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Original Article

Lymphocyte immunotherapy for recurrent spontaneous abortion in patients with negative blocking antibody

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Abstract: Randomized trials of lymphocytes immunotherapy (LIT) in the treatment for recurrent spontaneous abortion (RSA) have shown conflicting results. Current evidences to evaluate the effectiveness of LIT for RSA patients who lack of blocking antibody (BA) are systematic reviewed as a purpose. Literatures concern RSA patients with negative BA and who receiving LIT were searched and screened. Pregnancy rates for each treatment group were extracted, and the overall odds ratio (OR) and absolute treatment effect for LIT were calculated. According to the literatures, results of 14 randomized, double-blind trials comparing LIT with placebo for the treatment of RSA included in the meta-analysis were shown as follows: pregnancy rate is better in LIT (OR=5.72, 95% CI 4.42-7.40, P<0.00001) and the positive BA group (OR=1.58, 95% CI 1.36-1.82, P<0.00001) than in the NLIT negative BA group after LIT respectively. Moreover, low abortion rate was found in LIT group (OR=0.19, 95% CI 0.14-0.24, P<0.00001) and the positive BA group (OR=0.15, 95% CI 0.10-0.24, P<0.00001). Irrelevant to the source of lymphocytes, LIT displayed good therapeutic effect (OR=5.09, 95% CI 3.83-6.76, P<0.00001). This meta-analysis suggests that no matter lymphocytes from, LIT may be beneficial to the treatment of RSA patients with negative BA, improving the pregnancy rate and reducing the abortion rate.

Keywords: Lymphocyte immunotherapy, recurrent spontaneous abortion, idiopathic habitual abortion, blocking antibody

Introduction

In 1985, Beer etc [1] first ever reported the immunotherapy for alloimmune-related recurrent abortions. They extracted the lymphocytes from partner or healthy donor and administered to patients with alloimmune disorders as active immunotherapy. Since then, numerous studies on lymphocyte active immunotherapy for the treatment of recurrent miscarriage have been increasingly reported, although its clinical application is still controversial. For a long time, no uniform standard treatment for recurrent abortion has been followed on clinical settings, which seriously affect the cure effect and the patient's physical and mental health and quality of life. Hence, it is significant to find the pathogeny and explore a safe and effective treatment for recurrent spontaneous abortion (RSA) to improve the re-pregnancy rate and better outcome. Since a long time, active immunotherapy has been considered as an optimal treatment choice for recurrent miscarriage globally.

However, significant differences in therapeutic effect have been observed in different studies [2, 3]. With the rapid development of reproductive immunology, research data confirm that the most RSA is mainly due to immune factors, and it mainly affects 30~40 years old women, regardless of natural pregnancy or pregnancy through assisted reproduction technology. RSA has gradually become a social problem due to the delay of reproductive age. Scholars around the world are trying various immunotherapeutic to treat these patients because they increasingly realize the important role the immune factors play on the pathogenesis of RSA [4, 5]. The morbidity of recurrent miscarriage has been rising in recent years about l%~5% [6]. A study suggests that the negative rate of blocking anti-

body (BA) is about 99.28% on agnogenic recurrent miscarriage patients [7].

In the serum of normal pregnant women, there is a kind of lymphocyte specific immunoglobulin G (IgG) which inhibits lymphocyte reaction, shuts the matrix of placental trophoblastic cells from the mother's lymphocytes, prevents the helper T cell recognition of fetal antigens, and deters the immune system's attack on embryos. In the process of pregnancy, BA prevent the matrix cytotoxic T cells in the embryo's immune attacks, and thereby play a role of protecting fetus and maintaining the pregnancy [8]. The BA has the following features [9]: (1) BA can be detected in pregnant women serum at early pregnancy period, its level increases along with the weeks of pregnancy, and it disappear few weeks after delivery; (2) The activity of BA in the serum resembles IgG; (3) The IgG activity in the placental tissue resembles the serum IgG; (4) The B lymphocytes of male partner can inactivate the BA. In the modern immunology research, the formation of human embryonic genes from the paternal were seen as foreign invaders, which can produce rejection in matrix [10]. In normal pregnancy, pregnant woman produce BA, which preserve the embryonic growth and development. The embryos will be influenced by nature rejection when the pregnant woman lacks of BA. With lymphocyte active immunotherapy, partner's lymphocytes stimulate the patient's immune system, leading to the production of BA in patient's body [11]. When the woman become pregnant again, BAs could identify the homologous antigen and integrate with it, and then protect the embryos [12].

In 1992 [13], Hwang JL found that neither BA nor anti-paternal lymphocytotoxic antibodies are essential for successful pregnancy. They probably reflect the immunological response of the mother to exposure to fetal antigens. In 1998 [14], Peña RB study demonstrated that the presence of mixed lymphocyte reaction blocking factors (MLR-BFs) is not a prognostic criterion for the outcome of pregnancy after alloimmunotherapy, and consequently, it is not a good diagnostic tool for RSA of alloimmune cause. Until 2013, in Khonina NA [15] research, the data obtained demonstrate that lymphocytes immunotherapy (LIT) with the paternal lymphocytes in MLR-Bf negative women is accompanied by increased proliferative cell response to the paternal alloantigen and enhanced production of soluble suppressor activity factors (MLR-Bf) that is associated with improved pregnancy outcome in women with history of RSA. However, in 2014, a systemic review [16] concluded that paternal cell immunization or third-party donor leukocytes provide no significant beneficial effect over placebo in improving the live birth rate.

Although pilot studies are encouraging with high success rates, the results from controlled trials have been contradictory. The purpose of this study was to conduct a meta-analysis of the evidences in the literature so that the role of LIT in the treatment of recurrent abortion could be evaluated. Hence, this article describes the evaluation of effectiveness of LIT for RSA patients with negative BA by meta-analysis.

Materials and methods

Trials were identified using several search strategies. PubMed, China Biomedical Literature Database, WanFang Database, and VIP Database were searched for the terms "lymphocytes immunotherapy", "recurrent spontaneous abortion", "idiopathic habitual abortion", and "blocking antibody" from 1996 through March 2015. Authors and publishers of relevant abstracts and articles were contacted to obtain further details on these studies. Finally, peer consultation was sought for any remaining articles. Published reports of clinical trials were selected only if they met the inclusion criteria and if the outcome data were provided to enable pooling of data.

