Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
48(6) 1–13

DOI: 10.1177/0300060520911829
journals.sagepub.com/home/imr
SAGE

Predicting first-trimester outcome of embryos with cardiac activity in women with recurrent spontaneous abortion

Huixian Li[1,2,*], Shuang Qin[3,*], Fanfan Xiao[1], Yuhong Li[1], Yunhe Gao[4], Jiexin Zhang[2] and Qing Xiao[1,3]

Abstract

Objective: This study was performed to evaluate the capability of routine clinical indicators to predict the early outcome of embryos with cardiac activity in women with recurrent spontaneous abortion (RSA).

Methods: A retrospective cohort study of pregnant women with a history of RSA in a Chinese tertiary hospital was performed using unadjusted and multivariable logistic regression.

Results: Of 789 pregnant women with RSA, 625 (79.21%) had ongoing pregnancy, whereas 164 (20.79%) developed abortion before 20 full weeks of gestational age even after embryonic heart motion was detected. The final model had an area under the curve of 0.81 (95% confidence interval, 0.78–0.84) with a sensitivity of 74.39%, a specificity of 76.00%, and a false-positive rate of 52.32% at a fixed detection rate of 90%.

Conclusions: The combination of multiple routine clinical indicators was valuable in predicting the early outcome of embryos with cardiac activity in viable pregnancies with RSA. However, this model might result in a high false-positive rate with a fixed detection rate of 90%; other markers must be investigated to identify first-trimester RSA once positive embryonic heart motion is established.

[1]The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen, Guangdong, China
[2]Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong, China
[3]Department of Reproductive and Immunological Gynecology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong, China
[4]Department of Gynecology Outpatient, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong, China

*These authors contributed equally to this work.

Corresponding author:
Qing Xiao, The Eighth Affiliated Hospital, Sun Yat-sen University, No. 3025 ShenNan Road, Shenzhen, Guangdong 518033, China.
Email: 18922382088@163.com

Keywords
First-trimester recurrent spontaneous abortion, fetal ultrasound, biomarker, embryonic heart motion, predictive value, predictive algorithm

Date received: 11 December 2018; accepted: 13 February 2020

Introduction

Recurrent spontaneous abortion (RSA), defined as two or more spontaneous pregnancy losses with the same sexual partner, is a common obstetric complication of early pregnancy in humans.[1] In China, RSA affects roughly 5% of women of reproductive age, and nearly 80% of all RSAs occur in the first trimester (up to 14 weeks' gestation).[2,3]

Previous studies have demonstrated that the risk of miscarriage decreases once fetal heart motion can be detected by ultrasound during pregnancy.[4] There is only a 1% to 4% risk of miscarriage between weeks 6 and 11 for a pregnant woman without vaginal bleeding or other risk factors. However, the overall miscarriage rate of a viable fetus in a pregnant woman with a history of RSA can reach 15%.[5] Recurrent miscarriage is associated with multiple etiologies, including maternal thrombophilic disorders, parental chromosomal anomalies, various endocrine disturbances, and immune dysfunction;[6–8] however, nearly 50% of recurrent miscarriages occur for unknown reasons. The misuse and misinterpretation of predictors may inadvertently lead to harmful interventions for pregnancies that could have had normal outcomes.

It is critical to estimate the risk of miscarriage and predict the subsequent pregnancy outcome not only for expectant mothers but also for clinical treatment and perinatal care. Previous studies have demonstrated that pelvic ultrasonography and biochemical analysis of maternal serum or urine can allow earlier detection of pregnancy and lead to more accurate diagnosis of its complications.[9,10] However, these studies mainly delineated normal outcomes of miscarriage.[11–13] The findings of research focusing on predicting pregnancy outcomes in women with RSA after the presence of fetal heart motion are sparse and the results are often conflicting.[13]

The primary objective of this study was to evaluate a wider algorithm that includes maternal, ultrasonic, and biochemical variants after the first detection of embryonic heart motion (EHM) in predicting first-trimester RSA (FRSA). We also assessed the incremental prognostic value of ultrasonic and biochemical variants in distinguishing ongoing pregnancy from FRSA.

Methods

Study design and participants

The medical records of 4296 women who were admitted and treated for RSA in the Department of Obstetrics and Gynecology of Guangzhou Women and Children's Medical Center from 1 November 2010 to 1 November 2017 were initially evaluated. The inclusion criteria were a history of RSA with a singleton pregnancy and positive EHM detected by ultrasound before 14 full weeks' gestation. The exclusion criteria were irregular menstrual cycles, twin or multiple pregnancies, ectopic pregnancy, trophoblastic disease, induced abortion because of fetal dysplasia or reproductive history, and uncertainty of the early pregnancy outcome. Among the 4296 women, 789 pregnancies met the study criteria. This retrospective cohort study protocol

was reviewed and approved by the institutional ethics committee (No. 2018052203).

