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HYdroxychloroquine to improve Pregnancy outcome in women with AnTIphospholipid Antibodies (HYPATIA) protocol: A multi-national randomised controlled trial of hydroxychloroquine vs placebo in addition to standard treatment in pregnant women with antiphospholipid syndrome or antibodies

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Short title: HYPATIA study protocol

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Abstract: (202 words)

Background: Women with antiphospholipid antibodies (aPL) are at risk of adverse pregnancy outcomes, including recurrent first trimester pregnancy loss and late pregnancy complications such as pre-eclampsia, HELLP syndrome, premature delivery, intrauterine growth restriction, placental abruption and intrauterine death. Current standard care in obstetric antiphospholipid syndrome (APS) include aspirin and heparin, and has resulted in live-birth rates of approximately 70%. However, 30% continue to have pregnancy complications. Hydroxychloroquine (HCQ) is suggested as a new treatment approach, but no randomised controlled trials (RCT) have assessed its efficacy.

Aims: To assess pregnancy outcome in women with aPL treated with HCQ vs placebo in addition to standard treatment.

Methods: The HYdroxychloroquine to improve Pregnancy outcome in women with AnTIphospholipid Antibodies (HYPATIA) study is a phase IV multicentre RCT, in which pregnant women with persistent aPL will receive either HCQ or placebo in addition to their usual medication. The primary endpoint is a composite of aPL-related adverse pregnancy outcomes: one or more pregnancy loss(es) (either < 10 or > 10 weeks of gestation) and premature birth before 34 weeks due to any of pre-eclampsia, eclampsia, recognised features of placental insufficiency.

Discussion: The HYPATIA study is expected to provide evidence on the effect of HCQ in pregnant women with persistent aPL.

Keywords: antiphospholipid antibodies, antiphospholipid syndrome, obstetric antiphospholipid syndrome, pregnancy, hydroxychloroquine

Introduction

Antiphospholipid syndrome (APS) is characterised by the presence of persistent antiphospholipid antibodies (aPL) and clinical manifestations such as thrombosis, micro vascular events and/or pregnancy morbidity and mortality [1]. The obstetric morbidity in APS includes recurrent pregnancy first trimester loss, and aPL-related ischaemic placental dysfunction such as pre-eclampsia, eclampsia, HELLP syndrome (haemolysis, elevated liver function and low platelets), premature delivery, intrauterine growth restriction (IUGR), along with intrauterine death [1]. The aPL included in the classification criteria include lupus anticoagulant (LAC), immunoglobulin (Ig)G and IgM anticardiolipin antibodies (aCL), as well as IgG and IgM anti-β2glycoprotein I (aβ2GPI)[1].

The use of low dose aspirin and low molecular weight heparin (LMWH) is the current standard of prevention of aPL- related obstetric complication in women with aPL, and despite a limited body of evidence supporting this treatment, approximately 70% of women deliver a live viable infant [2-4]. However, 30% of women with aPL continue to have aPL-related pregnancy complications.

The prevention of obstetric complications of aPL therefore remains suboptimal, and other treatment options have been explored. The addition of first-trimester low-dose prednisolone to conventional treatment improved the rate of live births in refractory aPL - related pregnancy loss(es) up to 61% [5]. Other treatments, such as intravenous Ig, have failed to show benefit [6, 7]. Hydroxychloroquine (HCQ) in APS is receiving increasing international attention, and a group of experts recommended HCQ in addition to standard treatment in those with APS and with previous pregnancy failure on current treatment [8]. Furthermore, an international task force highlighted the need for clinical trials of HCQ in pregnant women with aPL and APS [9]. Herein we will review the available basic science and clinical evidence for using HCQ in the setting of aPL. Moreover, we will present the protocol for the first randomised controlled trial (RCT) of HCQ versus placebo in pregnant women with persistent aPL.