Selection criteria

This systematic review was limited to trials reporting random allocation to either LIT or placebo in women with RSA (*i.e.,* two or more losses) with BA. Trials which patients receiving LIT before or after conception were included. No distinction was made between trials on the basis of women ages, the number of abortions and pregnancies, and the concentration of the lymphocytes. Primary or secondary RSA patients did not use any drugs that affect immune function before LIT and does not distinguish the source of leukomonocytes. All included studies were randomized controlled

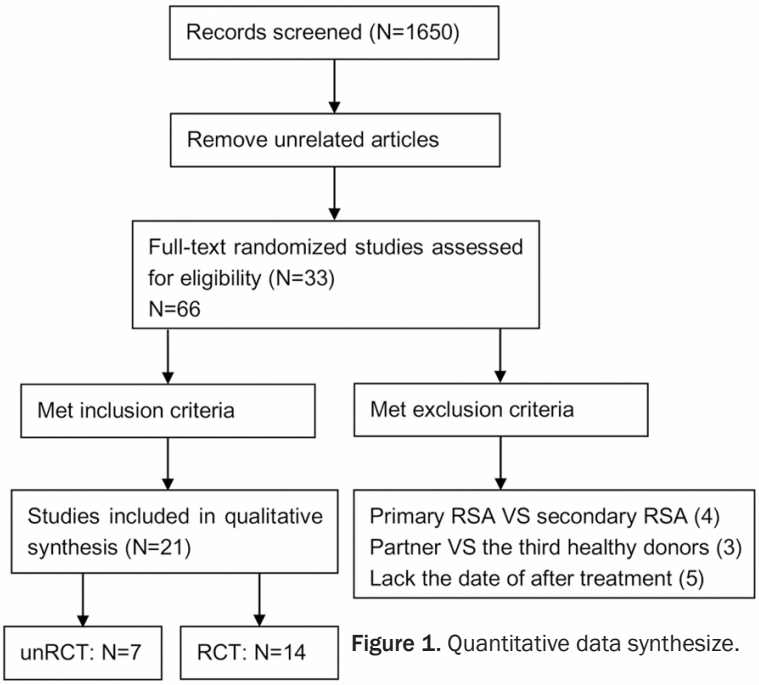

Figure 1. Quantitative data synthesize.

trials, quasi-randomized and prospective studies that compared the use of LIT with NLIT (routine tocolysis or no treatment) in RSA women with negative blocking antibody. The primary outcome of interest were the pregnancy rate, positive conversion rate, pregnancy rate of negative BA compared to positive BA after treatment, and abortion rate. The pregnancy rate of different sources of lymphocytes was assessed as a secondary outcome.

Exclusion criteria

Remove the literatures of retrospective cohort study or repeated reports, and some of which has no relevant indicators (un-randomized trials, lacking the detection of blocking antibody after LIT, joint drug therapy in LIT group). Excluded the object patients who were not mentioned the situation of blocking antibody.

Statistical analysis

This study is the comparison of the pregnancy rate, so we select a measurement method to analyze the data. Meta-analyses were conducted by Revman 5.2 (Cochrane Collaboration 2012). We performed several analyses to present pooled estimated effect sizes and use random effects models to incorporate heterogeneity within and between studies Data of each selected trials including author, year, selection and exclusion criteria, intervention measure and outcome indicators were extracted and summarized. The overall adjusted OR, and its 95% confidence interval (CI) were calculated to provide an estimate of the overall effect of treatment. A test of the homogeneity of treatment effect across all trials was performed using I^2. When the I^2< 50%, the fixed-effect model was used. Otherwise, the random-effect model was used.

Results

There were about 1650 published studies on the use of LIT in RSA patients. After reviewing titles and abstracts, 33 articles were assessed for eligibility. Then, after reading the full texts, 14 [17-30] publications met the selection criteria and included in this meta-analysis. Among these articles, SS-Xu's study lack of the data after lymphocytes treatment. We also incorporated the data into the calculation in the part of result. The reasons for the exclusion of 19 articles were the following: 5 articles lack complete data, 4 articles aimed at primary RSA compared to secondly RSA, and the other 3 articles were designed to compare the curative effect between partner group and healthy donor group. Finally, we excluded 7 non-randomized controlled trials. **Figure 1** shows the details of literatures selection process.

Validity assessment and data extraction

Each trial was strictly assessed by statistical method independently by two reviewers. And data extraction was accomplished by two completely independent investigators too. Each investigator abstracted data from each study and analyzed the data separately. Differences were resolved by common review of the data. We also searched the reference lists of retrieved studies, and did not apply any language restrictions. All articles were reviewed for pertinent references. Four patients from the LIT group and 9 from the NLIT group were missed to follow-up in the XQ-Liu's study [27]. Besides, the literature of SS-Xu lack of the data