Measurements

Causes and examination of RSA. To examine the cause of RSA, each woman routinely underwent medical and family history collection and gynecological examination. In addition, the patients were advised to undergo the following diagnostic evaluations. (1) Hysterosalpingography, ultrasound, or hysteroscopy. Incomplete uterine mediastinum, intrauterine adhesions, incompetent cervix, uterine fibroids, and other symptoms related to genital tract were recorded as "anatomic abnormalities." (2) Sex hormone tests, thyroid function tests, and blood glucose measurements were performed in the early stage of endometrial hyperplasia or in the luteal phase. Gestational diabetes, gestational hypertension, thyroid dysfunction, hyperprolactinemia, polycystic ovary syndrome, luteal defects, and other abnormalities were recorded as "endocrine abnormalities." (3) Screening of infectious factors. *Toxoplasma*, cytomegalovirus, herpes simplex virus, and other related pathogens were recorded as "infections." (4) Evaluation of thrombophilia. Anti-phospholipid antibodies were examined three times at a 6-week interval, and diagnoses were confirmed when a positive titer $\geq$2 times the upper limit was obtained. An elevated D-dimer concentration and antiphospholipid syndrome were recorded as "maternal thrombophilic disorders." (5) Autoimmune screens. For patients in whom no abnormalities were found by the above screenings, further examinations were conducted to determine whether blocking antibodies, antinuclear antibodies, or other types of antibodies were present. Abnormal results were recorded as "immune dysfunction." (6) Abnormal parental karyotypes, fetal karyotypes, and DNA analyses were recorded as "parental/fetal chromosomal anomalies." The above-listed probable causes of RSA and some additional causes that are not described in detail were determined by one senior expert.

Treatments. The patients' medical records and medical orders were reviewed, and the main drugs that they received were classified into the following three categories: progesterone supplementation, which included the use of progesterone, dydrogesterone, or traditional Chinese medicine alone or in combination; low-molecular-weight heparin (LMWH), which was usually used with aspirin; and intravenous immunoglobulin (IVIG).

Clinical parameters. Maternal factors, including age, gravidity, past spontaneous abortion, previous live birth, symptoms associated with pregnancy, gestational age (GA) and conception mode, were documented when the patients had a history of RSA. Gravidity was defined as the number of past pregnancies. The number of past spontaneous abortions was defined as the number of spontaneous pregnancy losses with the same sexual partner. The number of previous live births was defined as the number of times the patient had given birth to a live fetus with a GA of $\geq$24 weeks. The GA was estimated on the basis of the last menstrual period (LMP) combined with an ultrasound scan.

Ascertainment of symptoms. Symptoms of pregnancy were recorded after the LMP. Abdominal pain was recorded as present or absent. Vaginal bleeding was recorded as none, spotting, light, moderate, or heavy using the menstrual pictogram developed by Wyatt et al.[14]

Biochemical measurements. The serum β-human chorionic gonadotropin (β-hCG) and progesterone concentrations were tested regularly after conception was

confirmed. A standard protocol was adopted for serum sampling. The β-hCG and progesterone concentrations were monitored using an automated immunoassay technique (ADVIA Centaur Immunoassay System; Siemens Healthineers, Erlangen, Germany). Venous blood (3 mL) was collected at the first hospital visit and once every 1 to 3 weeks before a GA of 14 weeks. In the present study, we focused on the peak serum β-hCG and progesterone concentrations. The serum pregnancy-associated plasma protein A (multiple of the median) concentration was included in Down syndrome screening at a GA of 11 to 14 weeks.

Ultrasonic measurements. We searched the Clinical Data Repository database to identify women with a history of RSA who met the following criteria. First, women who had a singleton pregnancy with visible EHM by ultrasonography were selected. Second, follow-up was arranged for all pregnancies every 2 to 3 weeks combined with biochemical testing before 14 full weeks of gestation if the pregnancy was ongoing. For women who underwent more than one checkup, we used the earliest scan demonstrating a live embryo with EHM. In addition, ultrasound was performed to measure the crown–rump length (CRL) and assess the GA based on the CRL.[15]

Chromosome examination. Fluorescence in situ hybridization or chromosomal microarray analysis was used to perform chromosome examination.

Ascertainment of pregnancy. Pregnancy was established by a serum β-hCG concentration of $\geq$50 IU/L. Intrauterine pregnancy was confirmed by transvaginal or pelvic ultrasonography, which was performed every 2 to 3 weeks from exactly 5^{+0} weeks.

