A three-arm, multicenter, open-label randomized controlled trial of hydroxychloroquine and low-dose prednisone on recurrent spontaneous abortion with undifferentiated connective tissue diseases: Protocol for the immunosuppressant regimens for Living FEtuses (ILIFE) Trial

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Study protocol

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Abstract

Background Undifferentiated connective tissue disease (UCTD) is known to induce adverse pregnancy outcomes, and even recurrent spontaneous abortion (RSA) by placental vascular damage and inflammation activation. Anticoagulation can prevent pregnancy morbidities. However, it is unknown whether the addition of immune suppressant to anticoagulation can prevent spontaneous pregnancy loss in UCTD patients. The purpose of this study is to evaluate the efficacy of hydroxychloroquine (HCQ) and low-dose prednisone on recurrent pregnancy loss for women with UCTD. Methods The Immunosuppressant for Living Fetuses (ILIFE) Trial is a three-arm, multicenter, open-label randomized controlled trial of comparing hydroxychloroquine combined with low-dose prednisone and anticoagulation, hydroxychloroquine combined with anticoagulant, or anticoagulation alone in treating UCTD women with recurrent spontaneous abortion. Eligible patients will be randomly assigned to each of the tree arms with a 1:1:1 allocation ratio. The primary outcome is the rate of live births. Secondary outcomes include adverse pregnancy outcomes and progression of UCTD. Discussion This is the first multi-center, open-label, randomized controlled trial which evaluates the efficacy of immunosuppressant regimens on pregnancy outcomes and UCTD progression. It will provide evidence on whether the immunosuppressant ameliorates the pregnancy prognosis in UCTD patients with RSA and the progression into defined connective tissue disease.

Background

Undifferentiated connective tissue disease (UCTD) is defined as at least one symptom or sign suggesting connective tissue diseases (CTDs) and with presence of at least one of auto-antibodies, while not fulfilling any classification criteria of a defined CTD[1,2]. UCTD accounts for 20%–52% of patients with CTDs and is characterized by varying symptom onset time and antibody profiles. UCTD is more common in women, with a male-to-female ratio ranging from 1:5 to 1:17, predominantly at the reproductive age[2–5]. UCTD may evolve into definite CTDs like systemic lupus erythematosus (SLE), systemic sclerosis, primary Sjögren's syndrome, mixed connective tissue disease, systemic vasculitis, polymyositis and rheumatoid arthritis[6].

UCTD is the most common rheumatic disease diagnosed during the first trimester of pregnancy and plays a potential causal role of autoimmune disorders in the occurrence of pregnancy complications[7]. Antinuclear antibodies and inflammation in UCTD may impair platelet activity, unbalance coagulation/anticoagulation, increase the risk of thrombosis, cause collagen synthesis defect and endothelial dysfunction[8–10]. Therefore, it could increase uterine artery resistance, inhibit placenta development and remodelling, and thus increase the risk of pre-eclampsia, intrauterine growth retardation (IUGR), small for gestational age infant (SGA) and recurrent spontaneous abortion (RSA)[4,11–14]. Spinillo found that women with UCTD had a higher rate (39.0%) of at least 1 spontaneous pregnancy loss than healthy controls with an odds ratio of 5.92 (95% CI, 2.1–17.8)[14]. Other studies reported that the prevalence of adverse pregnancy outcomes ranged from 27% to 30% in women with UCTD[13,15].

In addition to adverse pregnancy outcomes, women with UCTD may experience a disease flare and even evolve into definite CTDs during the course of pregnancy and puerperium[16,17]. The incidence of disease flare was about 24%~32%, higher than the 11% in the non-pregnant patients[13,15]. An average of 30% of UCTD women eventually develop a well-defined CTD[17–20], especially during pregnancy, and require treatments with steroids and immunosuppressant like hydroxychloroquine (HCQ).