What is known about HCQ's action in the presence of aPL: basic science

Chloroquine and HCQ are weakly basic 4-aminoquinoline compounds. HCQ is the synthetic form of chloroquine and differs only by a hydroxyl group attached to a side chain resulting in a preserved efficacy with a less toxic side-effect profile [10]. The exact pharmacodynamics and action of HCQ *in vivo* remain to be fully uncovered, but most likely multiple molecular

pathways are involved. HCQ's detailed effects in systemic lupus erythematosus (SLE) have been described elsewhere [11]. Wallace et al have described HCQ's ability to antagonise Toll-like receptor-mediated immune response activation as one of the most important features in SLE [11]. It remains unknown which effects of HCQ are important in the immunomodulation of autoimmune diseases and also if different effects are required in different autoimmune diseases [12].

In retrospective clinical studies, HCQ was associated with a reduction in the risk of thrombosis in lupus patients and is discussed below. The mechanism by which HCQ might have an antithrombotic effect has been studied in a limited way. *In vitro* studies have showed that HCQ inhibits platelet aggregation and the release of arachidonic acid from aPL induced stimulated platelets [13]. Furthermore, Rand et al. showed that aPL can disrupt the physiological anticoagulant shield of annexin A5 (AnxA5), leading to exposure of pro-coagulant phosphatidylserine and subsequently triggering thrombosis. As a novel finding, the group could show that HCQ restores the disruption of natural anticoagulant AnxA5 in patients with aPL [14].

Tissue factor (TF) is the key initiator of *in vivo* coagulation and has been implicated in in the pathogenesis of APS [15]. The ability of aPL to induce TF expression was demonstrated *in vitro* studies using serum samples from patients with purified aPL, which were added to cells, showing up-regulation of TF on monocytes [16, 17], neutrophils [18] and endothelial cells [19]. A number of investigators have found that serum, plasma, purified total IgG, antiB2GPI from APS patients increases TF expression and pro-coagulant activity on monocytes [20-22]. Lopez- Pedrera et al showed that aCL IgG stimulate TF expression on circulating monocytes, acting through intracellular pathways including NFKappaB and MAP kinases [17]. In mice with aPL-induced fetal loss, neutrophils in turn can express TF through aPL–induced complement activation [23].

We have recently assessed the effect of using HCQ in patients with aPL and APS on plasma biomarkers including soluble TF, which has been shown to be increased in patients with APS [24]. We showed that the use of HCQ was effective to decrease soluble TF levels in patients with aPL and APS 12 weeks after the commencement of HCQ compared to baseline. This may be a mechanism contributing HCQ's antithrombotic effect [24].

Results from experiments in a *murine* model by Edwards et al. show that HCQ has the ability to reverse aPL-induced thrombosis [25]. The group observed that aPL-injected mice treated

with HCQ had a significantly reduced thrombus size and that thrombus duration was reduced compared to mice treated with placebo [25]. *In vitro* studies showed that aPL are directly pathogenic towards trophoblast cells, which is a mechanism cited as a possible cause of recurrent first trimester pregnancy loss(es) [26]. *In vitro* studies have shown that HCQ reversed the aPL-induced inhibition of the chemokine interleukin 6 (IL-6) [27]. IL-6 released by first trimester trophoblast cells had been shown to drive trophoblast migration (a vital process for implantation) in previous studies by the same group [28].

We and others have hypothesised that angiogenic molecules may play a role in the pathogenesis of obstetric APS, in light of their role in other placental dysfunction conditions such as pre-eclampsia [29, 30]. Elevated levels of circulating soluble fms-like tyrosine kinase 1 (sFlt1) is thought to cause the maternal expression of pre-eclampsia. Levels of sFlt1 and proangiogenic factors (such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF)) have been proposed as predictors of preeclampsia in healthy women [29, 30]. In 492 pregnant women from the prospective multicentre cohort, the PROMISSE cohort (PRedictors of pRegnancy outcome: BioMarkers In antiphospholipid Syndrome and Systemic lupus Erythematosus), Kim et al. found that the circulating pro and anti angiogenic factors such as sFlt1, PlGF and soluble Endoglin (sEng) measured between week 12-15 of gestation were predictive of adverse pregnancy outcomes in women with SLE and aPL [31].

