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Review

HIBISCUS: Hydroxychloroquine for the secondary prevention of thrombotic and obstetrical events in primary antiphospholipid syndrome☆

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ABSTRACT

The relapse rate in antiphospholipid syndrome (APS) remains high, i.e. around 20%–21% at 5 years in thrombotic APS and 20–28% in obstetrical APS [2, 3].

Hydroxychloroquine (HCQ) appears as an additional therapy, as it possesses immunomodulatory and antithrombotic various effects [4–16].

Our group recently obtained the orphan designation of HCQ in antiphospholipid syndrome by the European Medicine Agency.

Furthermore, the leaders of the project made the proposal of an international project, HIBISCUS, about the use of Hydroxychloroquine in secondary prevention of obstetrical and thrombotic events in primary APS. This study has been launched in several countries and at now, 53 centers from 16 countries participate to this international trial.

This trial consists in two parts: a retrospective and a prospective study.

The French part of the trial in thrombosis has been granted by the French Minister of Health in December 2015 (the academic trial independent of the pharmaceutical industry PHRC N PAPIRUS) and is coordinated by one of the members of the leading consortium of HIBISCUS.

1. Introduction

Antiphospholipid syndrome (APS) is characterized by the confirmed presence of antiphospholipid antibodies (aPL) and clinical manifestations such as thrombosis, and/or pregnancy morbidity and mortality [1]. APS may occur in the context of a background disease, autoimmune, mainly systemic lupus erythematosus (SLE) (secondary APS); when it remains isolated, it represents primary antiphospholipid

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syndrome (primary APS).

Nowadays, there is no drug which possesses the market authorization for the treatment of antiphospholipid syndrome (APS). There are international guidelines that recommend the use of oral vitamin K anticoagulants and/or of antiplatelet drugs for the prevention of thrombotic relapses and antiplatelet drugs and/or low molecular weight heparins for preventing obstetrical relapses.

Nevertheless, despite standard treatments according to international guidelines, the relapse rate remains high in antiphospholipid syndrome (APS), i.e. around 20%–21% at 5 years for thrombotic APS and 20–28% in obstetrical APS [2,3]. Survival rate in this population, most of them of young people, is low, i.e. at 90.1% at 10 years and 65% at 15 years [2,3].

New data on the pathogenesis of APS strengthen the need for additional or alternative therapies in this disease [4–13].

One of them appeared as one of the best candidates: Hydroxychloroquine (HCQ).

Several pharmacological mechanisms explain the therapeutic potential of Hydroxychloroquine in rheumatic and related diseases [14].

HCQ have various immunomodulatory, metabolic, cardiovascular, antithrombotic, and antineoplastic effects [15].

Task force report on antiphospholipid syndrome treatment trends underlined the need of concentrated efforts to perform large multicentric studies based on hydroxychloroquine use in primary and secondary prevention of relapses in APS [7].

Lastly, during the 15th International Congress on Antiphospholipid Antibodies (Istanbul 2016) and International Society of Thrombosis and Hemostasis (ISTH) congress (Berlin 2017) several relevant data focused on the interest of HCQ in APS [16].

1.1. Why HIBISCUS?

Only a major randomized study such as the HIBISCUS trial could allow formal conclusions on the potential major benefits of HCQ in increasing live birth rate in APS and decreasing thrombotic events recurrence. Therefore, only concentrated international efforts in this rare disease could allow to conclude on the benefit/risk balance of such a therapy and to obtain significant benefit data allowing to further apply for market authorization of HCQ in APS. HIBISCUS is a randomized versus placebo international trial, with an important number of participating centers which will allow a high potential of recruitment and the statistical power of analysis by subgroups.

Considering the high risk of relapse in APS despite treatment, the preclinical and clinical preliminary data concerning the benefits of hydroxychloroquine (HCQ) treatment in APS, our group has applied and recently obtained the orphan designation of HCQ in antiphospholipid syndrome by the European Medicine Agency.

HCQ is therefore designated by the European Commission decision from 12th January 2017 in the communitarian register of orphan drugs with the number EU/3/16/1820 and the academic sponsor is the University Hospital Angers (France).

Moreover, the leaders of the project made the proposal of an international project HIBISCUS about the use of Hydroxychloroquine in secondary prevention of obstetrical and thrombotic new events in primary APS.

Scientific protocol assistance for the international project HIBISCUS about the use of Hydroxychloroquine in secondary prevention of obstetrical and thrombotic new events in primary APS has been obtained from the European Medicine Agency on May 2017 (EurEMA/CHMP/SAWP/284691/2017 Procedure No. EMEA/H/SA/3509/1/2017/PA/II).

The project proposal has been launched during the Autoimmunity Congress (Leipzig April 2016), Europhospholipid Forum (Nancy April 2017), and, lastly, at the scientific session of Lupus anticoagulant SSC subcommittee ISTH (Berlin, July 2017) and Autoimmunity Congress (Lisbon, May 2018) [16].

Our consortium has decided to perform this study simultaneously in several countries.

Fifty three international centers from 16 countries (France, Italy, Spain, Israel, Portugal, Belgium, The Netherlands, Serbia, Russia, Romania, Hungary, Turkey, Canada, Tunisia, Argentina, China) joined the trial HIBISCUS; and the coordinating committee and the scientific advice committee's members are in the large majority members of the Europhospholipid group.

The participation of a high number of centers to this trial strengthens its feasibility and guarantees its success.

The French part of the trial about thrombosis has been granted by the French Minister of Health in December 2015 (the academic trial independent of the pharmaceutical industry PHRC N PAPIRUS) coordinated by one of the members of the leading consortium of HIBISCUS.

This trial has two parts: a retrospective study including data which focus on the effects of HCQ in primary thrombotic and obstetrical APS, and a prospective study (randomized in some countries and open label trial in some others).

We will further present the design of this trial based on the previous preclinical and clinical data.

2. Preclinical and clinical data in thrombotic APS

2.1. Preclinical

Experts underline that there is a need for additional and/or alternative therapies in APS and that HCQ could be one of the most interesting therapeutic options [4–9].

New mechanisms were recently described in APS [4,17]. The antibodies against the domain I of β2 glycoprotein I (β2GPI) are recognized as the main pathogenic subset in APS.

HCQ interacts with toll-like receptors [18]; inhibits calcium-dependent cell signaling [19] and reverses platelet activation induced by aPL [20].

HCQ significantly reduces the production of cytokines (IL-6, IL-8, IL-17 and IL-22 and TNF-α, Il-1, soluble CD8 and soluble IL2 receptors) and induces the normalization of complement activity [21,22].

In addition to the known mechanisms on platelets activity [20,23,24], intravascular red blood cell aggregation inhibition [25], reduction of APL IgG-B2GP1 complex binding to phospholipid layers [26]; protection of the anticoagulant shield formed by annexin V from damage by APL [27], cholesterol reduction, reduction of APL production, recent data have focused on new mechanisms of action of HCQ in preventing thrombosis [28–30].

It has been reported that upon binding, APL trigger intracellular mediators such as nuclear factor kappa B and mammalian target of rapamycin [4].

Hydroxychloroquine exerts not only immunomodulatory effects [15], but it also inhibits proinflammatory signaling pathways by targeting endosomal NADPH oxidase [28], and significantly reduces soluble TF levels in patients with aPL [29].