There is no consensus or guideline about treatments for recurrent spontaneous abortion (RSA) in pregnant women with UCTD. UCTD shares similar pathogenesis of placental vascular thrombosis as systemic lupus erythematosus +/- antiphospholipid antibody syndrome (APS) for which low-dose aspirin and low-molecular-weight heparin (LMWH) are used to prevent fetal losses and improve pregnancy outcomes[21,22]. Therefore, antiaggregation and anticoagulation was considered as a safe way reducing the risk of miscarriages among women with UCTD [23,24]. Despite low-dose aspirin and LMWH treatments, RSA women with UCTD still suffered from pregnancy morbidity[23,25].

Taking placental inflammation and imbalance of immune system of UCTD into consideration, immunosuppression is hypothesized to be a potential rescue. Immunosuppression may also be beneficial in reducing disease recurrence and progression. Currently, commonly used immunosuppressant includes low-dose corticosteroids, HCQ, azathioprine, cyclosporin, immunoglobulin[4]. Corticosteroids and HCQ used in nearly half of UCTD patients are considered as symptomatic treatment during non-pregnancy period. Low dose of corticosteroids were proved to inhibit T cells and Natural Killer (NK) cells, to improve the fertilization rate[26,27], and to decelerate the progression into rheumatic diseases. Several studies showed that prednisolone of 5–20mg per day can improve pregnancy outcomes[25,28–31]. However, there still lacks a large clinical trial to identify an optimal treatment. HCQ has been proved to decelerate the progress of UCTD into defined CTD in non-pregnant patients[32], decrease the risk of disease flare in pregnant SLE patients[33–35], increase the live birth rate, and reduce preterm delivery in antiphospholipid antibodies (aPL) positive patients[36], and prevent congenital heart block in neonates exposed to anti-SSA and anti-SSB antibodies[37]. It remains unknown whether HCQ could affect pregnancy outcomes of UCTD women. A study of 133 pregnancies of CTDs showed evidence for the safety of HCQ therapy during pregnancy but no differences in live birth rate [38]. Recently data from 66 UCTD patients with RSA treated by hydroxychloroquine plus low-dose prednisone combined with anticoagulation in our center seemed promising. There were 66 successful pregnancies (the live birth rate 97.1%) in 68 pregnancies.

Given that RSA in UCTD patients is mediated by autoimmune factors and may be improved by the addition of immune suppressant to anticoagulation. The objective of the ILIFE trial is to assess efficacy of low-dose corticosteroids and HCQ combined with anticoagulation in treating UCTD patients with recurrent pregnancy loss.

Methods/design

Study design

The ILIFE trial is a three-arm, multicenter, open-label, randomized controlled trial (RCT) to assess efficacy of low-dose corticosteroids and HCQ combined with anticoagulation in treating UCTD women with recurrent pregnancy loss.

The trial will be undertaken at rheumatology outpatient clinics in six university-affiliated, tertiary hospitals in China, including Shanghai Renji Hospital, the First Affiliated Hospital of Anhui Medical University, China-Japan Union Hospital of Jilin University, the Affiliated Wuxi No.2 People's Hospital of Nanjing Medical University, Jiangsu Province Hospital and Xiangya Hospital of Central South University. The study design is presented in the flowchart in Fig. 1. Standard protocol items: recommendations for interventional trials (SPIRIT) are provided as an additional file 1. This study was approved by institutional review boards (IRB) of study sites.

Recruitment

The study population consists of UCTD women who had experienced at least two miscarriages and try to conceive. Rheumatologists in each study site will check inclusion and exclusion criteria of UCTD women. A research assistant will present the study information during recruitment. Eligible patients who provide informed consents will be enrolled to the trial.

The anticipated duration of recruitment is 2 years, and the duration of participation of each patient is from 24 weeks to 72 weeks.