Ascertainment of pregnancy loss. Multiple methods based on the GA were used to distinguish ongoing pregnancy from pregnancy loss. FRSA was defined as the termination of pregnancy before 20 full weeks post-LMP GA. Pregnancy loss was ascertained by the absence of a previously positive EHM determined by more than two transvaginal or pelvic ultrasonography scans and incomplete or complete expulsion of the embryo after vaginal bleeding before 20 full weeks of gestation.

Data extraction

The Clinical Data Repository was queried for patients with a history of RSA treated for intrauterine pregnancy. To ensure accuracy, the data were independently collected by two authors.

Statistical analysis

Categorical variables are presented as number (percentage) and were compared using the chi-square test. Yates' continuity correction or the two-tailed Fisher's exact test was used when appropriate. Continuous variables are presented as median (interquartile range) and were compared using the Wilcoxon rank sum test for their non-Gaussian distribution. All individual FRSA-associated factors ($P < 0.10$) were added to backward stepwise logistic regression analyses to identify the best combination of predictors for miscarriage. Receiver operating characteristic (ROC) analysis was also conducted to assess the diagnostic performance of the selected independent predictors ($P < 0.05$). The areas under the ROC curve (AUCs) were compared with the DeLong method. The optimal cutoff values for defining miscarriage were calculated on the basis of maximizing Youden's index (sum of sensitivity + specificity − 1) of each index.

In the subgroup analysis divided by causes of RSA, all variables selected by the backward stepwise logistic regression for all patients were included. The magnitude of the increase in model performance was assessed by the change (Δ) in the AUC, absolute integrated discrimination improvement index, and non-categorical net reclassification improvement (>0). The false-positive rates (%) with various indicator combinations at different detection rates in the range of 50% to 90% were also calculated.

All probability values were two-sided, and values of $P < 0.05$ were considered significant. The statistical analyses were performed using SAS Windows software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Patients' clinical characteristics

Among 789 pregnant women with a history of RSA, 625 (79.21%) had ongoing viable pregnancy whereas 164 (20.79%) developed FRSA even after EHM was detected at 5 to 14 weeks of GA. The characteristics of the ongoing pregnancy group and FRSA group are presented in Table 1. No significant differences were found in the reproductive histories between the two groups with the exception of higher gravidity ($P < 0.001$) and a higher number of spontaneous abortions ($P = 0.002$) in the FRSA group. FRSA occurred more frequently among patients with abdominal pain and moderate/heavy vaginal bleeding than among patients without these symptoms ($P < 0.001$). The proportion of patients in whom the first EHM was detected by pelvic ultrasound was similar in both groups (68.80% vs. 62.20%). The GA based on the LMP was lower in the FRSA group ($P = 0.002$), indicating that EHM was found earlier in the FRSA group. The CRL and the CRL-based GA at which the first positive EHM was detected were smaller in the FRSA group ($P = 0.001$ and 0.002, respectively). The FRSA group also had a significantly higher prevalence of an inconsistent gestational sac diameter ($P < 0.001$). The patients in the FRSA group had lower β-hCG and progesterone concentrations than those in the ongoing pregnancy group ($P < 0.001$ for both).

With respect to the probable causes of FRSA, 99 (12.55%) patients had endocrine/hormonal imbalances, 98 (12.42%) had immune dysfunction, 73 (9.25%) had anatomic abnormalities, 61 (7.73%) had maternal thrombophilic disorders, 36 (4.56%) had infections, 6 (0.76%) had parental/fetal chromosomal anomalies, 217 (27.50%) had multiple possible causes, and 199 (25.22%) had an unknown cause. The main drug regimens administered to prevent miscarriages were as follows: 329 (41.70%) patients received single progesterone supplementation; 202 (25.60%) received a combination of progesterone, LMWH, and IVIG; 62 (7.86%) received a combination of progesterone and LMWH; 41 (5.20%) received a combination of progesterone and IVIG; 22 (2.79%) received other combinations; and 133 (16.86%) received none of the above-mentioned drugs. In addition, 34 (4.31%) patients underwent hysteroscopic surgery, 8 (1.01%) underwent cervical ligation surgery, and 7 (0.89%) underwent laparoscopic surgery before their current pregnancy. Of the patients who developed FRSA in our study, 91 (55.49%) underwent chorionic chromosome analysis, and 9 of these patients' samples were unqualified. Of the remaining 82 patients, 43 (52.44%) had chromosomal abnormalities (Table 1).

Predictive performance of the selective factors

Factors with a P value of <0.10 in Table 1 were added to backward stepwise logistic

Table 1. Maternal clinical characteristics and ultrasound and biochemical findings in the ongoing pregnancy and FRSA groups.

