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The impact of hydroxychloroquine on obstetric outcomes in refractory obstetric antiphospholipid syndrome

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ABSTRACT

Background: The use of low-dose aspirin (LDA) and heparin has improved pregnancy outcomes in women with antiphospholipid syndrome (APS). However, 20–30% still have adverse outcomes despite treatment. Recent retrospective studies showed a beneficial effect of hydroxychloroquine (HCQ) in APS due to its anti-inflammatory, immunomodulatory and antithrombotic properties. Data in refractory obstetric APS (OAPS) remain scarce and include heterogeneous populations with various concomitant treatments.

Objective: The objective of this study was to assess the impact on the obstetric outcomes of adding HCQ to classical treatments for women with refractory primary obstetric APS.

Methods: In a retrospective single-centre cohort study, we compared pregnancy outcomes in women with refractory primary OAPS (2004–2019) who received two different treatments in subsequent pregnancies. Group A received 400 mg HCQ + 60 mg enoxaparin + LDA, while Group B received 60 mg enoxaparin + LDA. The main outcome was live birth rates, while pregnancy complications (early and late pregnancy losses and placental-mediated complications) were the secondary outcome.

Results: A total of 101 pregnancies in 87 refractory primary OAPS patients were included. The rate of live-born babies in Group A (HCQ) was 97.1% (67/69) vs. 62.5% (20/32) in Group B (RR: 1.55 [95% CI, 1.19–2.1]; $p < 0.001$). Pregnancy complications in Group A were 8.7% (6/69) vs. 37.5% (12/32) in Group B (RR 0.22 [95% CI, 0.15–0.30]; $p < 0.001$).

Conclusion: Hydroxychloroquine was associated with a higher rate of live births and a lower prevalence of pregnancy complications in refractory primary obstetric APS. The addition of HCQ to classical treatment may present a promising approach that needs to be confirmed with prospective studies.

1. Introduction

Obstetric antiphospholipid syndrome (OAPS) is an autoimmune disease associated with a high rate (70%) of adverse obstetric outcomes including recurrent early pregnancy losses, foetal deaths, and preterm deliveries before 34 weeks due to preeclampsia or other causes of placental insufficiency in the presence of persistent antiphospholipid antibodies (aPLs) according to the Sydney criteria [1]. Conventional treatment with low-dose aspirin (LDA) and prophylactic-dose heparin has dramatically improved adverse outcomes [2–4]. However, 20–30% of these women still have an obstetric complication despite treatment, referred to as refractory primary OAPS [5–7]. Poor obstetric outcomes are associated with the presence of risk factors: a concomitant autoimmune disease, history of thrombosis or the presence of triple antibodies (+), lupus anticoagulant (LA) activity or complement reduction [8–12]. This urges the need to find the most efficacious additional treatment to improve adverse results [13–15]. Although there are no evidence-based recommendations yet, several pharmacological strategies are being used: increased heparin dosages, steroids, intravenous immunoglobulin, plasma exchange, pravastatin and hydroxychloroquine [16–21]. However, the best pharmacological approach is still debated.

In recent years, investigations have focused on hydroxychloroquine

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(HCQ), originally an antimalarial drug that has proven to be effective in systemic lupus erythaematosus (SLE) and other autoimmune diseases [22,23]. Currently, the use of HCQ is recommended by experts for refractory cases in antiphospholipid syndrome (APS) [24]. HCQ has been reported to be safe during pregnancy and breastfeeding [25,26]; serious side effects associated with hydroxychloroquine exposure are very rare and include cardiac and retinal toxicity [27–29]. The European Medicines Agency has licenced its use for the treatment of APS [30]. The rationale for HCQ use is based on the new understanding of the pathogenesis of obstetric APS, which involves placental thrombosis, inflammation, and defective placentation [31,32]. For many years, placental thrombosis was thought to be the main pathogenic mechanism [33,34]. However, recent data suggest that inflammation is one of the main mechanisms of aPL-induced maternal morbidity [35,36]. HCQ has been shown to reduce aPL binding to syncytiotrophoblasts, reduce inflammation and improve trophoblast fusion at the decidual level [37,38]. HCQ also improves Annexin V expression, a natural antithrombotic shield at the placental level [39,40]. Thus, hydroxychloroquine could be beneficial in APS treatment due to its anti-inflammatory, immunomodulatory and antithrombotic properties.