Current available evidence suggests that aPL can affect the maternal side of developing placenta by reacting with endometrial cells in the decidua, by which they induce a pro-inflammatory phenotype and interfere with the physiological implantation process causing first trimester loss [32]. These mechanisms are also thought to play a role in the failure of adequate vascularisation of developing placenta, ultimately resulting in complications such as pre-eclampsia. We wished to explore the possible beneficial effect of HCQ on angiogenic biomarkers, as this has not been explored before.

Some studies support the hypothesis that the complement system is activated and plays a role in the pathogenesis of thrombotic and obstetric APS [23, 33-36]. Our group and others have previously confirmed complement activation in patients with isolated aPL and APS (thrombotic and obstetric APS) compared to healthy controls [37, 38]. Our most recent *in*

vivo results assessing the effect of HCQ on complement activation markers C3-des-Arg and Bb in HCQ naive, non-pregnant aPL and APS patients at baseline and 12 weeks after the commencement of HCQ did not show any significant change in these values [24].

In mice exposed to complement inhibitors and knockout mice which have complement deficiencies there is inhibition of foetal loss and growth restriction mediated by aPL [23, 33], and also less aPL induced thrombus [34, 35]. Bertolaccini et al showed a possible protective effect HCQ of in a model of aPL-induced foetal loss [39]. In their mouse model, the fetuses died in 50% of pregnant aPL-injected mice and the survivors were growth-restricted and with smaller placentas compared to control mice. The administration of HCQ to mice exposed to aPL prevented fetal death, increased placental and fetal weight and decreased placental superoxide production (a marker of oxidative stress) [39]. In addition, the group reported HCQ-induced complement inhibition in APS patient serum, specifically looking at the common pathway complement activation product C5a-des-Arg after receiving 6.5 mg/kg HCQ for 6 months [39].

At an intracellular level, *in vitro* studies show that aPLs can induce endosomal NADPH (NOX) in endothelial cells and monocytes [40, 41]. NOX is an enzyme complex involved in pro-inflammatory signalling pathways [12]. Muller-Calleja et al recently published first *in vitro* data suggesting that HCQ significantly reduces the induction of endosomal NOX, which leads to a reduction of downstream gene activation [12]. This 'protective' mechanism of HCQ in preventing monocyte activation was confirmed in mice injected with human aPL [12].

What is known about HCQ's use in patients with systemic lupus erythematosus and APS: clinical studies

HCQ is traditionally an antimalarial drug and has since the 1950's been widely used in the treatment of patients with rheumatoid arthritis (RA) or systemic connective tissue diseases such as SLE. HCQ was the first drug licensed for the treatment of SLE, and is one of the two currently licensed medications in SLE [42]. The efficacy of HCQ in patients with SLE has been well described. A cohort study of 150 SLE patients showed that it improves damage free survival [43], whereas data of 518 SLE patients from the observational LUMINA cohort showed that its use is associated with a reduced accrual of new disease damage [44]. Ruiz-Irastorza performed a prospective observational cohort of 232 patients, showing an

improved survival in SLE patients taking HCQ [45]. The use of HCQ in SLE is also associated with a reduced risk of SLE flares. The Canadian Hydroxychloroquine Study Group reported from their randomised controlled trial (RCT), that discontinuation of HCQ was associated with a 2.5x relative risk of a clinical lupus flare and that the risk of severe disease flare was significantly higher in patients who had HCQ discontinued [46].