Inclusion criteria

Women who meet the following inclusion criteria will be eligible to participate in the study:

- At reproductive age (20–40 years old).
- Trying to conceive.
- Diagnosed with UCTD[2]: at least one symptoms or signs suggesting CTD and with at least one presence of auto-antibodies, including antinuclear antibody (ANA), anti-SSA antibody, while not fulfilling any classification criteria of a defined CTD.
- Diagnosed with RSA[39]: two or more failed pregnancies of unknown origin.
- Providing written informed consent.

Exclusion criteria

Women who meet any of the following criteria will be excluded from the study:

- Any known etiology of previous pregnancy loss:

1. Diagnosis of antiphospholipid antibody syndrome.
2. Known paternal, maternal or embryo chromosome abnormality.
3. Maternal endocrine dysfunction: corpus luteal insufficiency; polycystic ovarian syndrome; premature ovarian failure (follicle stimulating hormone, FSH ≥20uU/L in follicular phase); hyperprolactinemia; thyroid disease; diabetes mellitus; other hypothalamic–pituitary–adrenal axis abnormality.
4. Maternal anatomical abnormality: uterine malformation; Asherman syndrome; cervical incompetence; uterine fibrosis more than 5 cm.
5. Vaginal infection.

- Any known severe cardiac, hepatic, renal, hematological or endocrinal diseases:

  1. Alanine transaminase (ALT) or aspartate transaminase(AST) more than twice the upper limit of normal.
  2. Clearance of creatinine less than 30mL/min.
  3. Leucocytes less than 2.5*10^9/L, or Hemoglobine less than 85g/L, or Platelet less than 50~10^9/L.

- Any active infection:

  1. Active viral hepatitis including hepatitis B virus (HBV), hepatitis C virus (HCV).
  2. Active infection including V aricella-zostervirus(VZV), human immunodeficiency virus (HIV), syphilis or tuberculosis.

- Allergic to prednisone, hydroxychloroquine, LMWH or aspirin.
- Disease history as follows:

  1. Past history of digestive ulcers or upper gastrointestinal hemorrhage.
  2. Past history of malignancy.
  3. Past history of epilepsia or psychotic disorders.

- Woman unable to consent or impossible to follow-up.

Randomization

After obtaining the written informed consent, eligible patients will be randomized to one of the three treatment arms with a ratio of 1:1:1 stratified by site. Randomized numbers will be generated through SAS software at the trial methods centre located in Shanghai Renji Hospital. Opaque sealed envelopes contain identification numbers and the allocation information will be used for the randomization and allocation.

Treatments

Included patients are randomized to three treatment groups: 1) low-dose prednisone (10mg once daily orally), HCQ (100mg to 200mg twice daily orally), and anticoagulants using low-dose aspirin (50mg once daily orally) and subcutaneously administered LMWH once daily (enoxaparin 40mg or dalteparin 5000IU or nadroparin calcium 4100U); 2) HCQ (100mg to 200mg twice daily orally), and the same anticoagulant treatments as in the first group; and 3) the anticoagulant treatments only. Treatments are initiated before conception (recommended 3–6 months) and stopped at 6 weeks' post-gestation or miscarriage or after 24 weeks of treatment in the absence of pregnancy. Supplements such as folic acid, calcium tablet and vitamins are allowed throughout pregnancy.

Assessments and data collection

The assessments will be conducted at the screening and preparation phases and then routinely throughout the trial. The screening phase will last for 4 weeks. General profiles including age, height, weight, ethnicity, occupation, obstetric and rheumatology histories, medication uses, allergy and antecedents will be recorded during the screening phase. Following the screening phase is the 24 weeks pre-conception phase. If participants are not pregnant at week 24, the treatment and their participation will be terminated. Once pregnancy is confirmed, the pregnancy assessments will be launched and the index time is set to week 0. Pregnant women will be followed once every 4 weeks in the next 42 weeks gestation period. The last visit will be conducted at 6 weeks after delivery. The assessment will be terminated when spontaneous pregnancy loss or childbirth takes place. Children's observations will last for one year after the end of the study, including vision, hearing and growth parameters. The schedules for assessments and data collection are showed in Fig. 2. Data will be collected in paper case report forms (CRFs) stored at a secure place and double entered into the electronic trial database.