Long-term use of hydroxychloroquine reduces antiphospholipid antibodies levels in patients with primary antiphospholipid syndrome [30] and is associated with lower odds of persistently positive aPL in SLE patients [31].

Finally, hydroxychloroquine treatment might be useful to control type I IFN-related immune activation in PAPS [32].

2.2. Clinical

The data available from literature pertain mainly to secondary APS, as few data have been published for primary APS [30,33].

Our team has shown the role of hydroxychloroquine (added to standard oral anticoagulants) in reducing the incidence of new venous thrombosis in primary APS patients [33].

We have reported a striking significant difference in a preliminary study in primary APS patients in terms of thrombotic relapses in a comparative population treated with HCQ and AVK versus AVK alone. Therefore, after three years of treatment, we have observed new thrombotic events in 30% of patients from the group VKA alone; and no new thromboses in the group treated by HCQ and VKA [33].

To our knowledge, this was the first prospective study performed in primary APS.

A recent retrospective, propensity score-matched cohort study, which analysed the impact of HCQ on aPL titers and the incidence of thrombotic events in 57 APS APS patients treated with HCQ compared to 57 not exposed patients has shown the beneficial effects exerted by HCQ both in lowering aPl titers and on a significant reduction in the incidence of arterial events (0 vs 1.14%) [30].

A large panel of studies focuses on the benefits of HCQ as an anti-thrombotic in lupus and in APS secondary to SLE [15,34–46].

It has been reported that the use of HCQ was associated with a reduction in thrombotic risk [42–43,45–47]. HCQ administration was inversely correlated with the onset of thrombosis, kidney failure, hypertension and infection, and directly correlated with improved survival rates in lupus patients [48].

A case-control study on a population of lupus patients revealed by multivariate analyses, a 68% reduction of thrombotic risk (26–86%) under HCQ treatment, notably after correction for disease severity, monitoring time and disease duration (OR 0.32, 95% CI 0.14–0.74) [39].

These data were however controversial as the study of Tektonidou et al. failed to show any thrombotic risk reduction in the presence of HCQ [49]. However, this trial had several limitations, as 55% of APL+ patients possessed additional thrombosis factors, and even though it was a longitudinal study, the collected data were retrospective.

The rate of survival estimated over 15 years was 68% in patients naïve to antimalarials against 95% in patients treated by antimalarials [46].

These data account for a decrease of 77% of mortality with antimalarials.

A meta-analysis of 95 articles pertaining to randomized prospective studies and observational studies concluded that HCQ has multiple beneficial effects, including anti-thrombotic properties, with a moderate level of evidence [41].

The study of the international consortium “APS action” which intended to show a beneficial effect of HCQ in healthy people with positive aPL but no clinical events (aPL carriers), failed, as a relatively short follow-up was performed, and also as authors could not accurately assess the effectiveness of HCQ for primary thrombosis prevention in persistently PL-positive patients with no other systemic autoimmune diseases [50].

All these data are only preliminary and therefore there is a need of a large prospective study that we propose: a national French study financed in 2015 (PHRC N PAPIRUS) and further the international study HIBISCUS.

As nowadays most of SLE patients receive HCQ as basal treatment, only a study in primary thrombotic APS could provide the evidence of this treatment for the secondary prevention of thrombosis.

Thus, based on all these data dealing with the significant risk of thrombotic relapse, occurring under appropriate treatment, we propose the use of HCQ additionally to standard anticoagulant treatment for the prevention of thrombosis relapse in antiphospholipid syndrome.

3. Preclinical and clinical data in obstetrical APS

Experts accord in proposing HCQ as an additional therapy in refractory obstetrical APS [51–55].

3.1. Preclinical

The pathogenesis of obstetrical APS implies several mechanisms, such as prothrombotic state including inhibition of β2-GPI activity, platelet activation, tissue factor upregulation and annexin A5 resistance reducing anticoagulant activity of annexin A5 [10,56]. Trophoblast injury implies trophoblast cell apoptosis, impairment of invasiveness, and increased number of inflammatory cells and cytokines in placental bench.

Experimental models showed that HCQ may restore some defective biological functions induced by anti-phospholipid antibodies (aPL) on trophoblasts and some studies reported a protective effect on in vivo aPL-mediated placental and foetal neurodevelopmental abnormalities in an animal model of aPL-mediated foetal loss [57,58].

HCQ display a pleiotropic activity spanning from immunomodulation effect to anti-inflammatory and anti-thrombotic activities, all of them being potentially useful in APS. The well-known safety of HCQ in pregnancy encouraged for its use in pregnant women with autoimmune rheumatic disorders including APS and observational reports suggested a protective effect on obstetrical recurrences.

Since thrombosis is only one of the pathogenic mechanisms in obstetrical APS, the efficacy of HCQ is also related to other pharmacological effects.

Several in vitro studies demonstrated that hydroxychloroquine can reduce the aPL antibody binding to syncytiotrophoblasts and restore annexin A5 expression and functional anticoagulant activity [59]. Hydroxychloroquine could also antagonize aPL-mediated inhibition of trophoblast migration, invasion and differentiation [58,60,61]. Moreover, it has been reported that Hydroxychloroquine reversed the aPL-inhibition of trophoblast IL-6 secretion and partially limited aPL-inhibition of cell migration [58].

As anti-β2GP1 antibodies decrease trophoblastic differentiation via TLR4, it has been reported that this effect is restored by HCQ, suggesting its therapeutic interest in APS pregnancies [60]. The effect of Hydroxychloroquine on antiphospholipid antibody-induced changes in first trimester trophoblast function are beneficial [58,62]. Furthermore, it has been reported that HCQ is able to dramatically reduce the antiphospholipid antibodies levels in obstetrical APS [9].

3.2. Clinical studies

Observational studies and reports focussed on additional therapeutic strategies in refractory cases of obstetrical APS. Nowadays, the best therapy regimen for refractory obstetrical antiphospholipid syndrome remains to be determined: additional treatments with steroids, plasma exchanges and immunoglobulins, HCQ.

Among these alternatives, hydroxychloroquine (HCQ) is one of the most interesting therapeutic options.

Beneficial effects of HCQ therapy during pregnancy in SLE have been reported since long date [63]. HCQ reduces neonatal morbidity in women with SLE by significantly decreasing the rate of prematurity and intrauterine growth restriction [64] and is linked to a significantly higher live birth rate [51].

There are no randomized clinical trials on the use of HCQ to prevent recurrent miscarriages in APS patients.

There are several case reports [53,65] or case series, and among them our preliminary study, that reported the effects of HCQ in preventing obstetrical relapses. Most of the studies are retrospective and concern a heterogenous APS population (primary and secondary APS, asymptomatic APS and refractory obstetrical APS, with different additional and/or rescue treatments).

Therefore, for refractory APS few case reports and the obstetrical series of the Europhospholipid group strongly suggest the usefulness of HCQ in preventing obstetrical relapses [10,53,54,61,66,67].

Observational/retrospective studies on the protective role of HCQ on pregnancy complications in APS were recently published.

Ruffatti et al. reported in 194 pregnant PAPS patients attending 20 tertiary centers retrospectively enrolled that Hydroxychloroquine was found to be linked to a significantly higher live birth rate with respect to the other oral treatments [51]. Moreover, the high (400 mg) versus low (200 mg) doses of hydroxychloroquine ($p = .036$) and its administration before versus during pregnancy ($p = .021$) were associated with a significantly higher live birth rate [51].

