Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

D-Dimer Assay May Guide LMWH Treatment in Repeated Biochemical Pregnancy Losses in Women with Positive Antiphospholipid Antibody

Shi H. Bao, Sophia T. Frempong, Jia L. Ruan

Department of Reproductive Immunology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China

SUMMARY

Background: **Do D-dimer levels influence the pregnancy outcomes after treatment with low molecular weight heparin (LMWH) in women of recurrent miscarriage (RM), repeated biochemical pregnancy losses (BPL), and a positive test for antiphospholipid antibodies (aPLs)?**

Methods: **This study was a retrospective chart review of 569 RM patients who were identified as having a history of BPL and a positive aPL. These patients were grouped into three groups according to their treatment plan including those who received low dose aspirin (LDA) alone (group A), LDA plus LMWH after ovulation therapy (group B), and LDA plus LMWH after pregnancy confirmation (group C). We hypothesized that the administration of LMWH after ovulation increased the rates of live birth. D-dimer may predict the pregnancy outcome after treatment.**

Results: **The live birth rate of group B and group C is significantly higher than group A (86.96% and 66.80% vs. 52.89%, $p < 0.0001$, respectively). The live birth rate in group A, B, and C with elevated D-dimer is 36.92%, 90.52%, and 61.60% respectively. However, there is no significant difference in live birth rate among those who had normal baseline D-dimer.**

Conclusions: **These results suggest that LMWH therapy is more effective in improving the live birth rate when given after ovulation than after pregnancy confirmation. The plasma D-dimer assay can possibly guide LMWH treatment appropriately.**

(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190637)

Correspondence:
Shi Hua Bao, MD, PhD
Department of Reproductive Immunology
Shanghai First Maternity and Infant Hospital
Tongji University School of Medicine
West Gaoke Road No. 2699
201204 Shanghai
China
Phone: +86 21-20261439
Fax: +86 21-20161222
Email: baoshihua@tongji.edu.cn

Manuscript accepted August 5, 2019

KEY WORDS

biochemical pregnancy, recurrent miscarriage, antiphospholipid antibody, low molecular weight heparin, D-dimer

INTRODUCTION

Recurrent miscarriage (RM), the loss of two or more consecutive pregnancies, affects 1 - 5% of couples trying to conceive, and it is associated with genetic, anatomical, endocrine, immune, infective, thrombophilic, and unexplained etiologies [1]. Non-visualized pregnancy losses are increasingly diagnosed in women with early pregnancy failure. This is attributed to the availability of early pregnancy tests to timely detect biochemical pregnancy (BP) [2]. The International Com-

mittee for Monitoring Assisted Reproductive Technology (ICMART) and World Health Organization (WHO) defines BP as a condition in which human chorionic gonadotropin (hCG) is detected in maternal urine or blood, but clinical pregnancy is not yet achieved [3]. Therefore, a so-called clinical pregnancy is characterized by the confirmation of a visible gestational sac by an ultrasound examination. Studies report that 5 - 42% of women attending an ultrasound assessment in early pregnancy will be classified as having a BP [4-7]. In the infertile population, the average biochemical pregnancy rate was 13.8%. This was composed of a biochemical pregnancy rate of 13.8% in fresh IVF cycles and of 14.4% in frozen embryo transfer (FET) cycles [8]. Any woman having a history of BP needs follow-up to determine the final clinical outcome, which could be an ongoing viable intrauterine pregnancy, a biochemical pregnancy loss (BPL), an ectopic pregnancy (EP) or rarely a persisting BPL. Early intervention is not given since RM women with repeated BP are mostly ignored by gynecologists. However, Kirk et al. support the assumption that the majority of BPL are early intrauterine miscarriages [9], only 6 - 20% of BP are subsequently diagnosed with EP [10].

Antiphospholipid syndrome (APS) is an autoimmune disease that is characterized by the presence of antiphospholipid antibodies (aPLs) which result in thrombosis and morbidity during pregnancy. Clinically, aCLs, lupus anticoagulant (LAC) and anti-β2-glycoprotein I antibodies (aβ2-GPI) are considered to be the major aPLs found in APS [11,12]. The international Sapporo criteria, revised in 2006, emphasizes the diagnosis of APS when at least one of the major aPLs is detected on two or more occasions in a 12-week interval between measurements plus any association with a clinical condition, such as thrombosis or pregnancy complications. Pregnancy complications include unexplained stillbirths at $\geq$ 10 weeks of gestation, preterm delivery due to eclampsia, preeclampsia, or placental insufficiency, and two or more consecutive miscarriages [11]. APS is associated with RM [12] and defined by the American Society for Reproductive Medicine as two or more spontaneous pregnancy losses [1]. The incidence of aPLs in RM patients is between 15% and 20% [12]. It has been found that administration of low dose aspirin (LDA) and/or low molecular weight heparin (LMWH) in APS women can improve pregnancy and live birth rate [13-15]; however, whether these strategies could be beneficial to women with antiphospholipid antibodies who did not meet the laboratory standard for diagnosing APS due to a single positive antiphospholipid antibody result rather than persistent positivity is unknown [11]. There was no consensus on a treatment recommendation for recurrent pregnancy loss before 10 weeks of pregnancy when LDA alone or LDA plus LMWH were given. D-dimer is a reliable and sensitive index of fibrin deposition and stabilization. It can basically be used as an important marker for recurrent thromboembolism [16], and women with persistently negative D-dimer levels after cessation of anticoagulant therapy have low recurrence risk and can, therefore, stop anticoagulation therapy [16,17]. We assumed that D-dimer is a predictable factor for treatment outcome [18].