Indicators	Ongoing pregnancy (n = 625)	FRSA (n = 164)	Z/χ^2	*P* value
Maternal age, years	31.19 (28.13–33.77)	31.00 (28.35–33.89)	0.026	0.979
Gravidity	3 (3–4)	4 (3–4.5)	−5.244	**<0.001**
Past spontaneous abortions	2 (2–2)	2 (2–3)	−3.053	**0.002**
Previous live birth/Yes†	127 (20.32)	29 (17.68)	0.570	0.450
Conception			–	0.278*
Natural conception	606 (96.96)	162 (98.78)		
Assisted reproduction	19 (3.04)	2 (1.22)		
Abdominal pain/Yes†	60 (9.60)	34 (20.73)	15.340	**<0.001**
Vaginal bleeding			44.558	**<0.001**
Moderate/heavy bleeding	28 (4.48)	33 (20.12)		
None/spotting/light bleeding	597 (95.52)	131 (79.88)		
Examination method			2.580	0.108
Pelvic ultrasound	430 (68.80)	102 (62.20)		
Transvaginal ultrasound	195 (31.20)	62 (37.80)		
GA (LMP)	6.86 (6.14–8.00)	6.43 (6.00–7.29)	3.170	**0.002**
CRL	6.57 (6.14–8.00)	6.43 (6.00–7.14)	3.472	**0.001**
GA (CRL)	10 (6–18)	8 (5–12)	3.129	**0.002**
Inconsistent gestational sac diameter/Yes†	7 (1.12)	11 (6.71)	15.772	**<0.001****
Log10(β-hCG)#, IU/L	5.19 (4.99–5.31)	4.84 (4.58–5.07)	9.299	**<0.001**
Progesterone, nmol/L	74.10 (59.50–88.90)	56.05 (40.84–72.10)	7.832	**<0.001**
PAPP-A (MoM)			–	0.547*
Normal	610 (97.60)	162 (98.78)		
Low	15 (2.40)	2 (1.22)		
Cause			44.501	**<0.001**
Endocrine/hormonal abnormalities	88 (14.08)	11 (6.71)		
Immune dysfunction	79 (12.64)	19 (11.59)		
Anatomic abnormalities	41 (6.56)	32 (19.51)		
Maternal thrombophilic disorders	54 (8.64)	7 (4.27)		
Infection	21 (3.36)	15 (9.15)		
Parental/fetal chromosomal anomalies	5 (0.80)	1 (0.61)		
Multiple causes	181 (28.96)	36 (21.95)		
Unknown	156 (24.96)	43 (26.22)		
Drug treatment			37.954	**<0.001**
Progesterone	233 (37.28)	96 (58.54)		
Progesterone + LMWH + IVIG	181 (28.96)	21 (12.80)		
Progesterone + LMWH	46 (7.36)	16 (9.76)		
Progesterone + IVIG	37 (5.92)	4 (2.44)		
Other combinations of the above drugs	14 (2.24)	8 (4.88)		
None of the above drugs	114 (18.24)	19 (11.59)		
Surgery			8.946	**0.030**
Hysteroscopic	21 (3.36)	13 (7.93)		

(continued)

Table 1. Continued.

Indicators	Ongoing pregnancy (n = 625)	FRSA (n = 164)	Z/χ^2	*P* value
Cervical ligation	6 (0.96)	2 (1.22)		
Laparoscopic	4 (0.64)	3 (1.83)		
None of the above surgeries	594 (95.04)	146 (89.02)		
Chromosome examination of the chorion, n = 82			–	–
Abnormal	–	43 (52.44)	–	–
Normal	–	39 (47.56)	–	–

Data are presented as median (interquartile range) or n (%).

*Two-tailed Fisher's exact test. **Yates' continuity correction. #Base-10 log-transformed.

†The other group is "No," and the two groups contained all cases.

FRSA, first-trimester recurrent spontaneous abortion; GA, gestational age; LMP, last menstrual period; CRL, crown–rump length; β-hCG, β-human chorionic gonadotropin; PAPP-A (MoM), pregnancy-associated plasma protein A (multiple of the median); LMWH, low-molecular-weight heparin; IVIG, intravenous immunoglobulin.