Leroux M et al. evaluated the effect of HCQ on fetal preterm delivery and intrauterine growth restriction (IUGR) in a cohort of pregnant women with SLE including a small series positive for aPL [64]. HCQ reduced neonatal morbidity in all of the SLE women by significant decrease of the rate of prematurity and intrauterine growth restriction [64].

A total of 118 pregnancies were included over 11 years, 41 in the HCQ+ group and 77 in the HCQ- group. The rate of adverse fetal outcome was significantly lower in the HCQ+ group ($p = .001$) [64].

Two other studies included also primary APS and reported a decrease in pregnancy losses in the treated group; moreover, the addition of HCQ resulted in a significant increase in live births in the refractory group [10,67].

Hydroxychloroquine treatment was associated with a higher rate of live births (67% group A vs 57% group B; $p = .05$) and a lower prevalence of antiphospholipid antibodies-related pregnancy morbidity (47% group A vs 63% B; $P = .004$). The association of hydroxychloroquine with a lower rate of any complication in pregnancy was confirmed after multivariate analysis (odds ratio, 2.2; 95% confidence interval, 1.2–136; $P = .04$). Fetal losses at > 10 weeks of gestation (2% vs 11%; $P = .05$) and placenta-mediated complications (2% vs 11%; $P = .05$) were less frequent in group A than group B [67].

In the European multicentre study leaded by the Europhospholipid group, the outcome of pregnancies treated by hydroxychloroquine in patients with APS or asymptomatic antiphospholipid (aPL) antibodies carriers were analysed [10,66]. Thirty patients with APS with 35 pregnancies treated by hydroxychloroquine were analysed. Comparing the outcome of pregnancies treated by the addition of hydroxychloroquine to previous pregnancies under the conventional treatment, pregnancy losses decreased from 81% to 19% ($p < .05$), without differences in the associated treatments. The univariate analysis showed that the previous intrauterine deaths and higher hydroxychloroquine amount (400 mg per day) were the factors associated with pregnancy outcome. Considering 14 patients with previous refractory obstetrical APS ($n = 5$ with obstetrical and thrombotic primary APS and $n = 9$ with purely obstetrical APS), all with previous pregnancy losses under treatment (aspirin with LMWH in 11 cases and LMWH in 3 cases), the addition of hydroxychloroquine resulted in live born babies in 11/14 (78%) cases ($p < .05$) [10,54].

However, the effect of HCQ was not adjusted for the use of other medications such as aspirin, heparins or steroids [10]. Selected experts agreed that adding HCQ could be considered in selected cases or after failure of standard treatment with aspirin and a heparin [10]. Specifically, most experts considered adding HCQ in specific situations: women with previous thrombosis, and/or with placenta-mediated complications, when a high risk aPL profile or concomitant cardiovascular risk factors are present or in case of allergy/intolerance to aspirin [10,66].

Nevertheless, prospective well-designed studies are needed to conclude on the efficacy of HCQ in preventing miscarriages in APS and all experts are agreeing with this item.

Our group has recently performed a study in obstetrical APS [16]. There is only a preliminary prospective study about Hydroxychloroquine effects on preventing miscarriages in obstetrical primary APS refractory to standard therapy (curative doses low molecular weight heparins plus aspirin). The APS population was homogenous and consisted in refractory obstetrical APS with maximal standard treatment and fulfilling the Sidney criteria. 13/14 pregnancies were followed by a live normal birth in this preliminary report.

The available data in the literature about HCQ efficacy in obstetrical APS are summarized in Table 1.

These data suggest that the HCQ adjunction to treatment was successful in 11/14 cases (78%) in the retrospective series of Mekinian [10] et al. and 13/14 cases (93%) in our preliminary prospective trial [16].

Based on these data, on sporadically successful case reports and series of live birth when adding HCQ to standard therapy, we suggest a major beneficial effect of HCQ in preventing miscarriages in refractory primary APS patients.

A recent international trial HYPATIA: Hydroxychloroquine to Improve Pregnancy Outcome in Women with Antiphospholipid Antibodies (HYPATIA) Protocol: A Multinational Randomized Controlled Trial of Hydroxychloroquine versus Placebo in Addition to Standard Treatment in Pregnant Women with Antiphospholipid Syndrome or Antibodies was also launched in September 2017 [68].

We further propose the HIBISCUS trial: we hypothesize that HCQ added to standard therapy could significantly improve birth rate in primary obstetrical APS. The trial is based on the hypothesis of 80% obstetrical events for the entire studied APS population.

We expect a 15% increase of birth rate with HCQ treatment administered in addition to the standard treatment for APS, i.e. a 95% live rate birth in the HCQ group. The study will be performed as a randomized clinical trial or as an open trial in the different 16 participating countries of the consortium.

4. Design of the clinical trial HIBISCUS

HIBISCUS is an international multicentric, comparative, randomized, double-blinded, controlled versus placebo study. This is a phase III drug trial.

Patients will be distributed into groups according to a 1:1 ratio.

Therefore, this trial shall focus on a population of patients with primary antiphospholipid syndrome. Although initially we have designed for ethical reasons and to avoid stress during pregnancy an open label study, after the scientific advice of the European Medicine Agency

Table 1
Data with respect to HCQ efficacy in obstetrical APS.

Reference	Country	Methodology	Number of cases	Patients treated with HCQ
66	European The EUROAPS register led by the Europhospholipid group	Retrospective	247	3
10	European	Retrospective	30	14 including also asymptomatic aPL carriers without previous obstetrical manifestations
9	Italy	Case report	1	1
65	USA	Case report	1	1
16	France	Prospective	14	14
67	Italy and England	retrospective	96	31
64	France	retrospective	118	41
51	International	retrospective	194	

and discussion with the members of our national and International scientific consortium, we have finally decided to perform a double blinded randomized study, to get formal conclusions with significant statistical power. Furthermore, the patients will be informed that their participation to the trial is not a loss of chance, as they will be treated with the optimal standard of care therapy according to international guidelines.

4.1. Design of HIBISCUS trial in obstetrical APS: HIBISCUS-O

4.1.1. Main goal and main outcome measure

The main aim of this trial is to assess the number of live births in primary antiphospholipid syndrome in patients treated with Hydroxychloroquine added to standard treatment i.e. low molecular weight heparins at preventive dosage and aspirin, in a multicentre, prospective randomized, double-blind, versus placebo study.

Synopsis of obstetrical APS.