In order to evaluate whether different interventions can improve clinical pregnancy outcome in these women with the history of repeated BPL, positive antiphospholipid antibodies, and recurrent miscarriage, and whether the D-dimer test could be used as a marker to predict treatment outcomes and guide clinical decisions, we collected the data on pregnancy outcomes (including the rate of live birth, miscarriage, BPL, and EP) and D-dimer value. We compared the efficacy of LDA plus LMWH (LMWH given after ovulation or after conceptiion confirmation) versus sole administration of LDA. We also assessed the relationship between plasma D-dimer levels and the main outcomes.

MATERIALS AND METHODS

Study subjects

This study was approved by the institutional review board of Shanghai First Maternity and Infant Hospital. The database of all the consecutive patients who attended the Reproductive Immunology clinic of Shanghai First Maternity and Infant Hospital, between January 1st, 2012, and December 31st, 2015, were reviewed for inclusion in this study. All participant data were obtained with informed patient consent.

A flow diagram of the patient-selection process is shown in Figure 1. RM patients who also had a history of BPL and positive aPLs were selected for inclusion in this study. A diagnosis of recurrent miscarriage was based on two or more consecutive miscarriages before 12 weeks of gestation, confirmed by ultrasound. Repeated biochemical pregnancy loss was confirmed either by 3 or more positive HCG urine test results or a positive HCG blood test value but the pregnancy failed to progress to ultrasound confirmation. A comprehensive screening test to investigate the possible cause of the disease was performed. Conditions such as chromosomal abnormalities, endocrine, anatomical, thrombophilia or autoimmune diseases have been found to be associated with recurrent spontaneous miscarriage and repeated biochemical pregnancy losses. However, such patients having positive antiphospholipid antibodies were the target for our research study, which successfully included a total of 569 patients.

Therapeutic measures for anticoagulant treatment were recorded. This included administration of LDA, LMWH combination therapy, and time of therapeutic anti-coagulation achievement. According to the intended treatment scheme, we stratified the patients into three groups. Group A received an optimum daily dose of 75 mg LDA (aspirin, 75 mg/tablet; Shanghai Sine Pharmaceutical Co., Ltd, Pudong, China) after menstruation. Women classified as group B received 4,100 U of LMWH (Nadroparin injection, 4,100 IU/0.4 mL;

GlaxoSmithKline, Middlesex, UK) subcutaneously after ovulation and 75 mg LDA after menstruation. The women in Group C had a similar dose as those in group B but LMWH was administered as soon as conception is confirmed by blood or urine β-hCG test. The treatment course was demonstrated in Figure 2. Apart from anticoagulant therapy, no other treatment was given. All participants who received anticoagulant therapy provided written informed consent. The main outcomes observed after treatment were live birth, miscarriage, EP or BPL.

Laboratory tests

IgG and IgM isotypes of aCL and anti-β_2GPI antibodies were measured by a commercial enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Luebeck, Germany). The current classification criteria define clinically significant titers of aCL and anti-β_2GPI antibodies as > 40 GPL (IgG phospholipid) units and > 40 MPL (IgM phospholipid) units [12]. Lupus anticoagulant (LAC) activity was measured by prolongation of the dilute Russell's viper venom time ratio greater than 1.09 with at least 20% correction by washed, frozen/thawed platelets, consistent with published guidelines [11]. Subjects positive for aPL were classified as triple-positive (LAC^+, aCL^+, anti-β_2GPI^+, same isotype), double-positive (LAC^-, aCL^+, anti-β_2GPI^+, same isotype), and single-positive (LAC or aCL or anti-β_2GPI antibodies as the sole positive test). Other combinations (LAC^+, aCL^+, anti-β_2GPI^-, or LAC^+, aCL^-, anti-β_2GPI^+) were not found.

Plasma D-dimer was routinely assessed before pregnancy. The plasma D-dimer levels in human citrated serum were quantified using an automated latex-enhanced immunoassay with a HemoSIL D-dimer kit (Instrumentation Laboratory Company, Bedford, MA, USA), in accordance with the manufacturer's protocol in the Department of Laboratory Medicine of our hospital. This assay is a quantitative, fast, whole-blood method that was previously shown to perform well in the pregnant population [19]. The results are given in mg/L FEU (fibrinogen equivalent units). A value of 0.50 mg/L FEU was chosen as the decision threshold for exclusion of venous thromboembolism [20].

Statistical analysis

A detailed statistical analysis was performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). The primary outcomes of this study, live birth, miscarriage, EP, and BPL, were compared between treatment groups using Chi-square or Fisher's exact test, as required. We then applied univariate logistic regression analyses of age, BMI, the number of miscarriages, the number of previous BP, positive antiphospholipid antibodies, and treatment strategy and pregnancy outcomes. Multivariable logistic regression models were fitted while adjusting potential confounders. Adjusted OR and 95% CIs were obtained from these models. The pregnancy outcomes were therefore converted into two categorical variables (live birth and pregnancy failure) when univariate analysis and multivariate regression models were computed. The pregnancy failure, as a categorical variable, was merged with miscarriage, EP, and BPL. All p-values were two-sided and a probability of < 0.05 was considered to be statistically significant.