Table 1. Validity criteria for each selected trial

Author	Year	Age/Mean age (yr)	Group	Total (cases)	Successful pregnancy cases	Abortion (cases)	Criterion of cured (weeks)	After immunotherapy (cases)	Successful pregnancy (cases)	Abortion (cases)	Source of lymphocytes (partner/donor)	Lymphocytes concentration
Xiaofang Li	2014	22~35/23.31±3.50	LIT	53	46	6	14	BA (+) 14	37	3	Partner	(2~3)×10^7/ml
								BA (-) 13	8	5		
			NLIT	53	28	19						
Xiaoqin Liu	2012	24~40	LIT	61	51	6 (unknown 4)	12	BA (+) 50	45	5	Partner or donor	(2~4)×10^7/ml
								BA (-) 7	6	1		
			NLIT	52	10	32 (unknown 10)						
Xiaomei Liang	2011	25~35/28.4±4.9	LIT	64	52	12	12	BA (+) 44	40	4	Partner	(2~4)×10^7/ml
								BA (-) 20	12	8		
			NLIT	64	26	38						
Tianhong Wang	2014	21~37/29.8±3.2	LIT	25	19	6	12	BA (+) 18	16	2	Partner or donor	(2~4)×10^7/ml
								BA (-) 7	4	3		
			NLIT	25	10	15						
Yunxian Dai	2013	22~37/27.23±3.50	LIT	43	37	6	12	BA (+) 31	29	2	Partner	(2~2.5)×10^7/ml
								BA (-) 12	8	4		
			NLIT	43	24	19						
yunying Wang	2014	NLIT: 23~39/30±5.9	LIT	50	37	10 (Not pregnant 3)	12	BA (+) 43	35	8	Partner	(2~4)×10^7/ml
		NLIT: 24~37/31±4.9						BA (-) 7	2	5		
			NLIT	42	15	18 (Not pregnant 9)						
Hongbo Jing	2014	LIT: 22~42/32	LIT	60	48	12	12	BA (+) 46	40	6	Partner	(3~4)×10^7/ml
		NLIT: 22~45/33.5						BA (-) 14	8	6		
			NLIT	60	24	36						
Lin Su	2012	LIT: 20~42/29.5	LIT	42	33	9	16	BA (+) 32	28	4	Partner	Not mentioned
		NLIT: 22~43/30.5						BA (-) 10	5	5		
			NLIT	42	17	25						
Baozhu liang	2006	LIT: 21~42/29.5	LIT	50	42	8	12	BA (+) 36	32	4	Partner	Not mentioned
		NLIT: 24~45/31.5						BA (-) 14	8	6		
			NLIT	50	26	24						
Shuju Yuan	2014	25~34/30.9±1.2	LIT	38	31	7	12	BA (+) 28	25	3	Partner	(2~4)×10^7/ml
								BA (-) 10	6	4		
			NLIT	38	15	23						
Zhiyang Jiang	2014	22~45/30	LIT	40	33	7	12	BA (+) 30	28	2	Partner	Not mentioned
								BA (-) 10	5	5		
			NLIT	40	27	13						
Xiaoli Du	2015	LIT: 23~41/28.3	LIT	40	32	8	12	BA (+) 34	29	5	Partner or donor	Not mentioned
		NLIT: 19~43/29.1						BA (-) 6	2	4		
			NLIT	40	16	24						
Wei Cui	2015	LIT: 22~43/31.5	LIT	60	52	8	12	BA (+) 44	39	5	Partner	(2~4)×10^7/ml
		NLIT: 21~43/30.5						BA (-) 16	9	7		
			NLIT	60	31	29						

LIT: lymphocytes immunotherapy; NLIT: not lymphocytes immunotherapy. Times of abortion: All patients underwent more than 2 times abortion. After immunotherapy (cases): BA (+) positive blocking antibody, BA (-) negative blocking antibody. Unknown: lost to follow up or unclear. Donor: the healthy donor.

Table 2. Details of trials comparing LIT with NLIT for treatment of recurrent spontaneous abortion

First author of trial	Randomization Appropriate	Double-Blind	Methods for Blinding Appropriate	Description of Withdrawal or Dropout	Jadad Score
Baozhu-Liang 2006	Unclear	Unclear	Unclear	Yes	4
Xiaomei-Liang 2011	Unclear	Unclear	Unclear	Yes	4
Lin-Su 2012	Unclear	Unclear	Unclear	Yes	4
Xiaoqin-Liu 2012	Unclear	Unclear	Unclear	No	3
Yunxian-Dai 2013	Unclear	Unclear	Unclear	Yes	4
Yunying-wang 2014	Unclear	Unclear	Unclear	Yes	4
Hongbo-Jing 2014	Unclear	Unclear	Unclear	Yes	4
Tianhong-Wang 2014	Unclear	Unclear	Unclear	Yes	4
Xiaofang-Li 2014	Unclear	Unclear	Unclear	Yes	4
Shuju-Yuan 2014	Unclear	Unclear	Unclear	Yes	4
Zhiyang-Jiang 2014	Unclear	Unclear	Unclear	Yes	4
Xiaoli-Du 2015	Unclear	Unclear	Unclear	Yes	4
Shanshan-Xu 2015	Unclear	Unclear	Unclear	Yes	4
Wei-Cui 2015	Unclear	Unclear	Unclear	Yes	4

Jadad scoring: randomization appropriate, Double-Blind, Methods for Blinding Appropriate: yes, 2 points; no, zero points; unclear, 1 point; description of withdrawal or dropout: yes, 1 point; no, 0points. Low Quality 1-3 points, High Quality 4-7 points.

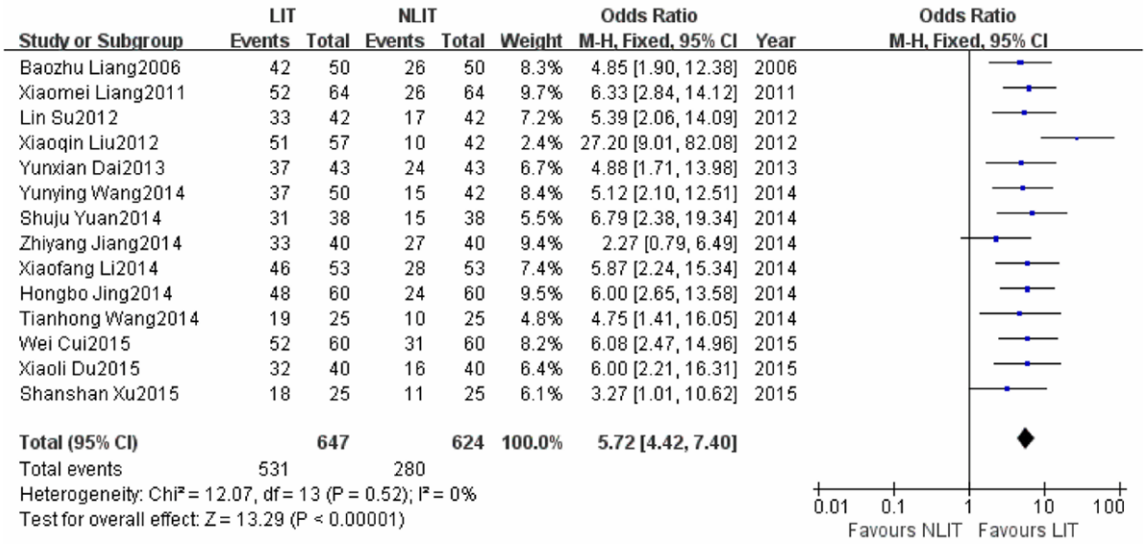

Figure 2. Odds ratios and proportions for pregnancy rate in LIT VS NLIT (OR=5.72, 95% CI 4.42-7.40, P<0.00001).

after lymphocytes treatment, so we just not list it in this part. The details of the included trials are listed in **Table 1**.