Very recently, in three retrospective studies, Mekinian, Sciascia, and Ruffati et al. [41–43] showed that the addition of HCQ to standard treatment was safe and improved live births and obstetric outcomes in women with APS or aPL. These studies included heterogeneous populations with obstetric and thrombotic APS or women with SLE who were not always refractory to classical treatment. Moreover, HCQ was sometimes associated with a variety of concomitant therapies that could bias the results. Currently, there are two ongoing randomized controlled trials called "HYPATIA" and "HIBISCUS" trying to assess whether the addition of HCQ to standard treatment is beneficial to pregnant women with aPL [44,45].

To date, data in women with refractory primary obstetric APS remain scarce [46]. The best pharmacological approach in refractory obstetric APS remains unknown and represents a distressing challenge for physicians that manage this high-risk population. The purpose of this retrospective study was to assess the impact of the addition of HCQ on obstetric outcomes in women with exclusively refractory primary obstetric APS. We selected 101 pregnancies out of eighty-seven women with obstetric APS and a previous pregnancy failure despite conventional treatment. In the subsequent pregnancies, we compared live birth rates and obstetric complications between two fixed pharmacological approaches: an increased enoxaparin dose + LDA versus the addition of HCQ to this scheme.

2. Methods

This was an observational, retrospective, single-centre cohort study. We compared the outcome of 101 pregnancies treated with the addition of HCQ versus conventional treatment with a higher enoxaparin dose in pregnant women with obstetric APS who had previously suffered an obstetric complication despite classical treatment.

The electronic clinical records of patients who attended the Thrombophilia and Pregnancy Loss clinic between January 2004 and December 2019 were searched. The Institutional Review Board of the Austral University Hospital approved the design of this retrospective observational study, and patients signed an informed consent form allowing their clinical records to be reviewed and data used for scientific analysis.

2.1. Inclusion criteria

After careful chart review, we selected Caucasian women (20–43 years old) with primary obstetric antiphospholipid syndrome (OAPS), according to the 2004 Sydney international consensus criteria [1], who had been refractory to classical treatment (LDA+ enoxaparin 40 mg) in their previous pregnancies and who, in subsequent pregnancies, received one of the two following pharmacological strategies: 100/150 mg aspirin + 60 mg enoxaparin daily or the addition of 400 mg enoxaparin daily to this scheme. To obtain a homogeneous population, we exclusively included women with primary obstetric APS. All patients suffered obstetric failure after an OAPS diagnosis was made despite conventional treatment; thus, these patients were considered to have refractory OAPS. **Refractory obstetric APS** was diagnosed when any of the following pregnancy complications occurred under classical treatment: early (<10 weeks) or late (>10 weeks) pregnancy loss of a normal foetus (karyotype, morphological or by ultrasound) or unknown cause; placental-mediated complications: preeclampsia (PE) (arterial blood pressure >140/90 mm Hg and proteinuria >3 g/24 h); intrauterine growth restriction (IUGR): postnatal birth weight less than the 10th percentile; abruptio placentae; or preterm delivery before 34 weeks due to PE or other cause of placental insufficiency. As we wanted to exclusively evaluate the refractory primary obstetric APS subgroup, we only included women who had obstetric (not thrombotic) complications under classical treatment.

2.2. Exclusion criteria

We excluded patients with any of the following criteria: women allergic to hydroxychloroquine; women with other autoimmune diseases that required concomitant treatments such as steroids or immunoglobulin; twin pregnancies; and women with thrombotic events; all of these subgroups are associated with other concomitant risks that could bias results.

Laboratory tests were performed at the same core laboratory. Lupus anticoagulant detection was performed using dilute Russell's viper venom and LA-sensitive activated partial thromboplastin time as screening tests and confirmed with appropriate tests according to international guidelines (Instrumentation Laboratory) [47]. Anticardiolipin (ACL) antibodies and anti-β2-glycoprotein I (β2GPI) antibodies were determined using ELISA (Orgentec Diagnostika). The cut-off values for the 99th percentile in our population were 20 UGPL/UMPL for IgG and IgM ACL and 20 U/ml for IgG and IgM anti-β2GPI antibodies.

For each patient, data were collected as follows: APS characteristics, age, weight, aPL profile, triple positivity, complement deficiency (C3, C4), APS + inherited thrombophilia, presence of other autoantibodies (antinuclear antibodies, anti-DNA, anti SS-Ro, Anti SS-La), and history of mother's morbidity (diabetes, smoking, chronic hypertension, history of thrombotic event).