regression analyses, which revealed that gravidity, abdominal pain, vaginal bleeding in early pregnancy, log10(β-hCG), and progesterone were independent predictors of FRSA ($P < 0.05$). In the ROC analysis of single parameters, the base-10 log-transformed peak serum β-hCG concentration had a maximum AUC of 0.74 (95% confidence interval [CI], 0.71–0.77; $P < 0.001$), which was significantly higher than that of the other indicators except progesterone. The log10(β-hCG) cut-off value was 5.07, indicating that patients with a maximum β-hCG concentration of 117,489.8 mIU/mL during 5 to 14 full weeks' GA were more likely to develop FRSA. The AUCs of gravidity, abdominal pain, vaginal bleeding, and progesterone were 0.62 (95% CI, 0.59–0.66; $P < 0.001$), 0.56 (95% CI, 0.52–0.59; $P = 0.030$), 0.58 (95% CI, 0.54–0.61; $P = 0.002$), and 0.70 (95% CI, 0.67–0.73; $P < 0.001$), respectively. The cut-off of gravidity and progesterone were three times and 61.80 nmol/L, respectively (Table 2). The final model had a better predictive value than that of each individual predictor, with an AUC of 0.81 (95% CI, 0.78–0.84) and Youden's index of 0.50. The model showed a sensitivity of 74.39% and a specificity of 76.00% in predicting FRSA with positive and negative likelihood ratios of 3.10 and 0.34, respectively.

In the subgroup analysis divided by causes of RSA, the combination of gravidity, abdominal pain, vaginal bleeding, base-10 log-transformed peak serum β-hCG, and progesterone for patients with infection had a maximum AUC of 0.93 (95% CI, 0.81–0.98; $P < 0.001$). The AUCs of other subgroups are shown in Table 2.

Combining biochemical indicators with the model that included gravidity, abdominal pain, and vaginal bleeding improved the discrimination of FRSA (0.69 [95% CI, 0.66–0.72] versus 0.81 [95% CI, 0.79–0.840]; $\Delta AUC = 0.12$; $P < 0.001$). The combinatorial model also showed significant incremental effects in the discrimination slope and reclassification based on analysis of the integrated discrimination improvement index (0.16; 95% CI, 0.13–0.19; $P < 0.001$) and categorical-free net reclassification improvement (0.86; 95% CI, 0.70–1.01; $P < 0.001$). It correctly up-classified 33% of FRSA cases and down-classified 53% of ongoing pregnancies when ultrasound and biochemical findings were entered into the final model. When

Table 2. Data from receiver operating characteristic curves.

Indicators	AUC (95% CI)	*P* value	Cut-off	Sensitivity/specificity	PLR/NLR
Gravidity	0.62 (0.59–0.66)	**<0.001**	>3	67.68/54.88	1.50/0.59
Abdominal pain	0.56 (0.52–0.59)	**0.030**	–	20.73/90.40	2.16/0.88
Vaginal bleeding	0.58 (0.54–0.61)	**0.002**	–	20.12/95.52	4.49/0.84
Log10(β-hCG)[#], mIU/mL	0.74 (0.71–0.77)	**<0.001**	≤5.07	76.07/67.43	2.34/0.35
Progesterone, nmol/L	0.70 (0.67–0.73)	**<0.001**	≤61.80	61.35/70.52	2.08/0.55
Model for all patients	0.81 (0.78–0.84)	**<0.001**	–	74.39/76.00	3.10/0.34
Subgroup analysis divided by causes of RSA*					
Model for patients with endocrine imbalances	0.81 (0.72–0.88)	0.040	–	9.09/100	100/89.90
Model for patients with immune dysfunction	0.78 (0.68–0.86)	**0.021**	–	21.05/97.47	66.67/83.70
Model for patients with anatomic abnormalities	0.83 (0.72–0.90)	**<0.001**	–	71.88/85.37	79.31/79.55
Model for patients with maternal thrombophilic disorders	0.88 (0.78–0.95)	**0.009**	–	42.86/96.30	60.00/92.86
Model for patients with infection	0.94 (0.81–0.99)	**<0.001**	–	93.33/100	100/95.45
Model for patients with multiple causes	0.79 (0.72–0.84)	**<0.001**	–	16.67/97.79	60.00/85.51
Model for patients with unknown cause	0.83 (0.77–0.87)	**<0.001**	–	44.19/96.79	79.17/86.29

[#]Base-10 log-transformed. *Subgroup analyses were not performed for patients with parental or fetal chromosomal anomalies because of an insufficient sample size.
AUC, area under the curve; CI: confidence interval; PLR: positive likelihood ratio; NLR: negative likelihood ratio; β-hCG, β-human chorionic gonadotropin; RSA, recurrent spontaneous abortion.

biochemical tests and medical records were gradually added into the predictive model, the false-positive rate varied from 9.92% to 60.16% with a fixed detection rate of 50% to 90% (Table 3).