Main aim	The main aim of this trial is to assess the number of live births in primary antiphospholipid syndrome in patients treated with Hydroxychloroquine added to standard treatment i.e. low molecular weight heparins at preventive dosage and aspirin, in a multicentre, prospective randomized, double-blind, versus placebo study
Secondary goals	- Analysis of adverse reactions and their severity in Hydroxychloroquine plus LMWH and aspirin group - Analysis of the time of occurrence of adverse effects - Analysis of maternal morbidity related to primary APS, including miscarriage as well as late pregnancy complications like fetal deaths and premature delivery related to eclampsia/pre-eclampsia - Analysis or fetal morbidity: intrauterine growth retardation and premature birth - Using standard basic tests, define predictive biological parameters of the occurrence of pregnancy morbidity events at inclusion and at the end of the trial (such as lupus anticoagulant positivity prior to heparin treatment, triple aPL positivity, thrombocytosis, complement C3, C4, CH50, C reactive protein, cholesterol, blood level of hydroxychloroquine at the end of the trial) - Measure of compliance to HCQ
Primary outcome measure	- The number and percentage of live births in each arm. The percentages of these events will be compared by appropriate statistical test (Chi2 test or Fisher exact method)
Secondary outcome measures	- The type and number of adverse reactions and their severity (minor/major) in Hydroxychloroquine group. The conventional definitions for severity will be used (i.e. death, life-threatening, hospitalization/prolongation of hospitalization, persistent or significant disability/incapacity, as defined by current pharmacovigilance standards. - The occurrence time of side effects measured every 3 months and at the end of the study. - Morbidity related to pregnancy morbidity events (hospitalization, temporary work incapacity, miscarriage, fetal deaths and premature birth), in each arm, as well as the confidence interval, will be evaluated after maximum 9 months of treatment (end of the pregnancy). - Predictive biological standard basic parameters of the occurrence of pregnancy morbidity event at the inclusion and at the end of the trial (such as lupus anticoagulant positivity prior to heparin treatment, triple aPL positivity, thrombocytosis, complement C3, C4, CH50, protein C reactive, lipid profile (total cholesterol, and HDL, LDL cholesterol), blood level of hydroxychloroquine at the end of the trial). - Dosage of blood level of hydroxychloroquine at the end of the trial will allow the measure of compliance to HCQ. End points will be assessed at the end of the study by statistical analysis
Study methodology/ scheme	This is a phase III drug trial, international multicentric, comparative, randomized, superiority, double-blinded, controlled with 2 compared groups (Hydroxychloroquine versus placebo) study. Patients will be distributed into groups according to a 1:1 ratio. Patients will be enrolled at multiple centres by internists, rheumatologists, nephrologists, angiologists, cardiologists, neurologists and vascular medicine and all other specialists. Patients monitored in the context of their disease (primary APS), or with primary APS newly diagnosed, will be invited to take part in the trial (incident and prevalent cases). Women with primary obstetrical APS willing a pregnancy will be informed about the study. When they will stop contraception and/or wish to start a new pregnancy for women without contraception, or the pregnancy is confirmed (but prior to 12 weeks of gestation) a pre-treatment with Aspirin 160 mg/day, that is the standard therapy of care will be started. This treatment will be continued all long pregnancy. Now when the pregnancy will be confirmed by BHCG dosage or echography attesting foetal cardiac activity, after informing again the patient and obtaining his/her signature on the informed consent form, the investigator will fill in a computerised pre-inclusion form, available 24/7 over the Internet via the e-CRF (secure access previously granted to each pre-declared investigating centre). • An ophthalmological visit will be scheduled prior to inclusion.

The patients will be randomized to additionally receive either LMWH at preventive dosage plus HCQ in arm A or LMWH at preventive dosage plus placebo in arm B. The experimental treatment will be HCQ. The standard treatment will be LMWH and Aspirin as the international guidelines indicate the use of LMWH at preventive dosage plus Aspirin in case of refractory obstetrical APS despite treatment with Aspirin.
Randomisation will be centralised and implemented by a computerised random allocation system coupled with the electronic case report form, such that patients included in the study can be easily and automatically randomized. The randomisation number will be identical to the treatment number displayed on the treatment kit (box) labels. The study treatment will be prescribed (sponsor prescription) and the inclusion confirmation will be faxed to the coordinating centre
The investigator shall perform several data checks and collections during the study. During each patient report, the inclusion and non-inclusion criteria will be checked. Therefore, at the inclusion visit it will be performed:

- Verification of patient eligibility criteria
- Confirmation of pregnancy by BHCG dosage or echography attesting foetal cardiac activity
- Verification of the absence of ECG conduction disorders
- Obtaining the patient's informed consent
- Obtaining the patient's clinical-biological information (standardised data sheet)
- Verification of normality of initial ophthalmic examination
- Fax to the Angers University Hospital central pharmacy for randomisation (numbered kits will be provided in advance in participating investigating departments)
- Initiation of treatment: HCQ or placebo, at a dose of 200 mg × 2/day taken once daily (adapted to 200 mg/day in women with a weight < 45 kilos).

Dispensation of the treatment every 3 months by the Pharmacy Department in each centre. Empty or not entirely empty flask will be contabilized and pills will be counted at each new dispensation.
Follow-up:

- Blood tests at M1, M2, M3 to check out the tolerance of HCQ (cell blood count CBC, liver enzymes: ASAT/ALAT). They will be performed during a monthly routine test for platelets dosage and antiXa activity (to follow LMWH tolerance and dosage range).
- Electrocardiogram (ECG) will be performed at inclusion in the study and at M1, M2, M3 as cardiovascular potential side effects of HCQ even though rare could appear early after the initiation of the treatment.
- Close monitoring of the pregnancy will be performed monthly by the obstetrician (these visits belong to the standard follow-up of patients with primary APS).
- A close monitoring of the fetus by echography during pregnancy to detect any adverse situation and characterize the safety of the fetus will be performed monthly by the obstetrician (these monitoring belongs to the standard follow-up of patients with primary APS).
- The observance and tolerance of the treatment will be followed up **every month** by the internist/rheumatologist/nephrologist or any other specialist co-investigator of the trial that insures the regular follow-up of the patient for the primary APS via an in site medical visit, and data will be completed on a simplified eCRF standardised file (a part of these visits belong to the standard follow-up of patients with primary APS).

*Although the specialists that insure the follow-up of the patients for the APS see them regularly in routine every 2–3 months during pregnancy, we have decided to schedule these visits monthly for monitoring the safety of the pregnant women and foetus.

- An ophthalmological visit will be scheduled during the sixth month of pregnancy and in case of any doubt on ophthalmological impairment the HCQ will be stopped.
- At the end of the pregnancy HCQ blood dosage will be performed during a routine monthly biological test.
- Long term follow up to discard any late onset effects (i.e. audition or visual defects) will be planned via a medical visit of the child at one year and at two years performed by pediatricians in each center, co-investigators in the trial.

All non-serious adverse event and/or abnormal results defined as critical to the evaluation of the safety of the test subjects, must be reported to the sponsor by the investigator, in accordance with the SAR reporting rules.
24 months for inclusion and maximum 9 mo (until delivery) of follow-up will be scheduled. Statistical analysis will be performed at the end of the trial, one intermediate analysis is planned after the inclusion of the first 40 patients and at each 40 patients included.
In summary biological and complementary tests during the study:

- *Profile on inclusion visit D0* consisting in full blood test and platelets, creatinine, liver tests (ASAT, ALAT), C reactive protein (CRP), cholesterol (total, HDL, LDL) hemostasis test, i.e. prothrombin time, TCA, fibrinogen; antiphospholipid

antibodies (lupus circulating anticoagulant, anticardiolipin antibody, anti-B2GP1 antibody), antinuclear antibody (ANA) assay with determination of anti-DNA and anti-ENA if anti-ANA antibodies positive. These blood tests are part of the normal follow-up for a patient suffering from APS. This follow-up shall be charged to the patient's health insurance.
- at M1, M2, M3 full blood count cells test (minor risk of agranulocytosis). These are also blood tests included in routine.
- at M1, M2, M3 ALAT and ALAT (minor risk of hepatitis) and ECG. The additional blood tests will be performed during a monthly routine blood test for platelets dosage and antiXa activity (to follow LMWH tolerance and dosage range).