As shown in **Table 2**, the methodological quality of each trial was assessed by the Jadad scale. The evaluation included the type of randomization procedure used and whether it was concealed, the use of blinding, the presence of co-intervention, the completeness of follow-up of study subjects, and whether a sample size calculation had been performed.

Meta-analysis of results from the 14 RCTs

As shown in **Figure 2**, 14 studies including 1271 patients were evaluated for the pregnancy rate of RSA patients treated with or without LIT. The successful pregnancy was 531 of 647 patients in LIT group, and 280 of 624 patients in NLIT group. No significant statistical heterogeneity was present among these studies (I^2= 0%, P=0.52). Hence, a fixed-effect modal was adopted for analysis. The results showed higher successful pregnancy rate in LIT group com-

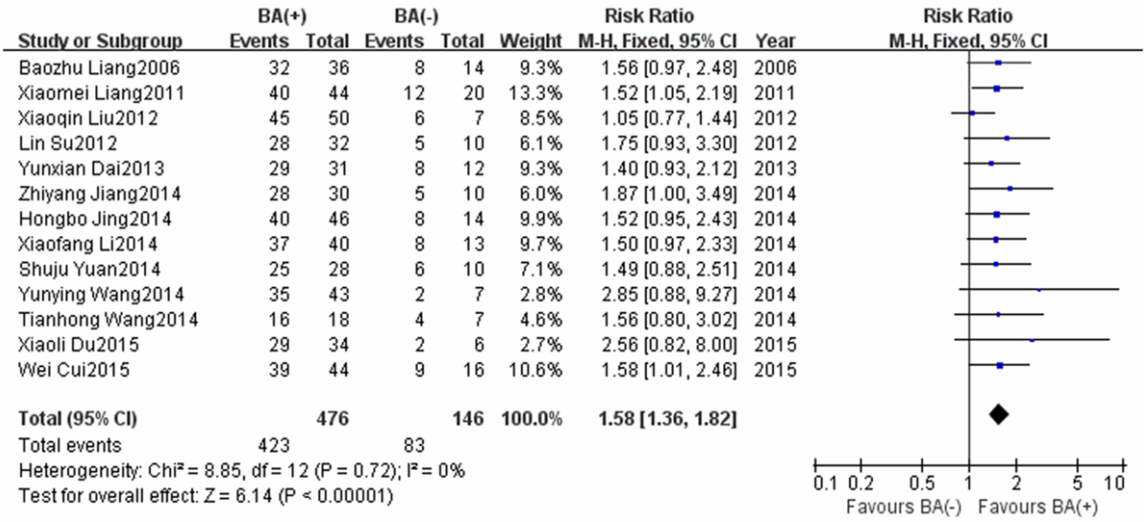

Figure 3. Odds ratios and proportions for pregnancy rate in BA (+) VS BA (-) (OR=1.58, 95% CI 1.36-1.82, P<0.00001).

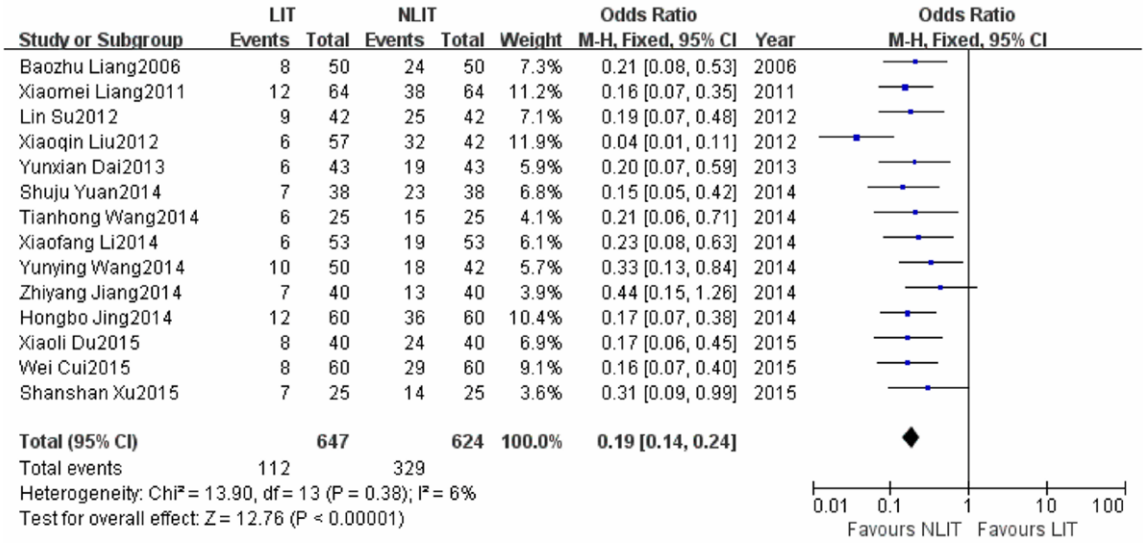

Figure 4. Odds ratios and proportions for abortion rate in LIT VS NLIT (OR=0.19, 95% CI 0.14-0.24, P<0.00001).

pared to NLIT group and it was statistically significant (OR=5.72, 95% CI 4.42-7.40, P<0.00001).

As shown in **Figure 3**, 13 studies were evaluated for the pregnancy rate in positive BA group compared to negative BA group. Overall, 423 of 476 patients in positive BA group and 83 of 146 patients in negative BA group were succeeded. No significantly statistical heterogeneity was present among these studies (I^2=0%, P=0.72). Hence, a fixed-effect modal was adopted for analysis. The results showed that after LIT, the pregnancy rate of positive BA group was higher than the negative BA group (OR=1.58, 95% CI 1.36-1.82, P<0.00001).