Discussion

Predicting the occurrence of FRSA has been proven to be quite challenging. In the present study, the combination of clinical parameters with biochemical measurements was more effective than any single parameter or measurement in predicting FRSA after EHM of a singleton pregnancy was detected. The present study confirmed that the addition of tests not only correctly up-classified FRSA cases but also correctly down-classified ongoing pregnancy cases. However, the model containing all statistically significant variables in this study still resulted in a 52.32% false-positive rate with a fixed detection rate of 90%.

Among the clinical parameters in this study, gravidity, spontaneous abortion, abdominal pain, and vaginal bleeding in early pregnancy were significantly associated with FRSA; these findings are compatible with previous studies.[16–18] The risk of FRSA increased as the number of pregnancies or spontaneous abortions increased. First-trimester vaginal bleeding is a common obstetric complication in approximately 15% to 25% of pregnancies.[19] Compared with no/spotting/light vaginal bleeding, moderate/heavy vaginal bleeding was significantly associated with a high risk of FRSA (odds ratio = 5.37). Hasan et al.[20] found that heavy bleeding with pain in the first trimester significantly increased the risk of miscarriage. In the present study, bleeding accompanied by pain was found in only 14 pregnancies, and no significant interactive effect of these two symptoms was found. In many cases, vaginal bleeding is a consequence rather than the cause of early miscarriage. To ensure that bleeding episodes in women who miscarried were not all clustered near the time of loss, we examined the time from the bleeding episode to the miscarriage for both heavy and spotting/light episodes. For moderate/heavy episodes, the median time from the end of the index episode to the miscarriage was 13 days (interquartile range, 6–46 days), indicating that the bleeding episodes were not all clustered near the time of loss.[20]

The univariate analysis of the ultrasonic indicators showed that EHM occurred earlier in the FRSA group. We have also noticed this phenomenon in the clinical setting. The primitive cardiac tube usually starts beating in a developing embryo 6 to 8 weeks after fertilization.[21] In practice, the time point at which EHM can be detected

Table 3. First-trimester false-positive rates with combinations of indicators at various detection rates.

	False-positive rate (%)		
Detection rate (%)	Model I	β-hCG + Progesterone	Model I + β-hCG + Progesterone
50	25.28	15.52	9.92
60	–*	21.92	13.92
70	–*	29.60	21.12
80	51.36	44.16	33.92
90	–*	60.16	52.32

*Invalid for the specified detection rate.

β-hCG, β-human chorionic gonadotropin.

depends on the frequency of maternity care, the risk level of the pregnancy, and the accuracy of the pregnancy dates. Ultrasound can detect an embryo's heartbeat as early as 6 to 7 weeks. However, the detection of EHM by ultrasound does not ensure the viability of an ongoing pregnancy.[22] Ultrasound findings are reportedly useful factors with which to predict miscarriage in early intrauterine pregnancies and in live embryos from assisted conceptions. In the present study, the FRSA group had more frequent and earlier clinical symptoms (such as vaginal bleeding), which might explain why the earlier detection of positive EHM possibly indicates a higher risk of miscarriage.[15] Before adjustment for clinical parameters, the CRL was an independent predictor of FRSA. However, after adding biochemical markers to the multivariate model, the diagnostic value was invalid. Nevertheless, ultrasound is still needed because pregnancy loss should be ascertained by more than two transvaginal or pelvic ultrasonography scans, and ultrasound examination can provide more information that was not included in our study.

In the present study, endocrine imbalances were the most single common cause of RSA, and more patients had two or more probable causes than a single cause. Supplementation with progestogen therapy probably reduces the rate of subsequent miscarriage for women with unknown causes of miscarriage, especially for women with unexplained recurrent miscarriages.[23] Most patients (67.33%, 134/199) with unknown causes were treated with progesterone in our hospital. The model for patients with infection had a maximum AUC of 0.94 (95% CI, 0.81–0.99) and the model for patients with immune dysfunction had a minimum AUC of 0.78 (95% CI, 0.68–0.86), which suggests that prediction might differ among subgroups of women with different causes of RSA. In China, karyotype analysis is more commonly performed using the parents' peripheral blood than the chorionic villus.[24] Forty-three patients (52.44%, 43/82) in the present study had chromosomal abnormalities as shown by chorionic chromosome analysis (Table 1). However, chromosome analysis of the parents' peripheral blood only reflected a very small number of patients' causes of FRSA (3.3%, 3/91). Chromosome aberrations were detected in only 4.1% (62/1510) couples in a study by Tunc et al.[25] We recommend chorionic chromosome analysis for patients with RSA.