RESULTS

Patient characteristics

A total of 765 RM women with the history of repeated BPL, and positive aPLs (obtained from a single antiphospholipid antibody result) were eligible for the study according to the inclusion and exclusion criteria. However, 196 women were excluded for reasons such as lost information, inability to conceive after referral, abnormal fetus karyotype causing miscarriage, failure to observe treatment protocol during the study, loss of D-dimer values, and specific allergies and other external reasons. A total of 569 participants aged 25 to 40 during treatment were available for analysis. In group A, 121 patients (21.27%) were treated with LDA alone after menstruation. In group B, 207 patients (36.38%) were intentionally treated with LMWH after ovulation and LDA after menstruation, whereas, in group C, 241 patients (42.35%) received their LWMH therapy immediately after pregnancy confirmation and LDA after menstruation. The baseline characteristic of women is presented in Table 1. According to our findings, there were no significant differences in the clinical characteristics such as; maternal age, BMI, numbers of previous miscarriages, BPL, previous live birth, and the level of baseline D-dimer among the three groups. There were no differences found in positive aPLs (aCL, and/or anti-β_2-GPI, and/or LAC) among the groups.

Pregnancy outcome stratified by treatment

In this study, the overall live birth rate was 71.18% (405/569). As summarized in Table 1, the live birth rate of both group B and group C were significantly higher than group A (86.96% and 66.80% vs. 52.89%, $p < 0.0001$). The miscarriage rate and biochemical pregnancy rate of both group B and group C after treatment were significantly lower than group A (2.42% and 9.96% vs. 15.7%, $p < 0.0001$; 6.28% and 15.77% vs. 23.14%, $p < 0.0001$). There were no significant differences among the three groups in terms of the incidence of ectopic pregnancy.

Pregnancy outcome stratified by the pre-pregnancy D-dimer level

To determine whether the D-dimer level could predict the pregnancy outcome, we compared live birth, miscarriage, EP, and BPL of the three groups to their baseline D-dimer levels. As previously defined, a baseline D-dimer level higher than 0.50 mg/L FEU is classified as positive, on the other hand, < 0.50 mg/L FEU is negative. As seen in Table 2, there were 65 (53.72%), 116

Table 1. Baseline patient characteristics and primary results for three groups.

Variable	Group A (n =121)	Group B (n = 207)	Group C (n = 241)	p-value
n	121 (21.27%)	207 (36.38%)	241 (42.35%)	
Median age (mean ± SD), years	31.62 ± 4.99	30.52 ± 4.49	31.30 ± 5.01	0.08
BMI (mean ± SD), kg/m^2	21.75 ± 3.06	21.53 ± 2.87	21.54 ± 2.85	0.76
Median number of previous miscarriages (mean ± SD)	5.10 ± 1.76	5.34 ± 1.85	5.24 ± 1.73	0.33
Number of previous BPL (mean ± SD)	3.07 ± 1.38	3.19 ± 1.30	3.41 ± 1.48	0.06
Previous live births only	16 (13.22%)	20 (9.66%)	26 (10.79%)	0.61
Number of patients positive for:				
aCL positive only	61 (50.41%)	112 (54.10%)	123 (51.04%)	0.91
anti-β_2-GPI positive only	31 (25.62%)	59 (28.50%)	71 (29.46%)	0.64
LAC positive only	15 (12.40%)	18 (8.70%)	20 (8.30%)	0.31
Double - positive	8 (6.61%)	12 (5.80%)	14 (5.80%)	0.95
Triple - positive	6 (4.96%)	6 (2.90%)	13 (5.40%)	0.41
Baseline D-dimer (mg/L FEU)	0.75 ± 1.60	0.66 ± 1.13	0.75 ± 1.20	0.74
Primary outcome after drug administration				
Live birth	64 (52.89%)	180 (86.96%)	161 (66.80%)	< 0.0001
Miscarriage	19 (15.70%)	5 (2.42%)	29 (9.96%)	< 0.0001
Ectopic pregnancy	10 (8.26%)	9 (4.35%)	21 (8.71%)	0.17
Biochemical pregnancy loss	28 (23.14%)	13 (6.28%)	30 (15.77%)	< 0.0001

Group A: LDA alone, Group B: LDA/LMWH (after ovulation), Group C: LDA/LMWH (after pregnancy confirmation).

Table 2. Pregnancy outcome stratified by baseline D-dimer level and treatment groups.

Variable/ D-dimer level	Group A (n = 121)			Group B (n = 207)			Group C (n = 241)			p-value
	Negative (n = 56)	Positive (n = 65)	p-value	Negative (n = 91)	Positive (n = 116)	p-value	Negative (n = 116)	Positive (n = 125)	p-value	
Live birth	40 (71.43%)	24 (36.92%)	0.0002	75 (82.42%)	105 (90.52%)	0.10	84 (72.41%)	77 (61.60%)	0.08	0.20 *, < 0.0001 $^\#$
Miscarriage	4 (7.14%)	15 (23.08%)	0.02	1 (1.10%)	4 (3.45%)	0.39	13 (11.21%)	16 (12.80%)	0.84	0.02 *, 0.0008 $^\#$
BPL	7 (12.50%)	21 (32.31%)	0.02	9 (9.89%)	4 (3.45%)	0.08	10 (8.62%)	20 (16.0%)	0.12	0.51 *, < 0.0001 $^\#$
Ectopic pregnancy	5 (8.93%)	5 (7.69%)	1.00	6 (6.59%)	3 (2.59%)	0.19	9 (7.76%)	12 (9.60%)	0.65	0.84 *, 0.10 $^\#$

Chi-square and Fisher's exact test were used as required. Group A: LDA alone, Group B: LDA/LMWH (after ovulation), Group C: LDA/ LMWH (after pregnancy confirmation). * - Compared the pregnancy outcomes of all three plasma D-dimer negative subgroups. $^\#$ - Compared the pregnancy outcomes of all three plasma D-dimer positive subgroups.