Although these blood tests could be scheduled only in the first month, we have chosen to perform them from M1 monthly until M3 to avoid any unknown side effects, considering the fragile population of pregnant women. Moreover, these tests will be performed during a routine monthly blood test, and no additional blood volume is needed.

- Monthly creatinine, urinary bandelette, C reactive protein, hemostasis test, i.e. TP, TCA, fibrinogen. This will be biological tests included in routine.
- At M3, M6 and M9 visit: the blood tests will be identical to those performed on inclusion visits (with the exclusion of lupus anticoagulant that would be no reliable due to the LMWH ongoing treatment).

All these blood tests are part of the normal follow-up for a patient suffering of APS. This follow-up shall be charged to the patient's health insurance.

- Hydroxychloroquine blood dosage will be exclusively performed at the end of pregnancy during a routine monthly blood test (to insure to the compliance to treatment, but also to not affect the double-blind character of the trial).

This will be an additional biological test, not included in routine.
The additional examinations will be in summary

1. Ophthalmic examination prior to inclusion and at 6 months
2. ECG at M1, M2, M3
3. ASAT/ALAT at M1, M2, M3 during a monthly blood test (without an additional blood volume requested)
4. Four additional clinical visits by specialists that insure the follow-up of the mother monthly for APS (instead of every 2–3 months as performed in routine follow-up).
5. Hydroxychloroquine blood dosage will be exclusively performed at the end of pregnancy
6. Two medical visits of the child (at one year and at two years) performed by pediatricians in each center, co-investigators in the trial.

Unblinding and statistical analysis shall be performed at the end of the trial, i.e. at 33 months. Intermediate statistical analyses every 40 patients included are planned.
An independent committee survey will check that there is no abnormal rate of events in one of the arms of the study every 40 included patients or at least once yearly, to check the clinical and biological tolerance of the experimental drug.
An independent committee of adjudication of obstetrical events will deliberate on the confirmation of obstetrical relapses.
As the trial involves interventional biomedical research, it shall be declared to the competent authorities in each country and shall be subject to IRB approval

Subject inclusion criteria

- Age > 18 years
- With primary* obstetrical antiphospholipid syndrome**according to Sidney criteria, with one of the following 2 criteria:

1. 3 or > 3 consecutive spontaneous abortions < 10 weeks of gestation with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal abnormalities excluded
2. or one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th weeks of gestation, with normal morphology detected by ultrasound or by direct examination of the fetus

- Willing to be pregnant or being pregnant < 12 weeks (pregnancy confirmed by BHCG dosage or cardiac fetal activity on echography)
- Patients are volunteers, informed and signed the consent form for participation in the study after receiving the information letter.
- *Primary APS refers to APS with no identified cause.

**According to the Sydney international criteria, obstetrical antiphospholipid syndrome is evidenced by the presence of an obstetrical clinical manifestation, combined with the presence of a biological criterion: presence of circulating anticoagulant and/or of anticardiolipin antibodies > 40 uGPL (> 99 percentiles) and/or anti-B2GP1 antibodies > 40 uGPL (> 99 percentiles). The presence of antiphospholipid antibodies (aPL) should be confirmed to 3 months for an APS be recognized.
Obstetrical manifestations are defined according to international criteria as

- 3 or > 3 consecutive spontaneous abortions < 10 weeks of gestation with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal abnormalities excluded

	• or one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th weeks of gestation, with normal morphology detected by ultrasound or by direct examination of the fetus • or one or more premature births of a morphologically normal neonate before the 34th week of gestation related to pre-eclampsia/eclampsia or recognized features of placental insufficiency The trial has taken the choice of using (> 99 percentiles) to insure the homogeneity and reliability of tests in the different co-investigator centers
Subject non-inclusion criteria	• Secondary APS • The following ophthalmological disease: ○ retinal disease contraindicating Hydroxychloroquine prescription ○ cataract ○ monophthalmia • Demonstrated history of HCQ intolerance/ allergy or HCQ contraindication • Known glucose-6-phosphate dehydrogenase deficiency • Chronic liver disease • Renal failure (creatinine clearance < 30 ml/min) • Chronic alcoholism • Patients with already increased QT values • Concurrent treatment with other products potentially increasing QT or with arrhythmogenic properties • Epilepsy • Haemolytic anaemia • Porphyria • Progressive ongoing cancer or hematological malignancy • Psychiatric disease not allowing the treatment • Lack of health insurance • *Participation to concomitant study with interventions leading to a bias to our study (other drugs, with synergistic or antagonizing or confounding effects). • History of heparin-induced thrombocytopenia (HIT) (with or without thrombosis) • Uncontrolled, active bleeding • Conditions in which coagulation tests cannot be performed at appropriate intervals • Known hypersensitivity to heparin or pork products • Any risk factor for hemorrhage (e.g., subacute bacterial endocarditis, blood dyscrasias, menorrhagia, dissecting aneurysm, major surgery, spinal anesthesia, hemophilia, GI ulcerative lesions, liver disease, impaired hemostasis) *In case of doubt with respect to this criterion the coordinator of the study will be contacted and will deliberate about the possibility to participate to a concomitant trial
Treatments/ strategies/ Procedures	The treatment for prevention of pregnancy morbidity relapses will consist in low molecular weight heparins at preventive dosage and aspirin plus HCQ in arm A and plus placebo in arm B. To insure the homogeneity of the study and to follow the standard practice procedures, we have decided that one singe dosage of Aspirin to be used 160 mg/day, and one type of LMWH: Lovenox (Sodic Enoxaparin) 4000 UI or 0.4 ml/sc/day. The Aspirin dose is the dose the most prescribed by experts. Lovenox is the standard LMWH in France most frequently used in preventive intention. One single subcutaneous injection 4000 UI (0.4 ml) will be administered daily until the last trimester of pregnancy (until at least 35th week of gestation and the latest to be stopped 12 h prior to delivery). Patients shall be randomized in a double-blind manner to receive either HCQ or the placebo. The ratio of patients randomized into the two groups shall be of 1:1. Randomisation will be implemented by a computerised random allocation system coupled with the electronic case report form, such that patients included in the study can be easily and automatically randomized. The randomisation number will be identical to the treatment number displayed on the treatment kit (box) labels. **The choice of a 400 mg dose** seemed appropriate as this is below the toxic dose (< 6.5 mg/kg/d) and is routinely used in cases of SLE and RA. This dose corresponds to an average of 6 mg/kg/day, the recommended dose for patients with no associated risk factors. Patients with such risk factors will be excluded from the trial. The doses used in the literature to demonstrate the effect of HCQ obstetrical relapse are equal to this dose. Critical review of the current recommendations for the treatment of systemic inflammatory rheumatic diseases during pregnancy and lactation [69,71] and the EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation [70,71] have been recently published. The report of the European League Against Rheumatism (EULAR) task force on use of anti-rheumatic drugs before pregnancy, and during pregnancy and lactation suggested that HCQ should be continued in pregnancy for maintenance of remission or treatment of a disease flare (Grade B of recommendation, strength of evidence according to GRADE Oxford 2°) [70,71]. Considering the drug's expected benefits associated with its use, we deemed it ethical to propose these doses over a 9-month period. The patients' other primary treatments will not be altered and will remain freely chosen by the investigator. The benefit-risk balance of this drug would appear acceptable at the individual and collective levels.