Women with vaginal bleeding, a more advanced GA, or a history of first-trimester miscarriages are more likely to undergo ultrasonic examinations.[26] Autoimmune disorders, the progesterone concentration, single measurement of the serum β-hCG concentration in early pregnancy, and the rate of rise and peak level of the serum β-hCG concentration are reportedly good outcome predictors for women with a history of RSA. The ROC analysis for β-hCG or progesterone alone showed moderate sensitivity and specificity, indicating that these two indicators are insufficient for identifying women who are likely to develop FRSA. The results of the final model illustrated that once EHM was demonstrated, all factors in our study still had limited value in identifying women who were likely to develop FRSA; this is similar to the findings reported by Pillai et al.[13] These authors demonstrated that serum hCG and progesterone, the most commonly used biomarkers, were not useful in predicting the outcome of a viable fetus in women with threatened miscarriage. Other markers, such as inhibin A and glycodelin, require further investigation to hopefully improve the prediction of outcomes in women with threatened miscarriage.[13,27]

The final model investigated the comprehensive value of a series of maternal characteristics and biochemical findings in predicting FRSA. This algorithm for

patients with recurrent abortion detected 74.39% of women who subsequently miscarried with a false-positive rate of 24.00%. The algorithm had limited value because many ongoing pregnancies might be identified as FRSA.

Recurrent miscarriage is frustrating for the physician and a heartbreaking experience for the patient. Vaginal bleeding and abdominal pain are common indications of early miscarriage, recurrence of miscarriage, or ectopic pregnancy. In cases of intrauterine pregnancy with a live embryo demonstrated by ultrasound examination, using a model to estimate the patient-specific risk of subsequent miscarriage would be beneficial for both the patient and the planning of follow-up. Notably, the 90% detection rate produced a false-positive rate of 52.32%, which would increase anxiety for women with ongoing pregnancies. We further searched for data regarding indicators such as inhibin A, cancer antigen 125, and estradiol; however, these were not included in systematic inspections and had no significant predictive value for FRSA. Therefore, more systematic inspections and markers are needed to minimize or avoid false-positive test results.

Strengths and limitations

This was a large retrospective cohort study of pregnant women with FRSA, and the study has several strengths. The Guangzhou Women and Children's Medical Center, a Chinese tertiary hospital, has a large number of patients and complete information collection. Information regarding the patients' reproductive history, symptoms, ultrasound findings, and biochemical parameters was extracted from the structured electronic medical record system. Each indicator was assessed for its value in predicting pregnancy outcomes with reproducible statistical methods. However, the study also had some limitations. Data regarding potential confounding factors such as the etiology and treatment regimens might have been absent, causing us to miss some etiological information and underestimate the types of medication because of the retrospective nature of the study. In addition, selection bias is an inherent problem of a 7-year retrospective cohort study. Although the regression analyses were adjusted for potential confounding factors, residual confounding may still persist. We did not collect data regarding the patients' body mass index or history of smoking, which might further improve the performance of the model. Future collection of cumulative follow-up data of all women in this cohort might provide the outcomes of all pregnancies following the diagnosis of unexplained FRSA.[28]

Conclusions

The combination of multiple routine clinical indicators used in this study was valuable in predicting the early outcome of embryos with cardiac activity in women with FRSA. However, our model resulted in a high false-positive rate with a fixed detection rate of 90%. Hence, other markers need to be investigated to improve the identification of FRSA once EHM has been established.

Acknowledgements

We thank Dr. Liang for providing his opinions and assistance with modifying the article. We also thank our colleagues from the Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center for their statistical support.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Ethics approval

The patients were diagnosed and treated according to national guidelines and agreements. The blood testing and recording of all other variables included in our analysis were essential to confirm the diagnosis and classify the patients. Therefore, we did not seek or obtain consent from each patient; rather, our project was approved by our research ethics committee.

Funding

This work was supported by the Natural Science Foundation of Guangdong Province, China (Grant No. 2015A030313689).

ORCID iDs

Huixian Li https://orcid.org/0000-0001-5722-8602
Qing Xiao https://orcid.org/0000-0002-8231-6951