(56.04%), and 125 (51.87%) baseline D-dimer positive cases found in group A, group B, and group C, respectively. In group A, the live birthrate of D-dimer negative subgroup was higher than the positive subgroup (71.43% vs. 36.92%, p = 0.0002). The miscarriage rate (7.14% vs. 23.08%, p = 0.02) and biochemical pregnancy rate (12.50% vs. 32.31%, p = 0.02) of D-dimer negative subgroup were lower than D-dimer positive sub-

Table 3. Prognostic factors of anticoagulant therapy outcomes; results for univariate analysis.

Variable	OR (95% CI)	p-value
Median age (years)	1.11 (1.08 - 1.22)	0.03
BMI (kg/m^2)	1.06 (0.61 - 1.19)	0.17
Number of previous miscarriages	1.10 (0.77 - 1.24)	0.29
Number of previous BPL	1.71 (0.55 - 1.91)	0.07
Previous live birth absence vs. presence	0.38 (0.15 - 1.06)	0.36
Types of antiphospholipid antibodies positive		
aCL positive only	Reference	
anti-β_2-GPI positive only	1.33 (0.49 - 3.63)	0.58
LAC positive only	1.54 (0.57 - 4.15)	0.39
Double - positive	1.12 (0.22 - 5.71)	0.88
Triple - positive	1.18 (0.91 - 1.52)	0.22
Baseline D-dimer level (mg/L FEU)	1.17 (1.02 - 3.44)	0.01
Treatment strategy		
LDA alone	Reference	
LDA plus LMWH initiated as soon as pregnancy confirmed	0.69 (0.35 - 0.96)	0.001
LDA plus LMWH initiated after ovulation	0.33 (0.18 - 0.91)	0.001

Table 4. Significant prognostic factors of anticoagulant therapy outcomes after multivariate analysis.

Variable	Non-adjusted I [a] (OR 95% CI)	p-value	Adjust II [b] (OR 95% CI)	p-value
Baseline D-dimer level	1.04 (1.01 - 3.08)	0.01	1.06 (1.04 - 3.12)	0.01
Treatment strategy				
LDA alone	Reference		Reference	
LDA plus LMWH initiated as soon as pregnancy confirmed	0.62 (0.33 - 0.97)	0.001	0.51 (0.26 - 0.98)	0.001
LDA plus LMWH initiated after ovulation	0.35 (0.20 - 0.80)	0.003	0.32 (0.17 - 0.79)	0.001

[a] - Adjust I model adjusted for: Age, BMI. [b] - Adjust II model adjusted for: Age, BMI, Number of miscarriages, Number of previous BPL, Positive antiphospholipid antibodies.

group in group A. Apparently, there was no significant difference in the rate of EP between D-dimer negative and positive subgroups in group A. Interestingly, group B and C also had no significant difference in the pregnancy outcome between the D-dimer positive subgroup and the negative subgroup. Comparing the negative D-dimer level among the three groups, there were no significant differences in their pregnancy outcome except for the rate of miscarriage (7.14%, 1.10% and 11.21%, $p = 0.02$). For women with positive D-dimer, with the exception of EP which showed no significant differences among the three groups, the live birth rates of group B and group C were significantly higher than in group A (90.52% and 61.60% vs. 36.92%, $p < 0.0001$). Conversely, the rate of miscarriage and BPL were significantly lower in group B and group C than in group A (3.45% and 12.80% vs. 23.08%, $p = 0.0008$; 3.45% and 16.0% vs. 32.31%, $p < 0.0001$). This shows that the treatment combination of LDA and LMWH in women with positive baseline D-dimer is effective in improving the outcome of pregnancy. A thorough analysis of the result revealed LMWH therapy to be more effective in improving the live birth rate after ovulation than giving the therapy after pregnancy confirmation.