Outcome measures

Primary outcome

The primary outcome is the rate of live births.

Secondary outcomes

The secondary outcomes include adverse pregnancy outcomes and progression of UCTDs:

- The rate of miscarriage.
- Premature birth (live birth between 28 and 37 weeks of gestations).

- Concerning the child: intrauterine growth retardation, gestational age and weight at birth, survival at 28 days, safety data at 42 days of life and congenital abnormality (congenital heart conduction block, neonatal lupus or malformation).
- Concerning the mother: eclampsia (new-onset hypertension after 20 weeks of gestation, with or without proteinuria more than 300mg/24h, with or without any organ damage with seizures), infection, gestational diabetes mellitus.
- Concerning the UCTD: activity of UCTD (at any time of gestation), progression of UCTD (post-partum evaluation).

Sample size

There is very limited evidence on the spontaneous pregnancy loss in pregnancies of women with UCTD and the reported risk rates for RSA varied substantially across studies. The live-birth rate of a randomized trial enrolling 364 women with a history of unexplained recurrent miscarriage was 69.1% in the group receiving aspirin plus nadroparin[40]. In another cohort study of women with positive aPL, HCQ can improve the live birth rate from 57% to 67%[41]. A double-blind placebo randomized control trial involving 160 patients with unexplained recurrent miscarriage showed that the addition of prednisolone to heparin and aspirin might be beneficial, with 70.3 % of women in the prednisolone group having successful pregnancy outcome versus 9.2 % in the placebo group[42]. Data from 68 pregnancies in our department showed that the live birth rate was 97.1% in UCTD women with RSA treated by hydroxychloroquine plus low-dose prednisone combined with anticoagulation.

Our primary comparison is between the combined use of prednisone, HCQ and anticoagulant with the anticoagulant only. Based on our pilot and limited published data, we hypothesize that the proportion of live birth rates with the treatment strategy using prednisone, HCQ, aspirin and LMWH is 85% vs 70% for anticoagulant only. We need to enrol 354 women (118 in each group) to allow us to detect the difference in spontaneous pregnancy loss between the treatment groups with a power of 0.8 at a two-sided P value of 0.05. Since not all women would get pregnant during the trial, we planned to include about 420 participants (140 in each group) to account for a 15% loss to follow-up[43].

Data analysis

For categorical data, frequencies and percentages will be presented. Continuous data will be presented as the mean and standard deviation or median and interquartile range. The primary outcome will be summarized as the proportion of live birth in each group. All data will be analyzed according to the intention-to-treat approach in which all randomized patients are included. Occurrences of the primary and secondary endpoints are compared between the treatment groups. Results are presented as risk ratios with corresponding 95% confidence intervals. A two-tailed $P< 0.05$ is considered statistically significant.

Trial management

A steering committee comprised of local principal investigator at each participating hospital will manage the trial. Screening and recruitment will be reviewed regularly at steering committee meetings; protocol deviations (e.g. late administration) will be distinguished from protocol violations (e.g., missed doses). Enrolment or adherence barriers will be addressed by implementing improvement strategies. Relevant clinical and laboratory data submitted to the steering committee will be checked for the completeness and accuracy. All investigators will undertake a standardized training for study procedures, data collection and adverse event reporting. The plans to promote participant retention and complete follow-up include: giving specific follow-up schedule and obtaining at least two phone contacts to ensure communication. The research assistant will contact participants by telephone regularly to improve adherence to medication and assessments. To maintain confidentiality, CRFs and other records will be identified by a coded number and initials only. Data tools and instruments are stored securely and access restricted to authorized study team members.