References

1. Hogge WA, Byrnes AL, Lanasa MC, et al. The clinical use of karyotyping spontaneous abortions. *Am J Obstet Gynecol* 2003; 189: 397–400; discussion 400–392.
2. Allison JL and Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. *Curr Opin Endocrinol Diabetes Obes* 2009; 16: 446–450.
3. Lin QD and Qiu LH. Pathogenesis, diagnosis, and treatment of recurrent spontaneous abortion with immune type. *Front Med China* 2010; 4: 275–279.
4. Bae S and Karnitis J. Triple ultrasound markers including fetal cardiac activity are related to miscarriage risk. *Fertil Steril* 2011; 96: 1145–1148.
5. Falco P, Milano V, Pilu G, et al. Sonography of pregnancies with first-trimester bleeding and a viable embryo: a study of prognostic indicators by logistic regression analysis. *Ultrasound Obstet Gynecol* 1996; 7: 165–169.
6. Vasiljevic A, Poreau B, Bouvier R, et al. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome and recurrent intrauterine fetal death. *Lancet* 2015; 385: 2120.
7. Sundermann AC, Velez Edwards DR, Bray MJ, et al. Leiomyomas in pregnancy and spontaneous abortion: a systematic review and meta-analysis. *Obstet Gynecol* 2017; 130: 1065–1072.
8. Larsen EC, Christiansen OB, Kolte AM, et al. New insights into mechanisms behind miscarriage. *BMC Med* 2013; 11: 154.
9. Doubilet PM, Benson CB, Bourne T, et al. Diagnostic criteria for nonviable pregnancy early in the first trimester. *N Engl J Med* 2013; 369: 1443–1451.
10. Spaggiari E, Czerkiewicz I, Sault C, et al. Impact of including or removing nuchal translucency measurement on the detection and false-positive rates of first-trimester Down syndrome screening. *Fetal Diagn Ther* 2016; 40: 214–218.
11. Papaioannou GI, Syngelaki A, Maiz N, et al. Ultrasonographic prediction of early miscarriage. *Hum Reprod* 2011; 26: 1685–1692.
12. Barnhart KT, Guo W, Cary MS, et al. Differences in serum human chorionic gonadotropin rise in early pregnancy by race and value at presentation. *Obstet Gynecol* 2016; 128: 504–511.
13. Pillai RN, Konje JC, Tincello DG, et al. Role of serum biomarkers in the prediction of outcome in women with threatened miscarriage: a systematic review and diagnostic accuracy meta-analysis. *Hum Reprod Update* 2016; 22: 228–239.
14. Wyatt KM, Dimmock PW, Walker TJ, et al. Determination of total menstrual blood loss. *Fertil Steril* 2001; 76: 125–131.
15. Musters AM, Koot YE, van den Boogaard NM, et al. Supportive care for women with recurrent miscarriage: a survey to quantify women's preferences. *Hum Reprod* 2013; 28: 398–405.
16. Axmon A and Hagmar L. Time to pregnancy and pregnancy outcome. *Fertil Steril* 2005; 84: 966–974.
17. Sapra KJ, Buck Louis GM, Sundaram R, et al. Signs and symptoms associated with early pregnancy loss: findings from a population-based preconception cohort. *Hum Reprod* 2016; 31: 887–896.

18. Strobino BA and Pantel-Silverman J. First-trimester vaginal bleeding and the loss of chromosomally normal and abnormal conceptions. *Am J Obstet Gynecol* 1987; 157: 1150–1154.
19. Lykke JA, Dideriksen KL, Lidegaard O, et al. First-trimester vaginal bleeding and complications later in pregnancy. *Obstet Gynecol* 2010; 115: 935–944.
20. Hasan R, Baird DD, Herring AH, et al. Association between first-trimester vaginal bleeding and miscarriage. *Obstet Gynecol* 2009; 114: 860–867.
21. Iruretagoyena JI, Davis W, Bird C, et al. Metabolic gene profile in early human fetal heart development. *Mol Hum Reprod* 2014; 20: 690–700.
22. Papaioannou GI, Syngelaki A, Poon LC, et al. Normal ranges of embryonic length, embryonic heart rate, gestational sac diameter and yolk sac diameter at 6-10 weeks. *Fetal Diagn Ther* 2010; 28: 207–219.
23. Haas DM, Hathaway TJ and Ramsey PS. Progestogen for preventing miscarriage in women with recurrent miscarriage of unclear etiology. *Cochrane Database Syst Rev* 2018; 10: CD003511.
24. Dai R, Pan Y, Fu Y, et al. Role of male genetic factors in recurrent pregnancy loss in Northeast China. *Eur J Obstet Gynecol Reprod Biol* 2018; 224: 6–11.
25. Tunc E, Tanriverdi N, Demirhan O, et al. Chromosomal analyses of 1510 couples who have experienced recurrent spontaneous abortions. *Reprod Biomed Online* 2016; 32: 414–419.
26. Lautmann K, Cordina M, Elson J, et al. Clinical use of a model to predict the viability of early intrauterine pregnancies when no embryo is visible on ultrasound. *Hum Reprod* 2011; 26: 2957–2963.
27. Senapati S, Sammel MD, Butts SF, et al. Predicting first trimester pregnancy outcome: derivation of a multiple marker test. *Fertil Steril* 2016; 106: 1725–1732.e3.
28. Kaandorp SP, van Mens TE, Middeldorp S, et al. Time to conception and time to live birth in women with unexplained recurrent miscarriage. *Hum Reprod* 2014; 29: 1146–1152.