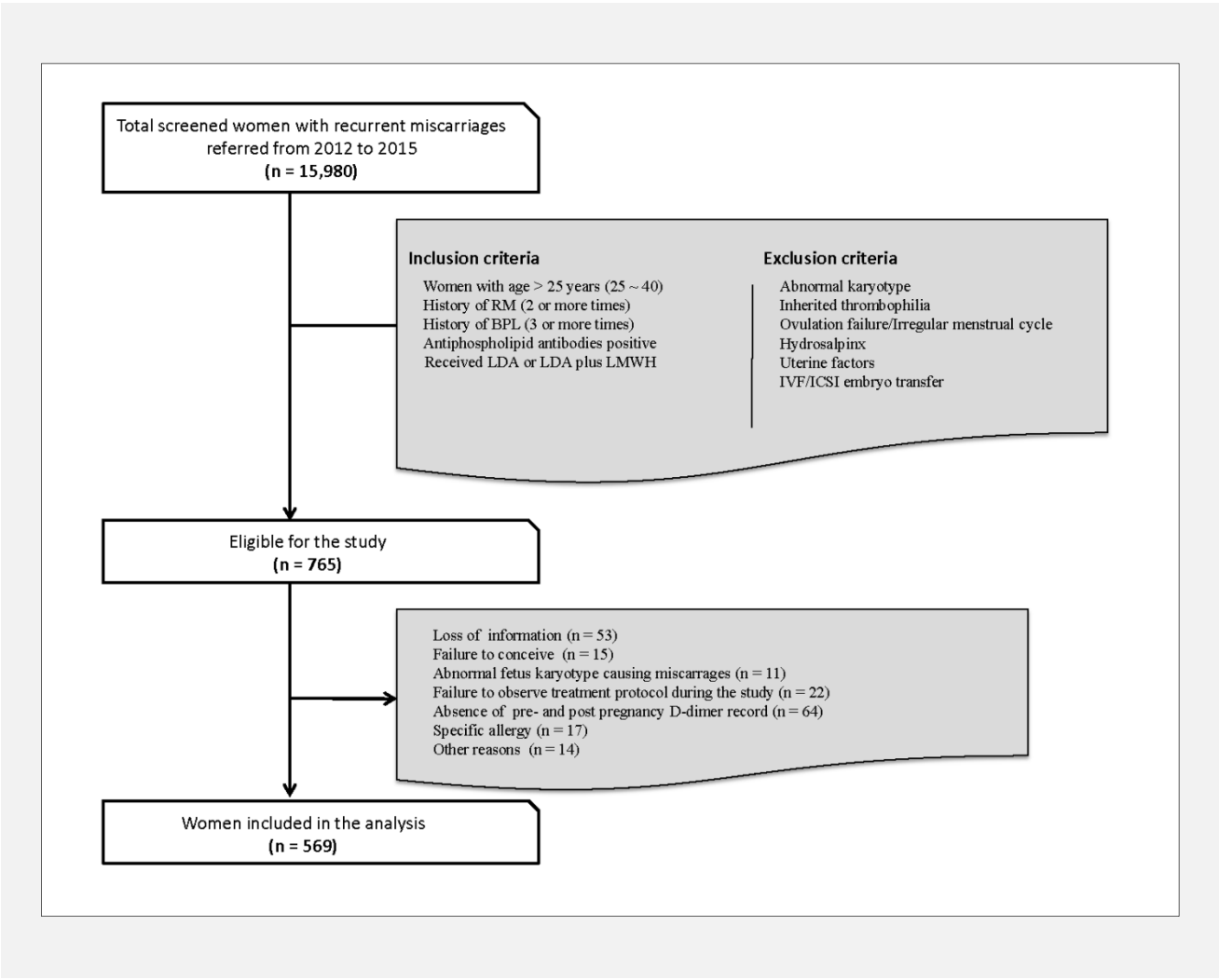

Figure 1. Flow chart of the study.

Univariate and multivariate analysis of predictors of pregnancy outcome

Univariate analysis comparing women who had a live birth and those who did not is presented in Table 3. Treatment strategies were closely associated with the live birth rate obtained. LDA plus LMWH therapy administered immediately after pregnancy was able to subsequently reduce the rate of pregnancy failure by 31% (OR 0.69, 95% CI 0.35 - 0.96; p = 0.001) compared to the administration of LDA alone. Meanwhile, LDA plus LMWH therapy when given after ovulation can reduce the failure rate by 67% (OR 0.33, 95% CI 0.18 - 0.91; p = 0.001). The age (OR 1.11, 95% CI 1.08 - 1.22; p = 0.03) and baseline D-dimer level (OR 1.17, 95% CI 1.02 - 3.44; p = 0.01) had a negative correlation with live birth rates. After adjustment for age and BMI in Adjust I model, and age, BMI, number of miscarriages, number of BPL and positive antiphospholipid antibodies in Adjust II model, a similar conclusion was obtained (Table 4). The baseline D-dimer level and treatment strategies (LDA alone vs. LDA plus LMWH, after pregnancy or ovulation) were independent factors associated with live birth rates.

DISCUSSION

Our data showed that the use of LDA plus LMWH could significantly increase the success rate of clinical pregnancy in RM women with BPL history and positive aPLs compared to using LDA alone. This supports the treatment combination of LDA and LMWH in aPLs positive or APS women as existing research evidence suggested [13-15,21,22]. To the best of our knowledge, it is the first time an evaluation was done on whether D-dimer test could be used as a marker to predict treatment outcomes and guide clinical decisions in women with coexisting conditions of RM, BPL, and positive