The antithrombotic effects of HCQ in patients with SLE was reported in data from the LUMINA cohort. In univariate analysis, including 442 SLE patients with aPL of whom 46 were identified having 51 recorded thrombotic events (over 1446 visits followed over a mean of 88 months), a protective effect of HCQ use was demonstrated (odds ratio [OR], 0.536). This finding is in line with an observational prospective cohort study of 232 SLE patients, in which Cox regression analysis showed that HCQ use was associated with a reduced risk thrombosis (seven events occurred while patients were taking HCQ, 7 further events happened after the patient had stopped HCQ whereas 28 events were reported in patients who had never taken HCQ, so yielding a hazard ratio [HR] of 0.28) [45]. Likewise, the antithrombotic effect of HCQ could also be demonstrated in several other cohort, case-control and retrospective studies [11]. Moreover, the safe use of HCQ in pregnancy and lactation has been extensively documented and systematically reviewed [47-49]. Most data on the use of HCQ in pregnancy stems from patients with SLE. Treatment of pregnant patients with SLE was first described more than three decades ago, and national and international clinical guidelines now recommend using HCQ in pregnant women with underlying autoimmune diseases requiring immunomodulation [50, 51].

The role of HCQ in APS is currently being investigated in thrombotic and obstetric APS. In a prospective non-randomised study of 40 patients with primary thrombotic APS without underlying SLE, Schmidt-Tanguy et al. studied the effect of 400 mg HCQ in addition to oral anticoagulation with vitamin K antagonists (VKA), target INR 2-3. None of the patients receiving HCQ along with standard anticoagulation had recurrent thromboembolic events, whereas 30% in the control group experienced a recurrent event (p=0.0086) [52]. Unfortunately, the first RCT assessing the role of HCQ role as primary prophylaxis in aPL positive patients has recently been terminated due to manufacturing shortage [53].

In retrospective studies of pregnant patients with aPL and or APS, we and others showed that HCQ is a candidate for preventing aPL-related adverse pregnancy outcomes [54, 55]. In a retrospective multicentre cohort consisting of 30 APS patients (and 35 pregnancies),

Mekinian et al reported that HCQ was associated with fewer first trimester miscarriages (pregnancy losses decreased from 81% to 19%, p<0.05) and improved live birth rates in refractory obstetric APS to 78% (p<0.05)[54]. In our retrospective observational cohort study of 96 women with 170 pregnancies, we also showed that HCQ treatment was associated with a higher rate of live births (67% in HCQ treated vs. 57% in untreated patients, p=0.05) and a lower prevalence of pregnancy morbidity (47% vs. 63%, p=0.004). Pregnancy duration was longer in patients receiving HCQ compared to those who did not receive HCQ (median 27.6 weeks, range [6-40] versus 21.5 weeks [6-40], p=0.03) and foetal losses beyond the 10 weeks of gestation were less frequent in women who were treated with HCQ (2% vs. 11%, p=0.05). Moreover, ischaemic placental mediated complications (pre-eclampsia, eclampsia and foetal growth restriction (FGR) were less prevalent in HCQ treated women than in the control group (2% vs 10.9%, p=0.05). There was a significantly higher rate of women undergoing spontaneous vaginal labour in HCQ women compared to women without HCQ treatment (37.3% vs. 14.3%, p=0.01). The association of HCQ with the absence of aPL-related complications in pregnancy was confirmed in multivariate analysis (OR 2.2; 95% CI 1.2-136.1; p=0.04) [55]. However, the design of this retrospective study was suboptimal, as the two groups were heterogeneous, with higher prevalence of SLE amongst those receiving HCQ. These clinical and animal studies, as well as in vitro and ex vivo data, suggest a potential role of HCQ in reducing pregnancy morbidity and mortality in women with aPL. In view of all of the available evidence, we proposed HYPATIA study (HYdroxychloroquine to improve Pregnancy outcome in women with AnTIphospholipid Antibodies) which is a randomised controlled multicentre trial of HCQ versus placebo in women with persistent aPL planning for pregnancy. Our hypothesis is that HCQ may improve aPL-related pregnancy complications [56].

Methods

Study design

The HYPATIA study is an investigator initiated multi-centre, double blind RCT of women with persistent aPL planning to fall pregnant. Patients will be randomised to HCQ or an identically looking placebo in addition to their usual medication.