The recorded pregnancy complications in the previous pregnancy with classical treatment included early or late pregnancy loss, PE, IUGR, and preterm delivery due to placental insufficiency. The data recorded from subsequent pregnancies included treatment characteristics, live births, and obstetric complications (early <10 weeks or late >10 weeks pregnancy loss; placental mediated complications: preeclampsia, intrauterine growth restriction, placental abruption; premature delivery before 34 weeks due to placental insufficiency and neonatal outcomes: gestational age and neonatal weight at delivery).

In the following pregnancies (index pregnancies) after obstetric failure had occurred despite classical treatment, women were divided into two groups according to their risk profiles and received one of the two fixed pharmacological treatments: Group A (400 mg HCQ + 100/150 mg aspirin + 60 mg enoxaparin daily) or Group B (100/150 mg aspirin + 60 mg enoxaparin daily). Both groups received 100 mg aspirin daily at 8 PM, begun preconceptionally and continued during pregnancy until the 36th week of gestation. Women at high risk of developing preeclampsia according to their first trimester PE screening (risk of PE before 34 weeks >1:150) were switched to 150 mg aspirin daily [48]. Both groups received enoxaparin 60 mg daily since pregnancy was confirmed (instead of 40 mg as used in their previous pregnancies), a real-life practice being used for many years in refractory cases. Additionally, Group A started with 200 mg HCQ once a day at least two months before pregnancy and 200 mg twice daily since pregnancy was

achieved and throughout the entire pregnancy period. Considering that there are no evidence-based recommendations regarding the effectiveness and/or dosage of HCQ in APS refractory cases, this scheme was based on the available data at the moment the study began and the authors' clinical experience in the field. Before receiving HCQ, patients were examined and had a normal visual field, normal liver enzymes and a normal electrocardiogram. None of the patients received another concomitant drug, such as steroids or a higher dose of enoxaparin other than 60 mg/daily.

2.3. Group A: exposed to hydroxychloroquine

Between January 2004 and December 2019, 69 pregnancies occurred in fifty-six patients with a median age of 35 (21–42 years) who were treated with HCQ + LDA + enoxaparin. Hydroxychloroquine was given to the highest-risk group. Indications for HCQ included women who had suffered the most severe obstetric complications in their previous pregnancies despite conventional treatment: foetal death beyond 20 weeks (stillbirth), preeclampsia, intrauterine growth restriction, preterm delivery due to placental insufficiency and those who had the highest-risk laboratory profile: triple positivity and complement deficiency. All the patients in this group were treated with 200 mg HCQ daily starting at least 2 months before pregnancy and 400 mg daily since pregnancy began, plus 60 mg enoxaparin daily since pregnancy was confirmed. Eleven patients were switched from 100 mg to 150 mg aspirin daily after the 12th week because they had a high risk of developing preeclampsia. None of the patients received steroids or immunoglobulin during the index pregnancy. Two patients developed cutaneous allergies and were excluded from the study.

2.4. Group B (not exposed to HCQ)

Between January 2004 and December 2019, 32 pregnancies occurred in thirty-one patients with a median age of 34 (21–42 years) who were treated with LDA + 60 mg enoxaparin daily. This group included women who had a history of less severe complications in previous pregnancies under conventional treatment and a lower-risk laboratory profile. Women in this group had undergone only pregnancy losses before 20 weeks. None of the patients in this group had suffered a stillbirth or any placental-mediated complication (PE, IUGR) under classical treatment. We included 1 woman with triple positivity who refused to take HCQ because she was concerned about its potential side effects. In their subsequent pregnancies, all of them were treated with 60 mg enoxaparin daily + 100 mg aspirin daily at 8 PM. Two patients (6%) were switched from 100 mg to 150 mg aspirin in the 12th week due to a high risk of developing preeclampsia according to their PE screening.

The primary outcome was the live birth rate. The secondary outcome included pregnancy complications: early and late pregnancy losses and placental-mediated complications, such as PE, IUGR, and abruptio placentae.