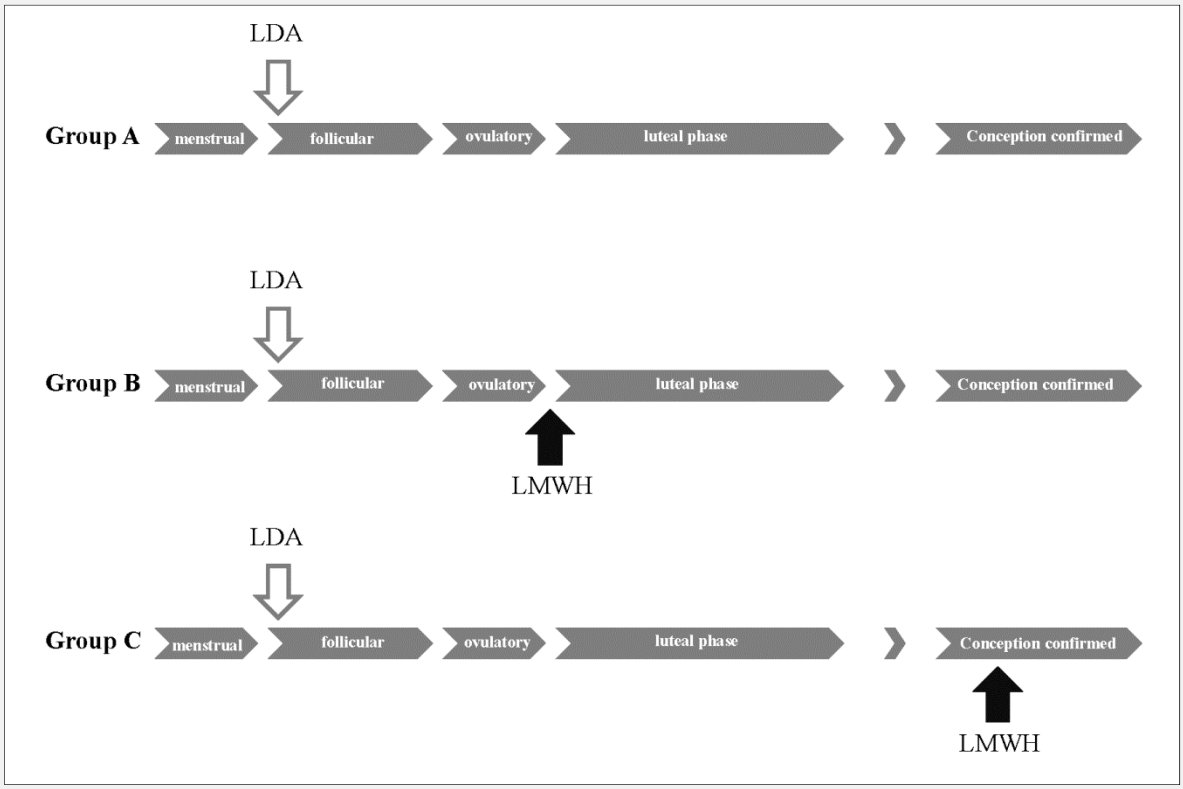

Figure 2. Treatment course of low dose aspirin (LDA) or LDA plus low-molecular-weight heparin (LMWH).

aPLs. We found that women who had an elevated basal D-dimer and were treated with LMWH plus LDA after ovulation seem to have a better outcome than those treated with LDA and/or LMWH after pregnancy. In clinical practice, LDA is used when antiphospholipid antibodies are sporadically positive and/or accompanied by increased platelet aggregation. LMWH is given in cases of sporadically positive anticardiolipin antibodies with an ongoing hypercoagulable process.

Based on our current understanding, it is particularly difficult to predict the risk of possible thrombosis in asymptomatic aPL carriers, except for the aPLs test. Moreover, there is no clear consensus on what constitutes a standardized laboratory test for thrombophilia status assessment in APS women [23]. It has been indicated that the changes of thrombophilia status may exist in APS women before pregnancy, and several recent studies have suggested that endogenous thrombin potential could be applied as a strategy to identify thrombotic complications in women with positive aPLs [24, 25]. D-dimer is widely used in the clinical field as a blood marker of hypercoagulable state but not endogenous fibrinolysis, and a positive result of D-dimer test could help diagnose women with arterial and/or venous thrombosis [26,27]. Persistent elevations of the D-dimer level or the presence of residual thrombosis provided further information to predict recurrence risk and help with treatment decisions [28]. We discovered that women with negative D-dimer values who were treated with LDA plus LMWH and those given LDA alone had a similar live birth rate; D-dimer positive women treated with LDA plus LMWH had an increased live birth rate and a negative D-dimer value during conception in some women. Di Nisio M et al. suggested that D-dimer plasma levels could become helpful in monitoring the LMWH effect in those women who had a reduction in D-dimer plasma levels. They may benefit the most from LMWH administration [29]. Stricker et al. [30] also demonstrated a significant fall in D-dimer values in women serially tested after commencing anticoagulation with unfractionated heparin (UFH) or LMWH for venous thromboembolism (VTE). Based on our previous study and various research studies conducted, it has been demonstrated that as gestation progresses D-dimer values increase; however, no significant differences were found during the first trimester [18]. In spontaneous pregnancies, BPLs are thought to be fairly common, involving as many as half of all pregnancies, but an accurate number is difficult to determine, this is because most women who experience a BPL never even realize they are pregnant until an attempt is made to conceive or undergo a series of early routine medical tests. Wom-

en treated with ART are routinely monitored for early detection of pregnancy by measuring serum β-hCG concentration. Also, women with the history of RM are often monitored closely and, therefore, the possibility of BPLs is less likely to be excluded from the diagnosis. Presently, the early discovery of BPLs in these women has already been established. Currently, no specific treatment is required for BP, the most important goal for a follow-up test is to ensure that the hCG levels have significantly declined to non-detectable levels in order to differentiate it from an EP.

However, it should be noted that BPL does not mean an EP. The prevalence of EPs among BPLs has been reported to be between 6 and 20% [9,31-34]. After the treatment therapy, we found that RM women with a history of BPL and positive aPLs who had a miscarriage, EP, or BPL were 9.31% (53/569), 7.03% (40/569), and 12.48% (71/569), respectively. Christiansen had reported that in women with a history of only BPLs, 27.1% of all pregnancies were EPs, whereas among RM women, the rate of EPs was only 3.6% [35]. Due to a lack of standardized protocols, the management of women with the history of BPL varies and may be stressful for women who are often subjected to repeated blood tests and scans before the final outcome of the pregnancy is known; and thus the subsequent occurrence of early intrauterine pregnancy losses could be due to treatment opportunity missed.

As far as BPL is concerned, its exact etiology remains unknown. Studies showed that factors such as age, chromosome anomalies, sperm DNA damage, oocyte and embryo quality, endometrial receptivity, endometrial pattern, and infertility factors may have something to do with the occurrence of BPL [36-42]. When considering the past, reproductive history of patient factors such as RM and the importance of BPL has not been well studied. The management of women with histories of BPLs should shift towards effective triage rather than identifying pregnancy location.

Although the etiologies above seem to be the most plausible reason, BPLs are associated with a high frequency of positive aPLs [43]; aPLs have been shown to have a direct effect on the implanting trophoblastic cells and the pre-implantation embryo, as well as to play a role in the pathophysiology of thrombosis [44]. Numerous trials have been performed to assess the efficacy of prophylactic anticoagulation in these cases, and also the LMWH treatment was supported by some studies in which a basal prothrombotic state outside of pregnancy was measured in women with previous RM and without known thrombophilia [45-47]. In this study, LDA and/or LMWH were given to women according to their doctor's prescription. No attempt was made to convince the women to use either one of these methods. We found that LMWH plus LDA could improve the clinical pregnancy outcome in women with a history of RM, BPL, and positive aPLs.