	A weight-adjusted dose regiment: 200 mg daily will be administered for patients weighing < 45 kg. The results of this trial could, at minimal cost and with highly favorable benefits to risks ratio, allow a significant improvement in the management of patients with primary APS and reduce the incidence of pregnancy morbidity and improve natality in this population
Number of Patients	Number of subjects required to answer the research question: 170 in each group, i.e. 340
Justification of the sample size	The increase in birth rate using HCQ added to standard therapy in refractory APS was suggested to us by the data of the literature showing a decreased relapse of obstetrical manifestations varying from 78 to 95%, and respectively an increased in birth rate of at least 15% [10,16,66]. The sample size calculation is based on the following justifications: as the number of live births in APS patients failing to maximal standard therapy with LMWH and aspirin is 80%; the clinical relevance of the proposed primary endpoint is numerically defined as an expected increase with 10%; i.e. 90% of live births in the experimental group. Although we could have chosen the low range of rate of live births i.e. 70–75% for the statistical analysis, and even though our approach would increase the size of the population, we have preferred to use the high range i.e. 80% of range of rate of live births in the control group to get formal undeniable conclusion on the balance benefit/risks of HCQ use in this fragile population with a sufficient statistical power. If the live birth rate is of 80% in the control group, assuming a 10% increase in the studied group in a primary APS population receiving HCQ then 308 patients (154 per group) must be included (Biostat TGV on unilateral formulation with a first order risk alpha of 0.05 and a statistical power of 80%). Considering 20% of drop-outs a number of 340 patients will be necessary (170 per group). We make this assumption based on all preclinical and clinical existing data and on all preliminary indirect data. Although we are aware of the limits of a 20% dropout, as the pregnant women will be informed that there is no loss of chance in the placebo arm as we follow international guidelines of therapy in refractory to Aspirin obstetrical APS, we are confident that the adhesion of pregnant APS women will be important. Patients will remain in the trial, even after discontinuation for adverse event or toxicology reasons to collect data facilitating interpretation of missing data (loss of patients, drop outs for safety) at the end of the trial (intention to treat protocol). The non-responder approach would be deemed conservative and preferred as the main method for managing missing values. Other methods dealing with missing data will be also included as sensitivity analyses. As an example, multiple imputation, hybrid methods where treatment-related drop-outs (i.e. due to lack of efficacy or safety events) are treated as no-responders and all non-treatment related dropouts are managed through multiple imputation, or observed cases only analysis, may also be included as sensitivity analyses
Statistics	**Descriptive statistics**: Collected variables shall be described globally and per group. Qualitative variables are expressed in population size and percentage. Quantitative variables are expressed in terms of mean ± standard deviation with 95% confidence interval, along with the 5th and 95th percentiles. These are, however, expressed in terms of median, minimum, maximum and 5th and 95th percentiles when normality is rejected. The Kolmogorov- Smirnov test will be used to check parameter normality and the Levene test will be used to determine equality of variances. The Student *t* test will be used to compare the distribution of quantitative variables between the two patient groups (Plaquenil/ placebo). If the test application conditions are not met, the Mann-Whitney non-parametric test will be used. The Chi-square or Fisher exact-test will be used to compare the distributions of qualitative variables between the two groups. **Goals analysis** *The analysis of the main* outcome measure shall consist in comparing the proportions of live births in the two groups. This comparison shall be performed by means of a Chi-square test, or a Fisher exact-test if the Chi-square application conditions are not met. If a difference between the two groups is demonstrated for at least one of the potential confounding variables, the main outcome measure shall be analysed by means of multivariate logistic regression to consider the adjustment factors. *The secondary* outcome measures will be analysed using the same methods as the *main* outcome measure. A survival analysis related to the risk of live births failure during follow-up associated with the exposure factor shall be performed and estimate by calculating the hazard ratio (uni- and multivariate Cox model). We will perform analysis based on type of antiphospholipid antibodies types and values (low, moderate and high) and type of previous obstetrical event (event prior to inclusion) and we will analyse the tendencies. **Intermediate analyses** will be performed at each 40-patient's inclusion. In this case complete descriptive statistics

	will be conducted. Comparative analysis will be driven if statistical power is sufficient. In case an increased number of adverse events recorded at each intermediary analysis in one of the arms of the study the trial will be stopped according to the supervisor committee advices
Research duration	Considering the low prevalence of primary obstetrical APS in the general population, the total patient inclusion period shall be of 2 years, taking trial duration to maximum 33 months. Thus: Inclusions: 24 months Follow-up: maximum 9 months (until delivery) A total duration of maximum 33 months

4.2. Design of HIBISCUS trial in thrombotic APS: HIBISCUS-T

This is a phase III drug trial, international multicentric, comparative, randomized, superiority, double-blind, controlled with 2 compared groups (Hydroxychloroquine versus placebo) study.

The study proposed is a phase III randomized double-blinded trial with two arms: patients in Arm A will receive vitamin K anticoagulants (VKA) and Hydroxychloroquine (HCQ). Patients in Arm B will receive VKA plus placebo. The inclusion period will be 24 months and Arm A and Arm B will receive treatment 24 months (total study duration 48 months). The patients will have supplementary 3 months survey after the end of the study.

4.2.1. The main aim

The main aim of this trial is to assess at 24 months the efficacy of treatment with Hydroxychloroquine in preventing new thrombotic events (venous and arterial) in primary antiphospholipid syndrome in patients treated with VKA with a target INR between 2 and 3, in a multicenter, prospective randomized, double-blind, versus placebo study. The main end points will be the number and the percentage of new thrombotic events in each arm at the end of the study

HIBISCUS trial in thrombotic APS: HIBISCUS-T.

Main aim	The main aim of this trial is to assess at 24 months the efficacity of treatment with HCQ in preventing new thrombotic events in primary antiphospholipid syndrome in patients treated with VKA, in a multicentre, international, prospective randomized, double-blind, versus placebo study
Secondary goals	- Analysis of adverse reactions and their severity in the HCQ plus VKA group. - Analysis of the time of occurrence of adverse reactions. - Using standard basic tests, define predictive biological parameters of the occurrence of thrombotic events at the inclusion, M12 and M24. - Measure of compliance to HCQ End points will be assessed at the end of the study by statistical analysis
Primary outcome measure	- The number and percentage of thrombotic events in each arm, as well as the confidence interval, will be evaluated after 24 months of treatment. The percentages of these events will be compared by appropriate statistical test (Chi2 test or Fisher exact method)
Secondary outcome measures	- The type and number of side effects and their severity (minor/major) in the HCQ group. - The occurrence time of side effects - Predictive biological standard basic parameters of the occurrence of thrombotic events at the inclusion, M12 and M24. - Dosage of blood level of hydroxychloroquine at the end of the trial will allow the measure of compliance to HCQ
Study methodology/ scheme	○ Drug trial (phase III) ○ International multi-centre trial ○ Comparative trial ○ Randomized ○ Controlled (control group) ○ With 2 compared groups (HCQ versus placebo) ○ Superiority ○ Double-blind (patient and investigators) Patients will be distributed into groups according to a 1:1 ratio. Patients monitored in the context of their disease (primary thrombotic APS), or with primary thrombotic APS newly diagnosed, will be invited to take part in the trial (incident and prevalent cases). During each patient report, the inclusion and non-inclusion criteria will be checked. After informing the patient and obtaining his/her signature on the informed consent form, the investigator will fill in a computerised inclusion form, available 24/7 over the Internet via the e-CRF (secure access previously granted to each pre-declared investigating centre). Randomisation will be centralised and implemented by a computerised random allocation system coupled with the electronic case report form, such that patients included in the study can be easily and automatically randomized. Study treatment will be prescribed and the inclusion confirmation to the coordinating centre will be faxed. The investigator shall perform several data checks and collections during the **inclusion visit**: - Verification of patient eligibility criteria - Verification of the absence of ECG conduction disorders - Verification of use/initiation of effective contraception (as the VKA are teratogenic). - Obtaining the patient's informed consent - Obtaining the patient's clinical-biological information (standardised data sheet) - Prescription of an initial ophthalmic examination (within one month of inclusion) - Fax to the central pharmacy for randomisation (numbered kits will be provided in advance in participating centres)