As shown in **Figure 4**, the abortion rate was 112 of 647 patients in LIT group and 329 of 624 patients in NLIT group. Among these studies, a low statistically significant heterogeneity was detected (I^2=6%, P=0.38). Hence, a fixed-effect modal was adopted for analysis. The results showed lower abortion rate in LIT group compared to placebo (OR=0.19, 95% CI 0.14-0.24, P<0.00001).

As shown in **Figure 5**, the abortion rate was 53 of 476 patients in positive BA group and 63 of 146 patients in negative BA group. No significant statistical heterogeneity was present among these studies (I^2=0%, P=0.99) and a fixed-effect modal was employed. From the outcome

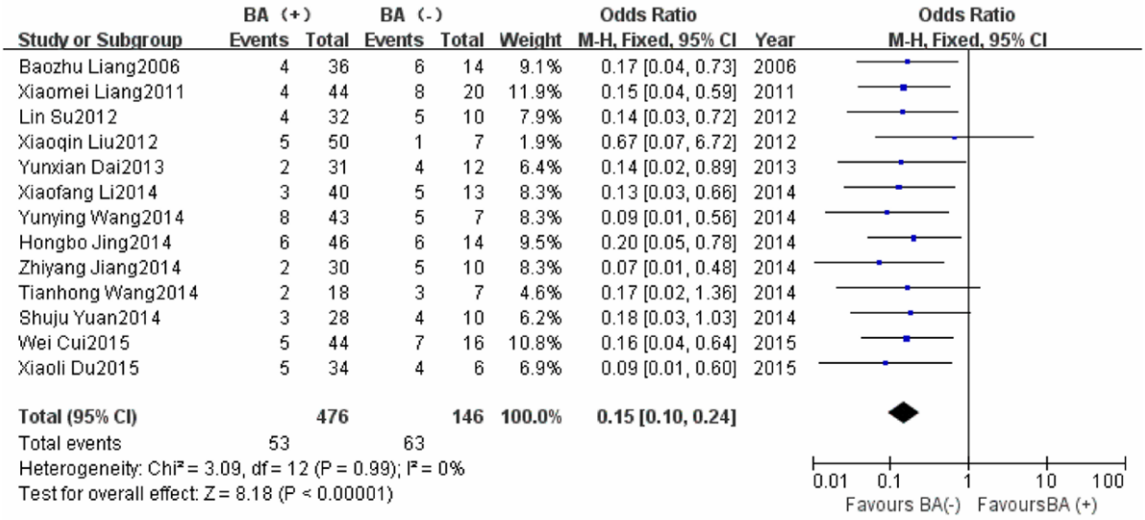

Figure 5. Odds ratios and proportions for abortion rate in LIT VS NLIT in BA (+) VS BA (-) (OR=0.15, 95% CI 0.10-0.24, P<0.00001).

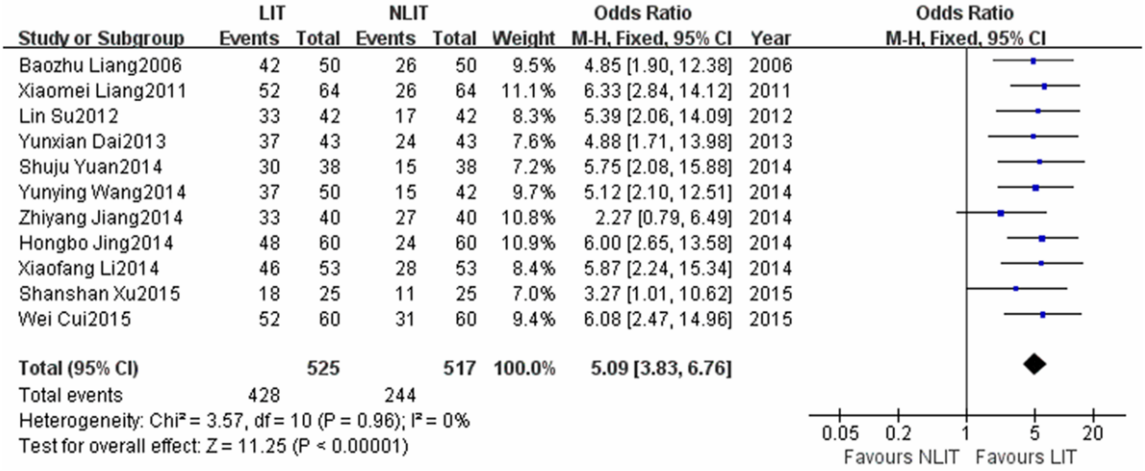

Figure 6. Odds ratios and proportions for pregnancy rate in LIT VS NLIT with partner lymphocytes (OR=5.09, 95% CI 3.83-6.76, P<0.00001).

of pooled estimates, the abortion rate in the positive BA group was higher than in the negative BA group (OR=0.15, 95% CI 0.10-0.24, P<0.00001).

Subgroup analysis of LIT

The source of the lymphocytes: partner versus (partner or healthy donor): For the source of partner lymphocytes, 10 studies met the standard to compare LIT with NLIT on RSA patients. Overall, 428 of 525 patients with LIT and 244 of 517 patients with NLIT get successful pregnancy. A fixed-effect modal was adopted for pooled analysis (I^2=0%, P=0.96). The results also showed a higher pregnancy rate in LIT group (**Figure 6**; OR=5.09, 95% CI 3.83-6.76, P<0.00001).

Only 3 studies involving 229 patients were tested for potential heterogeneity. The pooled analysis of these studies showed a significant association between LIT group and NLIT group (**Figure 7**; I^2=64%, P=0.06). A random-effect modal was adopted for analysis, and the results also showed a higher pregnancy rate in LIT group (OR=9.24, 95% CI 3.20-26.70, P< 0.0001). When we excluded the trail of XQ-Liu for sensitivity exclusion analysis, the heterogeneity markedly reduced from 64% to 0%, which indicated the potential sources of heterogeneity may come from this study. Similarly, final

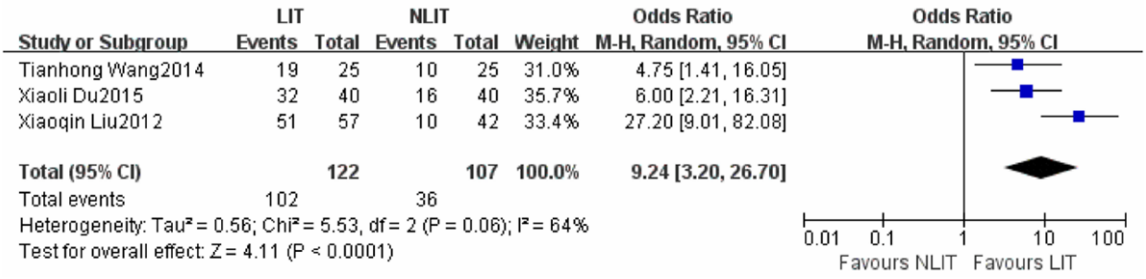

Figure 7. Odds ratios and proportions for pregnancy rate in LIT VS NLIT with partner or healthy donor lymphocytes (OR=9.24, 95% CI 3.20-26.70, P<0.0001).