Once a woman is enrolled into the HYPATIA study, she will be attending 3 monthly follow up visits until she falls pregnant (defined as pre-pregnancy visits). Patients will be starting to take the investigational medicinal product (IMP) as soon as they are randomised (i.e. before

conception). If no pregnancy is achieved within 12 months, she will be excluded from the study. These visits coincide with the usual routine follow up visits. On these visits the IMP will be repeatedly dispensed and compliance will be assessed. All data will be collected in an electronic case record file.

When a woman falls pregnant, she will contact the study team and attend her first pregnancy visit (first trimester visit). At this visit a new trial medication will be dispensed. Follow up visits for the HYPATIA study purpose will be scheduled once every trimester (defined as 1st, 2nd and 3rd trimester visits). Nevertheless, if the participant required closer monitoring, she will be followed up accordingly as per standard of care. Women will be instructed to stop trial medication on the day of delivery and will be asked to hand in the rest of the trial medication at their post-partum follow up. The post-partum visit shall be completed 6 weeks post-partum.

Patient recruitment

Potentially eligible women will be identified in routine outpatient clinics in our participating sites, which include: Guy's and St Thomas' NHS Foundation Trust, London, UK; Imperial College Health Care NHS Trust, London, UK; University College London NHS Foundation Trust, London, UK; Liverpool Women's Hospital NHS Foundation Trust; Oxford University Hospitals NHS Foundation Trust, Oxford, UK; Academic Medical Center Amsterdam, the Netherlands; University Hospital Torino, Italy; University Hospital Copenhagen, Denmark. Patients will receive full information about the HYPATIA study, and if they decide to take part, written informed consent will be obtained. Patients will have as much time as they need to consider their participation. Provided all eligibility criteria are fulfilled, study randomisation can be undertaken and the investigational medicinal product (IMP) will be dispensed.

Potential participants will be identified by their physician during routine outpatient visits to the trial sites. The HYPATIA study visit assessment schedule is outlined in Figure 1.

Inclusion criteria:

1. Women with known persistent aPL (ie. isolated persistent aPL or APS) who are planning pregnancy and consent to participate.

Exclusion criteria:

1. Women who are already pregnant

2. Allergy or adverse event to HCQ. Hypersensitivity to the active substance, 4-aminoquinoline or any of the compounds of the IMP or placebo.
3. Current treatment with HCQ
4. Age < 18 and > 45 years
5. Body weight < 45 kg
6. Psoriasis
7. Uncontrolled epilepsy
8. Anti-Ro antibodies
9. Renal replacement therapy
10. Other severe active co-morbidities (HIV, hepatitis B)
11. Porphyria
12. History of retinopathy
13. History of galactose intolerance, lactase deficiency or glucose-galactose malabsorption
14. Participation in any other IMP trial at the time of consent
15. Previous pregnancy failure on HCQ

Laboratory assessment for aPL diagnosis: For the purpose of the HYPATIA study aPL positivity is defined by the presence of a positive test for anticardiolipin antibodies (IgG/IgM isotypes > 95th percentile) and/or lupus anticoagulant and/or anti- beta 2 glycoprotein-I (IgG/IgM isotypes > 95th percentile), on two or more consecutive occasions more than 12 weeks apart. All participating sites will be expected to have established their own 95th percentile, and data on absolute values (for aCL and anti- beta 2 glycoprotein-I), lower and upper normal value will be collected in the electronic database. aPL are assessed according to standard established methods [57, 58].

Duration of trial

The HYPATIA study will last for a total of 36 months, which includes recruitment and follow-up of patients. The end of the trial is defined as database lock. Each individual will remain in the study as long as their time to conception (up to maximum 12 months), their full length of pregnancy until delivery. In total 328 pregnant women (sample size calculation is detailed below) will be included in the study. The trial flow chart is summarised in figure 2.