Actually, there are no comparative studies on the optimal timing of the start of anticoagulation in women with APS. In order to estimate whether improving endometrial blood coagulation after ovulation can enhance the clinical pregnancy rate, the LMWH was administered early enough in some women (i.e., after ovulation or after pregnancy). Our data showed that the best time for initiating anticoagulation treatment in repeated BPL women with RM was before pregnancy, especially for women with a high D-dimer level and positive aPLs. It is possible that to benefit fully, LMWH may require an adequate administration before the time of initial implantation.

CONCLUSION

There is no known medical literature to date describing the outcome of pregnancies in repeated BPL. Our data suggest that LMWH could subsequently improve pregnancy outcome in women with repeated BPL history. Starting with LMWH therapy after ovulation appears to be the best interventional treatment option. However, this is a single-center retrospective study, and clinical research needs larger numbers of samples, multi-center, randomized observation. It is suggested that more attention should be paid to BPL, especially in women with the history of RM. In the future, preliminary supportive prospective data could be useful to clinicians to further address BPL.

Financial Support:

This work was supported by the Research Grant from Shanghai Committee of Science and Technology under Grant (number 17411967800); the Joint Key Project of New Frontier Technology in Shanghai Municipal Hospitals under Grant (number SHDC12014129 and 2014ZYJB0003); and Shanghai municipal medical and health discipline construction projects under Grant (number 2017ZZ02015).

Declaration of Interest:

The authors did not report any potential conflicts of interest.

References:

1. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril 2012;98(5):1103-11 (PMID: 22835448).

2. Annan JJ, Gudi A, Bhide P, Shah A, Homburg R. Biochemical pregnancy during assisted conception: a little bit pregnant. J Clin Med Res 2013;5(4):269-74. (PMID: 23864915).

3. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92 (5):1520-4 (PMID: 19828144).

4. Larsen EC, Christiansen OB, Kolte AM, Macklon N. New insights into mechanisms behind miscarriage. BMC Med 2013;11: 154 (PMID: 23803387).

5. Banerjee S, Aslam N, Woelfer B, Lawrence A, Elson J, Jurkovic D. Expectant management of early pregnancies of unknown location: a prospective evaluation of methods to predict spontaneous resolution of pregnancy. BJOG 2001;108(2):158-63 (PMID: 1123 6115).

6. Condous G, Okaro E, Bourne T. Pregnancies of unknown location: diagnostic dilemmas and management. Curr Opin Obstet Gynecol 2005;17(6):568-73 (PMID: 16258336).

7. Cordina M, Schramm-Gajraj K, Ross JA, Lautman K, Jurkovic D. Introduction of a single visit protocol in the management of selected patients with pregnancy of unknown location: a prospective study. BJOG 2011;118(6):693-7 (PMID: 21332634).

8. Zeadna A, Son WY, Moon JH, Dahan MH. A comparison of biochemical pregnancy rates between women who underwent IVF and fertile controls who conceived spontaneously. Hum Reprod 2015;30(4):783-8 (PMID: 25678573).

9. Kirk E, Condous G, Bourne T. Pregnancies of unknown location. Best Pract Res Clin Obstet Gynaecol 2009;23(4):493-9 (PMID: 19345646).

10. Kirk E, Bottomley C, Bourne T. Diagnosing ectopic pregnancy and current concepts in the management of pregnancy of unknown location. Hum Reprod Update 2014;20(2):250-61 (PMID: 24101604).

11. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4(2):295-306 (PMID: 16420554).

12. Riera-Mestre A, Vidaller A. Diagnosis and Management of the Antiphospholipid Syndrome. N Engl J Med 2018;379(13):1289-90 (PMID: 30260602).

13. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. Am J Obstet Gynecol 1996; 174(5):1584-9 (PMID: 9065133).

14. Mak A, Cheung MW, Cheak AA, Ho RC. Combination of heparin and aspirin is superior to aspirin alone in enhancing live births in patients with recurrent pregnancy loss and positive anti-phospholipid antibodies: a meta-analysis of randomized controlled trials and meta-regression. Rheumatology (Oxford) 2010;49(2):281-8 (PMID: 19965971).

15. Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ 1997;314(7076):253-7 (PMID: 90 22487).

16. Palareti G. How D-dimer assay can be useful in deciding the duration of anticoagulation after venous thromboembolism: a review. Expert Rev Hematol 2015;8(1):79-88 (PMID: 25348806).

17. Palareti G, Cosmi B, Legnani C, et al. D-dimer to guide the duration of anticoagulation in patients with venous thromboembolism: a management study. Blood 2014;124(2):196-203 (PMID: 24879 813).

18. Bao SH, Sheng SL, Liao H, Zhou Q, Frempong ST, Tu WY. Use of D-dimer measurement to guide anticoagulant treatment in recurrent pregnancy loss associated with antiphospholipid syndrome. Am J Reprod Immunol 2017;78(6) (PMID: 29024233).

19. Khalafallah AA, Morse M, Al-Barzan AM, et al. D-Dimer levels at different stages of pregnancy in Australian women: a single centre study using two different immunoturbidimetric assays. Thromb Res 2012;130(3):e171-7 (PMID: 22704076).

20. Bombeli T, Raddatz Mueller P, Fehr J. Evaluation of an optimal dose of low-molecular-weight heparin for thromboprophylaxis in pregnant women at risk of thrombosis using coagulation activation markers. Haemostasis 2001;31(2):90-8 (PMID: 11684864).

21. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database Syst Rev 2005(2):CD002859 (PMID: 15846641).