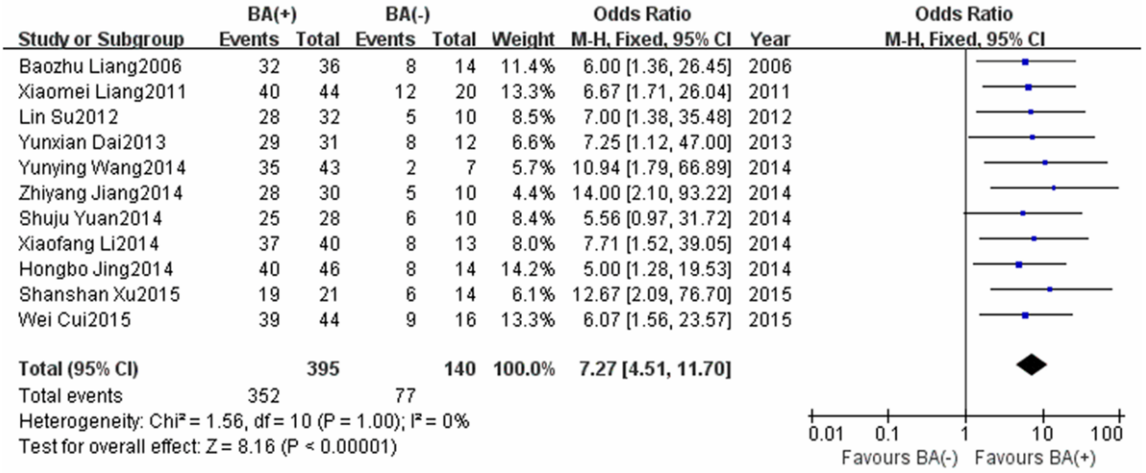

Figure 8. Odds ratios and proportions for pregnancy rate in BA (+) VS BA (-) with partner lymphocytes (OR=7.27, 95% CI 4.51-11.70, P<0.00001).

pooled estimate was still statistically significant (OR=5.46, 95% CI 2.52, 11.83, P<0.00001). After LIT, the results showed that the pregnancy rate in positive BA group was higher than the negative BA group, the results show in **Figure 8** (OR=7.27, 95% CI 4.51, 11.70, P<0.00001).

Publication bias

The potential publication bias was examined by the funnel plot and no significant publication bias was found. Here we just show one of them (**Figure 9**).

Discussion

RSA generally refers to three times or more of spontaneous abortion with a same male. Nowadays many scholars put forward saying that two consecutive spontaneous abortions are included in the category of RSA. The reason is that after two natural abortions, we need to be cautious enough to the pregnancy abortion rate, which can be as high as 50% above. The pathogeny of RSA is complex and some of the causal factors are chromosomal abnormality, genital tract anatomic abnormalities, endocrine disorders, infectious diseases, blood clots before status, and immunity. Besides, about 40% of cases are agnogenic. Due to the complex etiology of RSA, it remains refractory. There are interventions including immunotherapy, mononuclear cell immune therapy, anticoagulants (low molecular heparin or aspirin) treatment, intravenous drip immune globulin, and traditional Chinese medicine. However, for its effectiveness, scholars have different views [31].

In this meta-analysis, results mainly include the following aspects:

First, the comparison of pregnancy rates after immunotherapy. In this study, the pregnancy rate of immunotherapy group is higher than control group, though it is controversial to one of the literature [32]. However, in Gharesi Fard

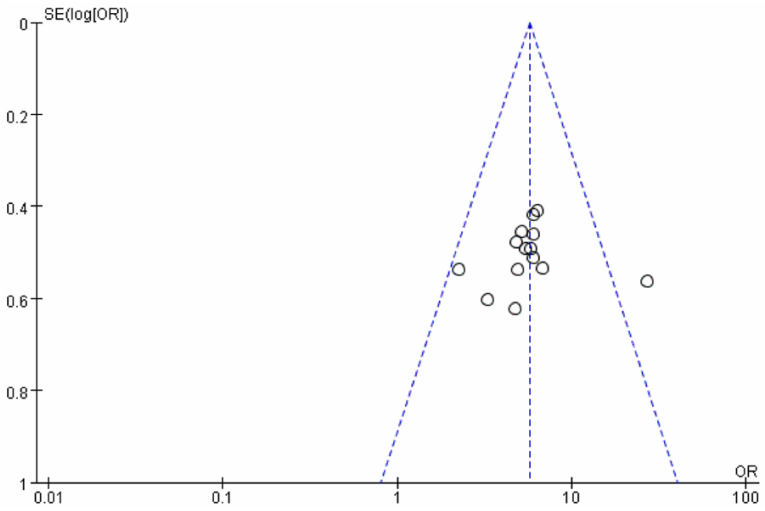

Figure 9. Funnel plot to assess publication and related biases.

B etc study, the success rate of lymphocyte active immunotherapy was reported to be 94.12%.

Second, the outcomes of positive BA compared to negative BA after immunotherapy. Higher pregnancy rate was observed in positive BA group. This is against with the literature data [33]. The pregnancy rate improved significantly in positive BA group compared to negative BA group which reasons may be attributed to immunoreactions. First, lymphocytes active immunotherapy is not only increasing the BA level of patients, but also improves the patients' immune status to develop embryos, reduces the toxicity of natural killer cells, and prevents the maternal immune reactions against embryos to increase the success rate of pregnancy. After immunotherapy, patients with negative BA can succeed pregnancy again, and the reason is active immunotherapy likely to induce the cellular immunity of patients [34]; However, after treatment, some of the positive BA patients fail to become pregnant which may due to embryo chromosomal abnormalities, gene mutation, and the early stages of viral infection. The influence of related factors is not yet clear. In Zare etc. study [35], it was found that in some of the RSA patients who lack of zinc, negative BA couldn't turn into positive one after immunotherapy; hence, they suggested these patients should take zinc before immunotherapy in order to improve the effectiveness of LIT.