Safety monitoring

An independent data and safety monitoring board (DSMB) will meet regularly to ensure patient safety and data quality. Vital signs, which include blood pressure, heart rate, temperature and respiration rate, side effects and adverse events will be monitored regularly throughout the trial. Safety monitoring and surveillance will be collected, processed, reviewed, evaluated, reported and discussed, including clinical pregnancy outcomes, identified risks of drugs, laboratory abnormalities and imaging changes. If serious adverse outcomes or rare possible risks are involved, a multidisciplinary Safety Management Team (SMT) will be established in each participating hospital and the results will be communicated with DSMB.

Discussion

UCTD is a systemic rheumatic disorder occurred mainly in women at the reproductive age and associated with a higher rate of recurrent spontaneous abortion[42]. To date, there has not yet been an optimal therapy for treating RSA of UCTD patients. Among all the possible causes of RSA in UCTD patients, immunological dysfunction is one of reversible backgrounds affecting pregnancy outcomes. It is recommended that pregnancy in patients with SLE or APS be treated using immunotherapy and the anticoagulation therapy. It is known that the immune system imbalance and impaired vascular function in UCTD could contribute to the pathogenesis of preeclampsia and placental vascular thrombosis as is the case in SLE or APS[9,43]. Therefore, immunotherapy may play a role in reducing the immune system dysfunction and vascular injury caused by UCTD, and then decreasing the adverse pregnancy outcomes. Meanwhile, immunosuppressant may prevent patients from disease recurrence and progressioninto defined connective tissue disease.

Based on limited published data, immunotherapy such as low dose corticosteroids and HCQ showed clinical benefit for RSA in patients with UCTD. There still lacks a large scale, well-designed clinical trial to assess the efficacy of immunotherapy in patients with RSA and UCTD. The ILIFE study is the first RCT to evaluate the efficacy of prednisone and HCQ in this patient population. This trial focuses on ameliorating the pregnancy outcomes, investigating the role of rheumatic diseases in pregnancy, and also exploring how pregnancy modifies the progress of UCTD. We expect that the ILIFE study will provide high quality evidence to inform treatment decision making for UCTD women with RSA.

Trial status

Patient recruitment will begin from August 2019 to August 2021. The trial will be completed by January 2023.

Protocol date and version: June 10, 2019; version 1.1.

Abbreviations

ALT: Aspartate transaminase; ANA: Antinuclear antibody; aPL: Antiphospholipid antibodies; APS: Antiphospholipid antibody syndrome; AST: Alanine transaminase; CRFs: Case report forms; CTDs: Connective tissue diseases; DSMB: Data and safety monitoring board; FSH: Follicle stimulating hormone; HBV: Hepatitis B virus; HCQ: Hydroxychloroquine; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; ILIFE: the Immunosuppressant for LIving FEtuses; IRB: Institutional review boards; IUGR: intrauterine growth retardation; LMWH: Low-molecular-weight heparin; NK: Natural Killer; RSA: Recurrent spontaneous abortion; RCT: Randomized controlled trial; SGA: small for gestational age infant; SLE: Systemic lupus erythematosus; SMT: Safety Management Team; SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials; UCTD: Connective tissue diseases; VZV: V aricella-zostervirus.

Declarations

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LLJ is the principal investigator. ZCY designed the study. YSY and NRN developed this study protocol and were also responsible for receiving ethics approval. NYN drafted the manuscript and the manuscript was revised by YSY. XF designed statistical elements of the trial. All authors are actively involved in carrying out the trial. ZCY will serve as study coordinator. YSY, WSL and LYK will participate in data collection. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Central ethical approval has been confirmed from the Institutional Review Board (IRB) of Shanghai Renji Hospital (ref approval no. KY–2019–056) and we will not begin recruiting at other centres in the trial until local ethical approval has been obtained. Any protocol amendments will require IRB approval. The protocol identification number on ClinicalTrials.gov is NCT03671174. We will conduct the study following the declaration of Helsinki and obtain informed consent from each participating patient before randomization. Study participation is voluntary and can be withdrawn at any time without provision of reason. We will disseminate our results through medical conferences and peer-reviewed clinical journals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Additional file