Primary outcome measures

The primary endpoint is a composite of three principal aPL-related adverse pregnancy outcomes: one or more pregnancy loss(es) (either < 10 weeks gestation or beyond 10 weeks of gestation of a morphologically normal foetus documented by ultrasound or by direct examination of the foetus) and premature birth of a morphologically normal neonate before 34 weeks due to any of pre-eclampsia, eclampsia, recognised features of placental insufficiency.

Premature birth for other reasons will not be included. The components of the primary endpoint will each be presented as secondary endpoints (below).

Secondary outcomes measures

The pre-defined secondary endpoints include: 1. Pregnancy loss < 10 weeks gestation, 2. Pregnancy loss > 10^{th} week of gestation of a morphologically normal foetus documented by ultrasound or by direct examination of the foetus, 3. Premature birth of a morphologically normal neonate < 34 weeks due to any of pre-eclampsia, eclampsia, recognized features of placental insufficiency, 4. Gestational age at delivery, 5. Birth weight, 6. Delivery by Caesarean section, 7. Apgar score < 7 at 5 min, 8. Neonatal morbidity (bleeding or thrombotic complications, infections, congenital abnormalities), 9. Days to hospital discharge following delivery (mother & child), 10. Thrombotic events in the mother during pregnancy and 6 weeks post-partum and 11. Days of neonate in special care.

Suspected unexpected serious adverse reactions (SUSARs) and other serious adverse events (SAEs) will be reported to the regulatory authorities and the research ethics committees, as appropriate. All SAE reports received from the study sites will be reviewed by independent medically qualified staff.

SAEs that do not require reporting: Pregnancy itself is well known to potentially causing nausea and tiredness due to several physiological adjustments of the body especially in the first trimester. Any symptoms thought to be related to the physiological changes in pregnancy do not require reporting.

In patients with aPL we expect pregnancy complications. Therefore, aPL related pregnancy complications, such as miscarriage < 10 weeks' gestation, miscarriage > weeks 10, premature birth < 34 weeks due to pre-eclampsia, eclampsia or fetal growth restriction

(FGR) and stillbirth shall not be reported as SAE. For these events we have designed a special section in the electronic database 'adverse pregnancy outcomes', which shall be completed in this case. Any other adverse events will be reported.

Randomisation

Randomisation will be performed by an independent individual at King's Clinical Trials Unit (King's CTU), using a computer-based minimisation process, adjusting for thrombosis (yes/no), previous adverse pregnancy outcomes (yes/no) and previous pregnancy (yes/no).

The investigators and trial subjects will remain blinded to treatment. The trial pharmacist will be blinded too. Unique pack numbers for the trial medication will be generated for the active and placebo products and will be sent with randomisation emails. The trial statistician will know only A vs B, with the meaning of A & B held by King's CTU.

Statistical analysis

Sample size

The sample size was calculated based on our audit data. Retrospective data from St Thomas' Hospital showed that treatment with HCQ was associated with a higher rate of live birth (67% in women with SLE and aPL treated with HCQ versus 57% in women in the control group p=0.05) and a lower prevalence of aPL related pregnancy morbidity (47% versus 63% p=0.004)[8]. The trial is powered to detect a 16% reduction in pregnancy morbidity, which is the main outcome of the study (total sample size 328). A minimum of 328 women will therefore be randomised. The calculation was made on the following: significance alpha = 0.05, power 1-beta 80%, percentage cross 5%.

Primary statistical analysis: Percentages in both arms of the study will be compared using binomial regression with a log link, adjusting for the minimization variables to give risk ratios. Risk differences will be estimated similarly. Continuous measures will be compared using linear regression adjusting for minimization variables and baseline measurement of the outcome (where available). Estimates will be given with 95% confidence intervals.

Results will be considered significant at the 5% level. However, attention will be paid to the totality of results and the exact size of the P-value. Smaller values will be treated as stronger

evidence against the null hypothesis. The intention-to-treat principle will be followed in the main analysis, but a secondary per-protocol analysis will also be performed to provide additional information on the nature of the treatment effect.

Three subgroup analyses are planned: by previous thrombosis; previous adverse pregnancy outcomes; previous pregnancy. In each case, the primary outcome will be analysed in each subgroup, and an interaction test carried out for evidence of a difference in treatment effect between the groups.