Third, after immunotherapy, the positive rate of BA was obviously improved. Some studies reported that with the increasing times of LIT, positive BA rate increases gradually [32, 36] and immunotherapy for 1 to 2 periods may lead to higher BA positive rate. So BA may become one of the predictors of recurrent miscarriage.

Fourth, the abortion rate was lower in the LIT group compared to NLIT group. The results are identical to the positive BA compared to negative BA group. This result demonstrated that immunotherapy can reduce the abortion rate.

Finally, the sources of lymphocytes were taken into subgroup analysis. There was no significant difference between two groups (partner compared to partners or healthy donor). The results show that no matter the lymphocytes from, LIT lead to good response. To the generating of BA, partner lymphocytes are superior to the healthy donor's in the immunotherapy. However, more data and further research are needed to support this view.

The production mechanism of BA is complicated and undefined. Liang P etc. [37] found that LIT alters the proportion and function of most peripheral blood lymphocyte subsets. Some of these alterations may be beneficial for pregnancy maintenance. Whereas, they may also be potential markers for predicting subsequent abortion. Modern medical research shows that the embryo formation involves combination male and female genes (1:1), similar to the process of homograft. And genes from male will be seen as foreign substances in the female's body, which leads to serious maternal immune attack on embryos, further affects the normal development of embryos and cause miscarriage. In current clinical practice, lymphocytes from the male partner are injected into the patients (LIT), which causes an immune response to produce BA, so it play an important role in next pregnancy.

Limitations of this meta-analysis

In this meta-analysis, although the rigorous searching strategy and explicit selection and

exclusion criteria were utilized, above-mentioned significant findings should be interpreted with caution because of following several important limitations. First, the sources of the lymphocytes are mainly from the partner, and it may lead to different results if the sources are different. Second, there are difference in auxiliary examination level, the diagnosis, and nursing methods. Third, the national culture, living environment, psychological factors, basic diseases of pregnant women, and genetic differences between individuals can affect the results of the study. Fourth, we do not have statistical data for some of the complications such as infectious diseases, graft versus host disease, bleeding tendency, thrombocytopenia, osteoporosis, prenatal pre-eclampsia and fetal developmental delay, newborn thrombocytopenia, and intracranial bleeding. In addition, BA extraction, production and detection index lack of agreement. Patients are given priority to naturally conceive; however, there is yet existence of assisted reproductive technology, which is not clear. And it is important to identify the abortion times. Furthermore, although the patient's age and number of abortions have no statistical significance across tested RCTs, we lack of data to analysis in dividing. Because all of the selected articles used the same therapeutic doses, we could not distinguish the effects between different doses of lymphocytes.

In this meta-analysis, only RCTs with Chinese patients are screened, which may lead to ethnic variation. Besides, recurrent abortion becomes the patient's psychological and mental burden which may also affect the pregnancy. Immunotherapy, if effective, will benefit the patients. It also reduces the unnecessary abortion harm to the body and the physiological and economic burden. In addition, due to these limitations, further studies are needed to confirm whether the effect of drug treatment can be imparted to patients in different countries.

In summary, this meta-analysis revealed that the immunotherapy is beneficial for RSA patients with negative BA. After treatment, BA positive rate increased significantly. In pregnant woman, pregnancy success rate in positive antibody group is significantly higher than the negative one. After immunotherapy, with the increasing of BA, the maternal immune tolerance, the survival of fetus, and the pregnancy success rates all increase. This meta-analysis suggests that there is still considerable risk of miscarriage after immunotherapy. Using male partner lymphocytes to treat the recurrent abortion with negative BA is a simple and economical method, with no obvious adverse reaction. This intervention has a good clinical application value and good prospects for patients.

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Disclosure of conflict of interest

None.

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References

[1] Beer AE, Semprini AE, Zhu XY and Quebbeman JF. Pregnancy outcome in human couples with recurrent spontaneous abortions: HLA antigen profiles; HLA antigen sharing; female serum MLR blocking factors; and paternal leukocyte immunization. Exp Clin Immunogenet 1985; 2: 137-153.

[2] Nonaka T, Takakuwa K, Ooki I, Akashi M, Yokoo T, Kikuchi A and Tanaka K. Results of immunotherapy for patients with unexplained primary recurrent abortions--prospective non-randomized cohort study. Am J Reprod Immunol 2007; 58: 530-536.

[3] Qin W, Yang N and Wang Y. Different sources of immunotherapy in unexplained recurrent miscarriage. Guangdong Medical Journal 2010; 31: 64.

[4] Cramer DW and Wise LA. The epidemiology of recurrent pregnancy loss. Semin Reprod Med 2000; 18: 331-339.

[5] Takakuwa K, Kanazawa K and Takeuchi S. Production of blocking antibodies by vaccination with husband's lymphocytes in unexplained recurrent aborters: the role in successful pregnancy. Am J Reprod Immunol Microbiol 1986; 10: 1-9.