- Initiation of treatment: HCQ or placebo, at a dose of 200 mg × 2/day taken once daily (200 mg/day in patients with a weight < 45 k).

Dispensation of the treatment every 6 months by the Pharmacy Department in each centre. Empty or not entirely empty flask will be counted, and pills will be counted at each new dispensation.

Ophthalmic examination will be performed within one month after inclusion (central visual field).

Follow-up:

Blood tests at M1, M2, M3 to check out the tolerance of HCQ (cell blood count CBC, and liver enzymes ASAT/ALAT).

An Electrocardiogram (ECG) will be performed at M1.

Visits at M6, M12, M18 and M24 with clinical examination (that belongs to the standard follow-up of patients with primary APS), recording of new events related and unrelated to the trial, monitoring of therapeutic observance, tolerance and any changes in adjacent treatment. Biological tests at these visits will be performed in routine as standard follow-up of care of patients with primary APS.

These 5 consultations (4 for the follow-up and one for inclusion) are part of the normal follow-up for a patient suffering from APS. This follow-up shall be charged to the patient's health insurance.

To insure the compliance and tolerance of treatment, and to register any eventual side effects **phone call** will be scheduled every three months between 2 visits to check the tolerance and observance of the treatment (M3, M9, M15, M21).

Ophthalmic examination: visual field and acuity at M12, and electroretinography (ERG) at M24.

Biological tests:

- ***Profile on inclusion visit D0*** consistent in full blood test and platelets, creatinine, liver tests (ASAT, ALAT), C reactive protein (CRP), hemostasis test, i.e. prothrombin time (TP), TCA, fibrinogen; antiphospholipid antibodies (lupus circulating anticoagulant, anticardiolipin antibody, anti-B2GP1 antibody). To these tests will be added an antinuclear antibody assay with determination of anti-DNA and anti-ENA if positive. These blood tests are part of the normal follow-up for a patient suffering from primary APS. This follow-up shall be charged to the patient's health insurance.
- **at M1, M2, M3** full blood test and platelets and ASAT, ALAT (minor risk of agranulocytosis and serious hepatitis). There are additional blood tests not included in routine.
- At **M6, M12, M18 and M24** visit tests will be identical to those performed on inclusion.
- Hydroxychloroquine blood dosage will be exclusively performed at **M24** (to insure to the compliance to treatment, but also to not affect the double-blind character of the trial).

This will be an additional biological test, not included in routine.

As all patients will be receiving VKA, treatment (**target INR**) follow-up shall be performed on an outpatient basis, as for all patients receiving VKA.

A minimal monthly INR will be performed, and the recording of the INR will be done on patient **diary book** delivered at the inclusion in the study.

These results will be included in the final trial analysis, as the INR level has a major impact on the risk of further thrombotic accidents.

In summary additional examinations are:

- Ophthalmic examination within one month of inclusion, then yearly (M1, M12, M24). A central visual filed will be performed at inclusion, a visual field and acuity at M12, and electroretinography (ERG) at M24.

Unblinding and statistical analysis shall be performed at the end of the trial, i.e. at 48 months. No intermediate statistical analyses are planned as the number of events at one year could be too low in the two arms of the study. An independent committee survey will check that there is no abnormal rate of events in one of the arms of the study every 100 included patients or at least once yearly, to check the clinical and biological tolerance of the experimental drug.

An independent committee of adjudication of thrombotic events will deliberate on the confirmation of thrombotic relapses.

As the trial involves interventional biomedical research, it shall be declared to the competent authorities in each country and shall be subject to IRB approval.

Subject inclusion criteria

- Age > 18 years
- With primary* venous antiphospholipid syndrome**, with past thrombosis, with an indication of oral anticoagulants
- Patients are volunteers, informed and signed the consent form for participation in the study after receiving the information letter.
- *Primary APS refers to APS with no identified cause.
  **According to the Sydney international criteria, antiphospholipid syndrome is evidenced by the presence of a thrombotic clinical manifestation, combined with the presence of a biological criterion: presence of circulating anticoagulant and/or of anticardiolipin antibodies > 40 uGPL (> 99 percentiles) and/or anti-B2GP1 antibodies > 40 uGPL (> 99 percentiles). The presence of antiphospholipid

	antibodies (aPL) should be confirmed to 3 months for a APS be recognized. The trial has taken the choice of using (> 99 percentiles) to insure the homogeneity and reliability of tests in the different co-investigator centres
Subject non-inclusion criteria	• Secondary APS • The following ophthalmological disease: ○ Retinal disease contraindicating hydroxychloroquine prescription ○ Cataract ○ Monophthalmia • Demonstrated history of HCQ intolerance/ allergy or HCQ contraindication • Known glucose-6-phosphate dehydrogenase deficiency • Chronic alcoholism • Patients with already increased QT values • Concurrent treatment with other products potentially increasing QT or with arrhythmogenic properties • Epilepsy • Haemolytic anaemia • Porphyria • Chronic liver disease • Renal failure (creatinine clearance < 30 ml/min) • Ongoing pregnancy or breast feeding • Progressive ongoing cancer or hematological malignancy • Psychiatric disease not allowing the treatment • Lack of health insurance • *Participation to concomitant study with interventions leading to a bias to our study (other drugs, with synergistic or antagonizing or confounding effects). • Uncontrolled, active bleeding • Conditions in which coagulation tests cannot be performed at appropriate intervals • Any risk factor for hemorrhage (e.g., subacute bacterial endocarditis, blood dyscrasias, menorrhagia, dissecting aneurysm, major surgery, spinal anesthesia, hemophilia, GI ulcerative lesions, liver disease, impaired hemostasis). • *In case of doubt with respect to this criterion the coordinator of the study will be contacted and will deliberate about the possibility to participate to a concomitant trial
Treatments/ strategies/ procedures	Patients shall be randomized in a double-blind fashion to receive either Plaquenil or the placebo. The ratio of patients randomized into the two groups shall be of 1:1. Randomisation will be implemented by a computerised random allocation system coupled with the electronic case report form, such that patients included in the study can be easily and automatically randomized. The randomisation number will be identical to the treatment number displayed on the treatment kit (box) labels. **The choice of a 400 mg dose** seemed appropriate as this is below the toxic dose (< 6.5 mg/kg/d) and is routinely used in cases of SLE and RA. This dose corresponds to an average of 6 mg/kg/day, the recommended dose for patients with no associated risk factors. Patients with such risk factors will be excluded from the trial. The doses used in the literature to demonstrate the effect of HCQ on thrombotic relapse are at least equal to this dose. HCQ requires a certain time before being effective. Considering the drug's harmlessness and of the expected benefits associated with its use, we deemed it ethical to propose these doses over a 24-month period. The anti-thrombotic treatment shall consist in VKA with a therapeutic goal freely chosen by the investigator. The patients' other primary treatments will not be altered and will remain freely chosen by the investigator. The benefit-risk balance of this drug would appear acceptable at the individual and collective levels
Number of Patients	Number of subjects required to answer the research question: 350 in each group, i.e. 700
Justification of the sample size	The sample size calculation is based on the following justifications: Based on previously published data concerning the risk of thrombotic relapse in APS, in our trial hypothesis we estimated this risk of relapse under treatment at 10% over 2 years, according to the results of prospective and retrospective studies and of the average relapse rates in APS. On the other hand, with HCQ treatment, the decrease of thrombosis events in secondary APS varies between 50% and 100%. We therefore consider that a reduction from 10% in the placebo-oral anticoagulant arm to 5% in the HCQ-oral anticoagulant arm is reasonable and justified in the context of this disease. Ensuring an alpha risk of 0.05 and an 80% test power, calculations (website biostatTGV) indicate a number of subjects required equal to 334 patients in each group. Moreover, and in an independent way, to obtain a confidence interval (at 95%) of relapse with oral anticoagulants HCQ between 0% and 6% (centered on 3% with a 3-point error margin), a sample of 302 patients is required. The choice of 350 patients in each group can satisfy the two previous conditions and guarantee the operational feasibility of the study. To consider any drop-outs, the final number of included patients shall be of 700 (350 per group)
Statistics	The trial biostatistician will perform a statistical analysis of all randomized and evaluated patients (intent to treat analysis). **Descriptive statistics**: Collected variables shall be described globally and per group.

