. Bates SM, Greer I a, Middeldorp S, Veenstra DL, Prabulos A-M, Vandvik PO. (2012) VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 141(2 Suppl):e691S-736S.

[16]. George L, Granath F, Johansson ALV, Olander B, Cnattingius S. (2006) Risks of repeated miscarriage. Paediatr Perinat Epidemiol 20(2):119-126.

[17]. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. (2000) Maternal age and fetal loss: population based register linkage study. BMJ 320(7251):1708-1712.

[18]. Smith ML, Schust D. (2011) Endocrinology and Recurrent Early Pregnancy Loss. Semin Reprod Med 29(6):482-490.

[19]. Bradley LA, Palomaki GE, Bienstock J, Varga E, Scott JA. (2012) Can Factor V Leiden and prothrombin G20210A testing in women with recurrent pregnancy loss result in improved pregnancy outcomes?: Results from a targeted evidence-based review. Genet Med Genet Med 1414(11):39-50.

[20]. Lim BC, Ariens RA, Carter AM, Weisel JW, Grant PJ. (2003) Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. Lancet 361(9367):1424-1431.

[21]. Dossenbach-Glaninger A, van Trotsenburg M, Oberkanins C, Atamaniuk J. (2013) Risk for Early Pregnancy Loss by Factor XIII Val34Leu: The Impact of Fibrinogen Concentration. J Clin Lab Anal 27(6):444-449.

[22]. Ogasawara MS, Aoki K, Katano K, Ozaki Y, Suzumori K. (2001) Factor XII but not protein C, protein S, antithrombin III, or factor XIII is a predictor of recurrent miscarriage. Fertil Steril 75(5):916-919.

[23]. Wells PS, Anderson JL, Scarvelis DK, Doucette SP, Gagnon F. (2006) Factor XIII Val34Leu variant is protective against venous thromboembolism: A HuGE review and meta-analysis. Am J Epidemiol 164(2):101-109.

[24]. Song J, Chen F, Campos M, et al. (2015) Quantitative Influence of ABO Blood Groups on Factor VIII and Its Ratio to von Willebrand Factor, Novel Observations from an ARIC Study of 11,673 Subjects. Miyata T, ed. PLoS One 10(8):e0132626. doi:10.1371/journal.pone.0132626.

[25]. Dossenbach-Glaninger A, van Trotsenburg M, Krugluger W, et al. (2004) Elevated coagulation factor VIII and the risk for recurrent early pregnancy loss. Thromb Haemost 91(4):694-699.

[26]. Marietta M, Facchinetti F, Sgarbi L, et al. (2003) Elevated plasma levels of factor VIII in women with early recurrent miscarriage. J Thromb Haemost 1(12):2536-2539.

[27]. Abu-Heija A. (2014) Thrombophilia and Recurrent Pregnancy Loss: Is heparin still the drug of choice? Sultan Qaboos Univ Med J 14(1):e26-36.

[28]. Gallagher EJ. (1998) Clinical Utility of Likelihood Ratios. Ann Emerg Med March 31:391-397

[29]. Greenland P. (2008) Comments on "Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond" by M.J. Pencina, R.B.D' Agostino Sr, R.B. D' Agostino Jr, R.S. Vasan, Statistics in Medicine (DOI:10.1002/sim.2929). Stat Med 27(2):188-190. doi:10.1002/sim.2976.

[30]. Attia J. (2003) Moving beyond sensitivity and specificity: using likelihood ratios. Aust Prescr 26:111-113.