[6] Ena-Peng RY. Significance and Change of Blocking Antibody before and after Active

Immunotherapy in Unexplained Recurrent Spontaneous Abortion. Journal of Practical Obstetrics and Gynecology 2010; 26: 773-775.
[7] Zhang YL, JG, Jin Y, Yao RJ, Liu WE. The detection of blocking antibody clinical significance in spontaneous abortion patients. Practical Preventive Medicine 2009; 6: 1917-1918.
[8] Shankarkumar U, Pradhan VD, Patwardhan MM, Shankarkumar A and Ghosh K. Autoantibody profile and other immunological parameters in recurrent spontaneous abortion patients. Niger Med J 2011; 52: 163-166.
[9] Liu JL. The effect of lymphocyte from husband for treatment of recurrent spontaneous abortion. Contemporary Medicine 2011; 11: 104-105.
[10] Huang L JC, Huang LJ. Clinical observation of immunotherapy for recurrent abortion. Jilin Medical 2012; 5: 927-928.
[11] Tian C JL, You Y, Niu YQ. Clinical comparison study on three kinds of therapy for unexplained recurrent spontaneous abortion. Modern Journal of Integrated Traditional Chinese and Western Medicine 2012; 14: 1487-1489.
[12] Wu TH YZ, Yi B, Li GG, Chen WN, Chen CM, Zeng Y, Liang PY. Study on the correlation of HLA-DQ alleles and unexplained recurrent miscarriage. Chinese Journal of Immunology 2013; 9: 955-959.
[13] Hwang JL, Ho HN, Yang YS, Hsieh CY, Lee TY and Gill TJ 3rd. The role of blocking factors and antipaternal lymphocytotoxic antibodies in the success of pregnancy in patients with recurrent spontaneous abortion. Fertil Steril 1992; 58: 691-696.
[14] Pena RB, Cadavid AP, Botero JH, Garcia GP, Gallego MI and Ossa JE. The production of MLR-blocking factors after lymphocyte immunotherapy for RSA does not predict the outcome of pregnancy. Am J Reprod Immunol 1998; 39: 120-124.
[15] Khonina NA, Broitman EV, Shevela EY, Pasman NM and Chernykh ER. Mixed lymphocyte reaction blocking factors (MLR-Bf) as potential biomarker for indication and efficacy of paternal lymphocyte immunization in recurrent spontaneous abortion. Arch Gynecol Obstet 2013; 288: 933-937.
[16] Wong LF, Porter TF and Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev 2014; 10: CD000112.
[17] Liang BZ ZT, Li C, Li YP. Study of immunotherapy with lymphocytes in women with recurrent spontaneous abortion. Maternal and Chid Health Care of China 2007; 25: 3561-3562.
[18] Jing HB ZW, Wang L. Clinical observation of 60 cases of recurrent spontaneous abortion treated with lymphocyte active immunotherapy. J Medical Forum 2014; 7: 170-171.
[19] Su L YS, Ou S, Chen P. The efficacy of active immunotherapy for recurrent spontaneous abortion. Jilin medical 2012; 9: 1822-1823.
[20] Xu SS. Clinical analysis of lymphocyte injection treatment for 50 recurrent spontaneous abortion patients with negative blocking antibody. Chin J Mod Drug Appl 2015; 2: 20-21.
[21] Yuan SJ. Immunotherapy effect analysis of recurrent miscarriage. Cardiovascular Disease Journal Of integrated traditional Chinese and Western Medicine 2014; 6: 105.
[22] Wang TH. Efficacy analysis of lymphocyte immunotherapy in treatment of reccurent misscarrige. China Medicine and Pharmacy 2014; 8: 197-199.
[23] Cui W YL, Li J, Jinju X, Weihong Z, Congwen S. Analysis of the effect of lymphocyte immunotherapy for reccurent spontanous abortion. Current Immunology 2015; 3: 230-232.
[24] Li XF JZ. The study of lymphocyte immune therapy in the treatment of negative blocking antibody in recurrent spontaneous abortion patients. Medcal Information 2014; 20: 225-226.
[25] Du XL. The Investigation of the Clinical Efficacy of Recurrent Spontaneous Abortion Using Lymphocyte Active Immunotherapy. Medical Innovation of China 2015; 11: 131-133.
[26] Liang XM. Evaluation of the efficacy of immunotherapy for recurrent spontaneous abortion. Journal of North Pharmacy 2011; 5: 51-52.
[27] Liu XQ LT. Level of the blocking antibodies in women with recurrent spontaneous abortion and the effect of immunotherapy with lymphocytes. Chinese Journal of Healthy Birth & Child Care 2012; 5: 276-278.
[28] Dai YX. The Research Research of Lymphocyte Immune Treatment for Recurrent Abortion. Chinese and Foreign Medical Research 2013; 3: 27-28.
[29] Wang YY. Clinical analysis of 50 cases of recurrent spontaneous abortion treated with active immunotherapy. Chinese Journal of Trauma and Disability Medicine 2014; 103-104.
[30] Jiang ZY. The clinical analysis of Immunotherapy for recurrent spontaneous abortion. Hebei Medical Journal 2014; 7: 986-987.
[31] Zhang JP CY. Controversy on diagnosis and management of recurrent spontaneous abortion. Chinese Journal of Obstetrics & Gynecology and Pediatrics (Electronic Edition) 2015; 3: 292-296.
[32] Zhao XF SJ, Cai T, Luo LZ, Ou YZ. Clinical research of blioking antibodies test and lymphocyte immunotherapy for reccurrent spontanous abortion patient. International Journal of Laboratory Medicine 2013; 2: 156-157.

[33] She H YH, Lv F, Wang P. Clinical Analasis of Lymphocytes Active Immunotherapy In the Treatment of reccurent Spontanous Abortion. China J Obstet Gynecol Pediatr (Electron Ed) 2014; 5: 98-101.

[34] Komlos L, Vardimon D, Notmann J, Ben-Rafael Z, Hart J, Klein T, Levinsky H and Halbrecht I. Mixed maternal-paternal lymphocyte cultures before and after immunotherapy for recurrent spontaneous abortions. Am J Reprod Immunol 1996; 35: 30-33.

[35] Zare A, Saremi A, Hajhashemi M, Kardar GA, Moazzeni SM, Pourpak Z, Salehian P, Naderi M, Safaralizadeh R and Nourizadeh M. Correlation between serum zinc levels and successful immunotherapy in recurrent spontaneous abortion patients. J Hum Reprod Sci 2013; 6: 147-151.

[36] Yu HL XD, Chao L, Chen C, Han YL. Effects on blocking antibody turning positive: Number of immunotherapy and injection methods with partner ymphocyte. Chinese Journal of Obstetrics and Gynecology 2013; 12: 903-906.

[37] Liang P, Mo M, Li GG, Yin B, Cai J, Wu T, He X, Zhang X and Zeng Y. Comprehensive analysis of peripheral blood lymphocytes in 76 women with recurrent miscarriage before and after lymphocyte immunotherapy. Am J Reprod Immunol 2012; 68: 164-174.