	Qualitative variables are expressed in population size and percentage. Quantitative variables are expressed in terms of mean ± standard deviation with 95% confidence interval, along with the 5th and 95th percentiles. These are, however, expressed in terms of median, minimum, maximum and 5th and 95th percentiles when normality is rejected. The Kolmogorov- Smirnov test will be used to check parameter normality and the Levene test will be used to determine equality of variances. The Student t test will be used to compare the distribution of quantitative variables between the two patient groups (HCQ/ placebo). If the test application conditions are not met, the Mann-Whitney non-parametric test will be used. The Chi-square or Fisher exact-test will be used to compare the distributions of qualitative variables between the two groups. *Goals analysis* *The analysis of the main* outcome measure shall consist in comparing the proportions of thrombotic events in the two groups. This comparison shall be performed by means of a Chi-square test, or a Fisher exact-test if the Chi-square application conditions are not met. If a difference between the two groups is demonstrated for at least one of the potential confounding variables, the main outcome measure shall be analysed by means of multivariate logistic regression to consider the adjustment factors. *The secondary* outcome measures will be analysed using the same methods as the *main* outcome measure. A survival analysis related to the risk of thrombosis during follow-up associated with the exposure factor shall be performed and estimate by calculating the hazard ratio (uni- and multivariate Cox model). We will perform analysis based on type of antiphospholipid antibodies types and values (low, moderate and high) and type of previous thrombotic event (event prior to inclusion) and we will analyse the tendencies. Comparative analysis will be driven if statistical power is sufficient. In case an increased number of adverse events recorded at each 100 patients' inclusion, in one of the arms of the study the trial will be stopped according to the supervisor committee advices
Research duration	Considering the low prevalence of primary APS in the general population, the total patient inclusion period shall be of two years, taking trial duration to 4 years. HCQ requires a certain time before being effective. Considering the drug's harmlessness and of the expected benefits associated with its use, we deemed it ethical to propose these doses over a 24-month period. In light of the published data, the planned follow-up period is of 24 months, as this appears to be the shortest duration allowing us to draw conclusions and to achieve a degree of trial significance (follow-up > 2 years in most trials). For the same reasons, no intermediate analyses are planned. Thus: Inclusions: 24 months Follow-up: 24 months, i.e. A total duration of 4 years.

In case of pregnancy of women with primary APS enrolled in the HIBISCUS T study, the participation to the HIBISCUS O trial will be then proposed.

5. Trial feasibility

Considering the prevalence of primary APS in the general population, regarding the number of patients to be included and the number of participating international centres, including the national SLE and APS tertiary centre, along with the duration of the inclusion period (24 months), the trial seems to be perfectly feasible.

The participation to the elaboration of the project of the French lupus and other autoimmune disease patients' association (AFL+), FI2AR rare disease French network, CRI-IMMIDIAT*E*-FCRIN, E-CRIN, EATRIS, Orphan Dev, for data management, national registry, biobanking and databases setting, the expertise of scientific international Europhospholipid committee, the creation of web platform for APS patients and the communication and valorization means are some of the strengths of the study. A communication campaign will be launched through Patients' organizations, expert centers, and social networks to optimize and facilitate the recruitment.

The participating centers possess the necessary skills, along with significant experience in the field of APS, with a considerable enrolment potential. Patients will be enrolled by co-investigator clinicians involved in the trial, with the subsequent possibility of adding unplanned centers wishing to include further patients, in the eventuality of a low recruitment frequency. Patients monitored in the context of their disease, or with APS newly diagnosed by the participating departments, will be invited to take part in the trial (incident and prevalent cases). Potential additional clinical centres can be added.

6. Conclusion

Only a major randomized study such as the trial that we propose could allow formal conclusions on the potential major benefits of HCQ in thrombotic and obstetrical in APS, and on the benefit/risk balance of such a therapy.

As all actual treatments recommended by international guidelines are not satisfactory in terms of prevention of relapses, we are persuaded that HCQ could bring a significant benefit both in terms of efficacy and safety in APS patients.

This high-impact clinical study will allow formal conclusion on the efficacy of HCQ in APS, and, based on all data on potential significant benefit, will further lead to an application for market authorization of HCQ in this indication.

Declaration of interest

None.

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The French part of the trial has been registered on ClinicalTrials.gov on 30th may 2018 (Identifier: NCT03540810).

Scientific protocol assistance about the use of Hydroxychloroquine in secondary prevention of obstetrical and thrombotic new events in primary APS trial has been obtained from the European Medicine Agency on May 2017 (EurEMA/CHMP/SAWP/284691/2017 Procedure No. EMEA/H/SA/3509/1/2017/PA/II).

Notes

Dr. Cristina Belizna, Professor PL Meroni, Professor R Cervera equally have designed the trial.

Professor PL Meroni, Professor R Cervera equally contributed to the coordination of this work.

All authors and collaborators have reviewed and edited the draft version of the article and approved the final version. All authors have approved the final article submitted.

Take home messages

- Relapse rate in antiphospholipid syndrome (APS) is high despite standard current therapies
- Survival rate in this population is 90.1% at 10 years and 65% at 15 years
- There is a need for additional or alternative therapies in this disease
- An international multicenter trial on Hydroxychloroquine for the secondary prevention of relapses in primary antiphospholipid syndrome is launched
- Fifty three centers from 16 countries participate to this international trial

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