22. Alalaf S. Bemiparin versus low dose aspirin for management of recurrent early pregnancy losses due to antiphospholipd antibody syndrome. Arch Gynecol Obstet 2012;285(3):641-7 (PMID: 218 42174).

23. Negrini S, Pappalardo F, Murdaca G, Indiveri F, Puppo F. The antiphospholipid syndrome: from pathophysiology to treatment. Clin Exp Med 2016 (PMID: 27334977).

24. Devreese K, Peerlinck K, Hoylaerts MF. Thrombotic risk assessment in the antiphospholipid syndrome requires more than the quantification of lupus anticoagulants. Blood 2010;115(4):870-8 (PMID: 19965629).

25. Devreese KM. Antiphospholipid antibodies: evaluation of the thrombotic risk. Thromb Res 2012;130 Suppl 1:S37-40 (PMID: 23026657).

26. Perrier A, Bounameaux H. Cost-effective diagnosis of deep vein thrombosis and pulmonary embolism. Thromb Haemost 2001;86 (1):475-87 (PMID: 11487038).

27. Eichinger S, Hron G, Kollars M, Kyrle PA. Prediction of recurrent venous thromboembolism by endogenous thrombin potential and D-dimer. Clin Chem 2008;54(12):2042-8 (PMID: 18948369).

28. Galioto NJ, Danley DL, Van Maanen RJ. Recurrent venous thromboembolism. Am Fam Physician 2011;83(3):293-300 (PMID: 21302870).

29. Di Nisio M, Klerk CP, Meijers JC, Buller HR. The prognostic value of the D-dimer test in cancer patients treated with and without low-molecular-weight heparin. J Thromb Haemost 2005;3(7): 1531-3 (PMID: 15978112).

30. Stricker H, Marchetti O, Haeberli A, Mombelli G. Hemostatic activation under anticoagulant treatment: a comparison of unfractionated heparin vs. nadroparin in the treatment of proximal deep vein thrombosis. Thromb Haemost 1999;82(4):1227-31 (PMID: 10544903).

31. Barnhart KT, Sammel MD, Rinaudo PF, Zhou L, Hummel AC, Guo W. Symptomatic patients with an early viable intrauterine pregnancy: HCG curves redefined. Obstet Gynecol 2004;104(1): 50-5 (PMID: 15229000).

32. Bignardi T, Condous G, Alhamdan D, et al. The hCG ratio can predict the ultimate viability of the intrauterine pregnancies of uncertain viability in the pregnancy of unknown location population. Human reproduction (Oxford, England) 2008;23(9):1964-7 (PMID: 18544580).

33. Kirk E, Condous G, Van Calster B, Van Huffel S, Timmerman D, Bourne T. Rationalizing the follow-up of pregnancies of unknown location. Hum Reprod 2007;22(6):1744-50 (PMID: 17468 257).

34. Morse CB, Sammel MD, Shaunik A, et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: exceptions to the rules. Fertil Steril 2012;97(1):101-6.e2 (PMID: 22192138).

35. Christiansen OB. Biochemical pregnancies-shall they count in the recurrent miscarriage diagnosis? Journal of Reproductive Immunology 2011;90(2):155. https://www.researchgate.net/publication/251513894_Biochemical_pregnancies-shall_they_count_in_the_recurrent_miscarriage_diagnosis

36. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update 2002;8(4):333-43 (PMID: 12206468).

37. Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertil Steril 2003;79(3):577-84 (PMID: 12620443).

38. Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008;23 (12):2663-8 (PMID: 18757447).

39. Dickey RP, Olar TT, Taylor SN, Curole DN, Harrigill K. Relationship of biochemical pregnancy to pre-ovulatory endometrial thickness and pattern in patients undergoing ovulation induction. Hum Reprod. 1993;8(2):327-30 (PMID: 8473442).

40. Troncoso C, Bosch E, Rubio C, Remohi J, Simon C, Pellicer A. The origin of biochemical pregnancies: lessons learned from preimplantation genetic diagnosis. Fertil Steril 2003;79(2):449-50 (PMID: 12568866).

41. Dor J, Seidman DS, Ben-Shlomo I, Levran D, Ben-Rafael Z, Mashiach S. Cumulative pregnancy rate following *in vitro* fertilization: the significance of age and infertility aetiology. Hum Reprod 1996;11(2):425-8 (PMID: 8671236).

42. Cai QF, Wan F, Huang R, Zhang HW. Factors predicting the cumulative outcome of IVF/ICSI treatment: a multivariable analysis of 2450 patients. Hum Reprod 2011;26(9):2532-40 (PMID: 21771773).

43. Coulam CB, Roussev R. Chemical pregnancies: immunologic and ultrasonographic studies. Am J Reprod Immunol 2002;48(5):323-8 (PMID: 12516655).

44. Tartakovsky B, Bermas BL, Sthoeger Z, Shearer GM, Mozes E. Defective maternal-fetal interaction in a murine autoimmune model. Hum Reprod 1996;11(11):2408-11 (PMID: 8981121).

45. Kher A, Bauersachs R, Nielsen JD. The management of thrombosis in pregnancy: role of low-molecular-weight heparin. Thromb Haemost 2007;97(4):505-13 (PMID: 17393011).

46. Laude I, Rongieres-Bertrand C, Boyer-Neumann C, et al. Circulating procoagulant microparticles in women with unexplained pregnancy loss: a new insight. Thromb Haemost 2001;85(1):18-21 (PMID: 11204573).

47. de Saint Martin L, Duchemin J, Bohec C, et al. Increased thrombin generation measured in the presence of thrombomodulin in women with early pregnancy loss. Fertil Steril 2011;95(5):1813-5.e1 (PMID: 21130429).