No formal interim analyses are planned. The Data Monitoring Committee will aim to meet in person at least every 6 months, and will consider the results so far. They are empowered to request that the trial stops if there is overwhelming evidence for or against one treatment, such that to randomise further patients would be unethical.

Competent authority approval in the UK has passed the initial assessment and ethics approval is currently awaited. As a European multicentre trial we are following the 'voluntary harmonisation procedure'[59].

Discussion

We hypothesise that the addition of HCQ to the standard of care treatment in pregnant women with aPL will improve aPL-related adverse pregnancy outcomes. Current data to suggest a role of HCQ in these patients is inadequate; hence, we have designed an adequately powered RCT [54, 55].

We have shown that with a rigorously applied local protocol around 70% of pregnant women with aPL/APS will deliver a viable infant [2]. The current management includes LMWH and/or low-dose aspirin. As mentioned above, this does not prevent all maternal, foetal and neonatal complications. Our retrospective study suggests that HCQ has a beneficial effect in women with aPL, as the treatment with HCQ was associated with a higher rate of live births (67% vs. 57%, p=0.05) and a lower prevalence of aPL-related pregnancy morbidity (47% vs. 63%, p=0.004) [55]. Despite the heterogeneity in the two groups in terms of SLE prevalence and previous pregnancy history, our results support the concept that women with aPL may benefit from treatment with HCQ during pregnancy to improve pregnancy outcome.

We have also conducted a systematic review of the evidence of HCQ in obstetric APS, which confirmed a lack of evidence [8]. We therefore performed an expert-based clinical judgement consensus, which is an accepted approach to address a specific and clinically relevant question in an area were best clinical practice is uncertain. The experts agreed that HCQ could be considered in selected cases of patients with obstetric APS or after failure of standard treatment with aspirin and any heparin-based agent. Moreover, the majority of experts considered adding HCQ in specific scenarios, such as women with previous thrombosis or previous ischemic placental medicated complications [8] .

The ideal RCT to answer the question of the benefit of HCQ in pregnancy in those with aPL, would be to investigate women with refractory obstetric APS, i.e. those who have failed conventional treatment, and then to randomise this group to HCQ versus placebo. However, after reviewing our patient group and discussing with all our trial centres, we calculated that it would take 10 years to recruit adequate women to a study of this design. We have hence chosen to study women with aPL rather than women with refractory obstetric APS, because it not only practically feasible but also supported by the results of our previous retrospective study.

Some may argue that that the inclusion of women with aPL above the 95th percentile as opposed to the defined 99th percentile in the Miyakis criteria is a fault in the study [1]. However, it is our and others experience, that 'low level aPL are highly relevant in the setting of obstetric APS [60, 61]. This is in line with the observation of Ruffatti et al, that women with purely obstetric APS have lower aCL antibody titres compared to patients with thrombotic APS [62]. Moreover, retrospective data and data from a cohort study suggest that low-titre aCL, defined as those between the 95th and 99th percentiles, are still of clinical significance for women with obstetric APS [63, 64].

Although this study includes women planning to fall pregnant, and will therefore be perceived by some as a difficult study. However some of our HYPATIA study team members have experience in successfully conducting RCT's in which women were recruited pre-conceptionally[65]. We are involving centres that are expert tertiary centres for managing women with aPL and managing them preconception and throughout pregnancy. Recruitment estimates of number of patients that will be entered into the study have been obtained from each centre and exceed the proposed number by about 20% and we fully expect completion of the study in the allotted time.

HYPATIA is the first trial to randomise HCQ versus placebo in pregnant women with persistent antiphospholipid antibodies and our results will provide an evidence-base for the decision to use of HCQ in these patients.

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Beverley Hunt and Karen Schreiber have designed the trial. All authors and collaborators have reviewed and edited the draft version of the manuscript and approved the final version. All authors have approved the final manuscript submitted.

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