Additional file 1: SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents. (DOC 139kb)

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Figures

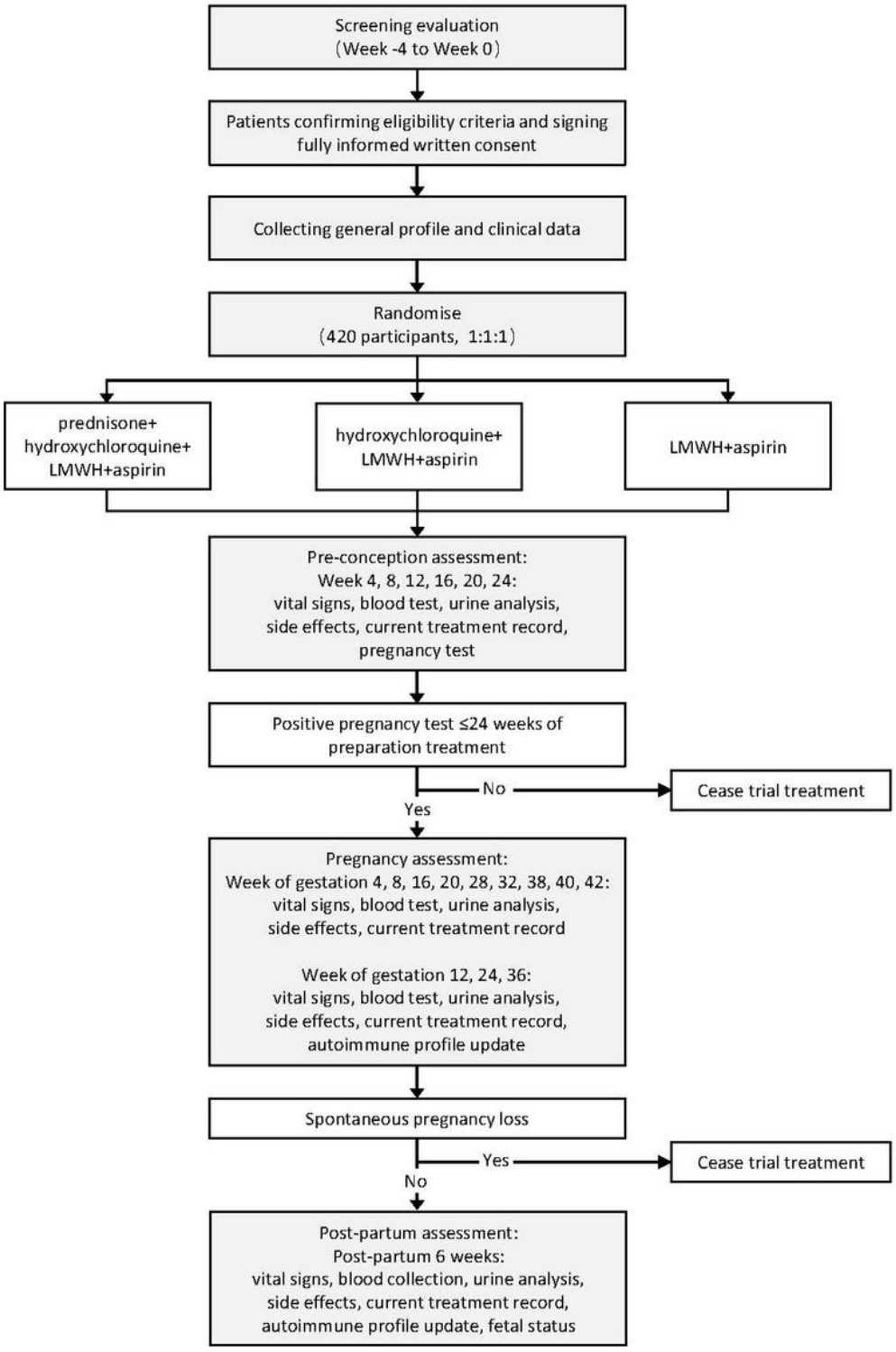

Figure 1

Trial Design Schema

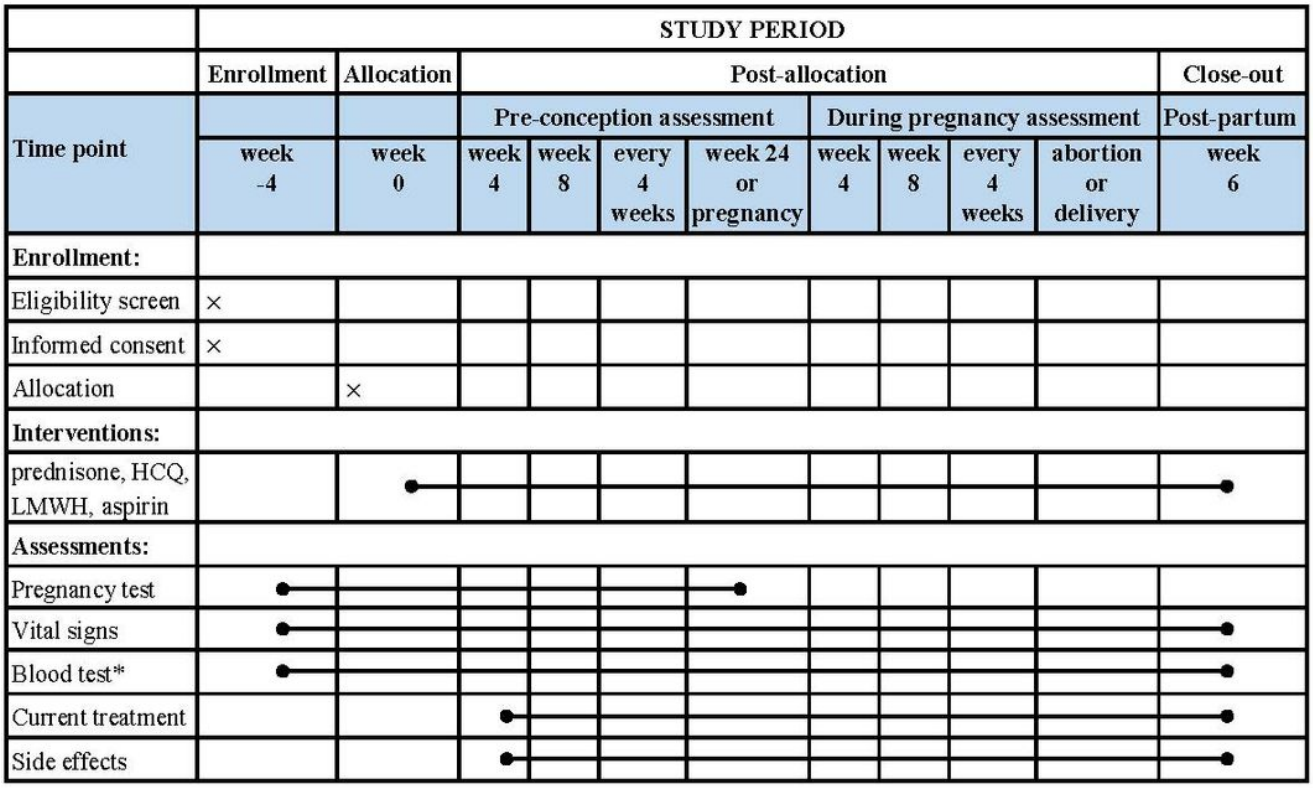

Figure 2

Time schedule of enrollment, interventions, and assessments. * Blood count, Biochemistry, Autoimmune profile, Coagulation profile, et al.

Supplementary Files

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- supplement1.doc