2.5. Statistical analysis

To identify potential confounders, we evaluated the association of each variable with the groups receiving or not receiving HCQ and the main outcomes (live births and obstetric complications: early or late pregnancy loss, PE, IUGR, abruptio placentae). Continuous variables were analysed using *t*-tests or Wilcoxon rank tests according to their distribution; categorical outcomes were evaluated with X^2 and Fisher exact tests. We included age, weight, aPL profile (LA, ACA, Anti ß2 GP1), triple positivity, complement deficiency, APS + inherited thrombophilia, the presence of other autoantibodies (antinuclear antibodies, anti-DNA, anti SS-Ro, Anti SS-La, anti SM), and the history of the mother's morbidity (diabetes, smoking, chronic hypertension, and history of thrombotic events).

Then, after obtaining the crude association between HCQ and the main outcomes, variables showing a p value <0.2 in the initial screening were included one by one in a multiple regression model to adjust for potential confounders. In addition, since the exposure of interest was an intervention not randomly assigned, we also used propensity score (PS) matching to adjust for group differences and reduce confounding bias [49–51]. The PS is a measure of the probability that an individual is in the "treated" (HCQ) group given her background characteristics. Conditional on the PS, it is expected that the distribution of observed baseline covariates will be similar between treated and untreated subjects. Finally, to estimate the treatment effect of HCQ, we also used inverse probability weighting regression adjustment (IPWRA) as a strategy for causal inference in the context of an observational study.

3. Results

Between 2004 and 2019, 148 out of 754 pregnancies in women with APS at our facility had an adverse obstetric outcome despite conventional treatment, and 128 of them decided to have a subsequent pregnancy. Our final cohort included 101 pregnancies from 87 patients with refractory primary obstetric APS. As shown in Fig. 1, in the subsequent pregnancies after failure with classical treatment, 69 pregnancies (Group A) were treated with 400 mg HCQ + LDA + LMWH 60 mg/day, while 32 pregnancies (Group B) received LDA + LMWH at 60 mg/day.

We excluded 8 patients with pregnancy losses with abnormal karyotype foetuses. Other causes of exclusion were concomitant treatments with steroids or immunoglobulin (n = 11); therapeutic heparin dose (n = 5); associated APS (SLE, rheumatoid arthritis, Sjögren) (n = 6); allergy to HCQ (n = 2); women who received HCQ but did not have a previous obstetric failure under conventional treatment (n = 2); and women who did not meet strict criteria for obstetric APS (n = 7).

As described in Table 1, the patients' clinical characteristics were similar in both groups. They were all Caucasian women with primary obstetric APS who had been refractory to classical treatment in their previous pregnancies. No significant differences were seen between the groups with regard to age, weight, cardiovascular risks or history of thrombosis. Group A had a higher-risk laboratory profile: lupus anticoagulant (92.8% in Group A vs. 67.7% in Group B), triple positivity (12.5% vs. 3.2%) and complement deficiency (14.2% in Group A vs. 0% in Group B) were more prevalent in Group A than in Group B, although they failed to reach statistical significance.

Table 2 shows that a history of pregnancy complications in a previous pregnancy under classical treatment was more severe in Group A: 13 patients (23.2%) had suffered a late pregnancy loss >10 weeks under conventional treatment, 7 had stillbirths vs. 6 patients (19.3%) who had a pregnancy loss before 20 weeks in Group B. Nine patients in Group A (14.7%) had a history of placental-mediated complications (preeclampsia, intrauterine growth restriction) despite classical treatment. Group B included only women with pregnancy losses before 20 weeks under classical treatment; there were no stillbirths and no placental-mediated complications in this group's previous pregnancies. There was no significant difference observed in the prevalence of early pregnancy loss <10 weeks between the two groups (67.8% in Group A vs. 80.6% in Group B).

As we can see in Table 3, in the subsequent pregnancies (index pregnancies) after failure with standard treatment, Group A (the highest risk group) was treated with 400 mg HCQ + 60 mg enoxaparin + LDA, and Group B was treated with 60 mg enoxaparin + LDA. Hydroxychloroquine exposure was associated with a **higher rate of live births**: 97.1% (67/69) in Group A vs. 62.5% (20/32) in Group B, $p < 0.001$, and a **lower incidence of pregnancy complications**: 8.7% (6/69) in Group A vs. 37.5% (12/32) in Group B, $p < 0.001$. When analysed individually, there was a significantly lower incidence of early pregnancy losses before 10 weeks in Group A: 2.8% (2/69) vs. 34.3% (11/32) in Group B; $p < 0.001$. One foetal death at the 12th week occurred in Group B (3.1%). There were no stillbirths and no placental abruption. There were

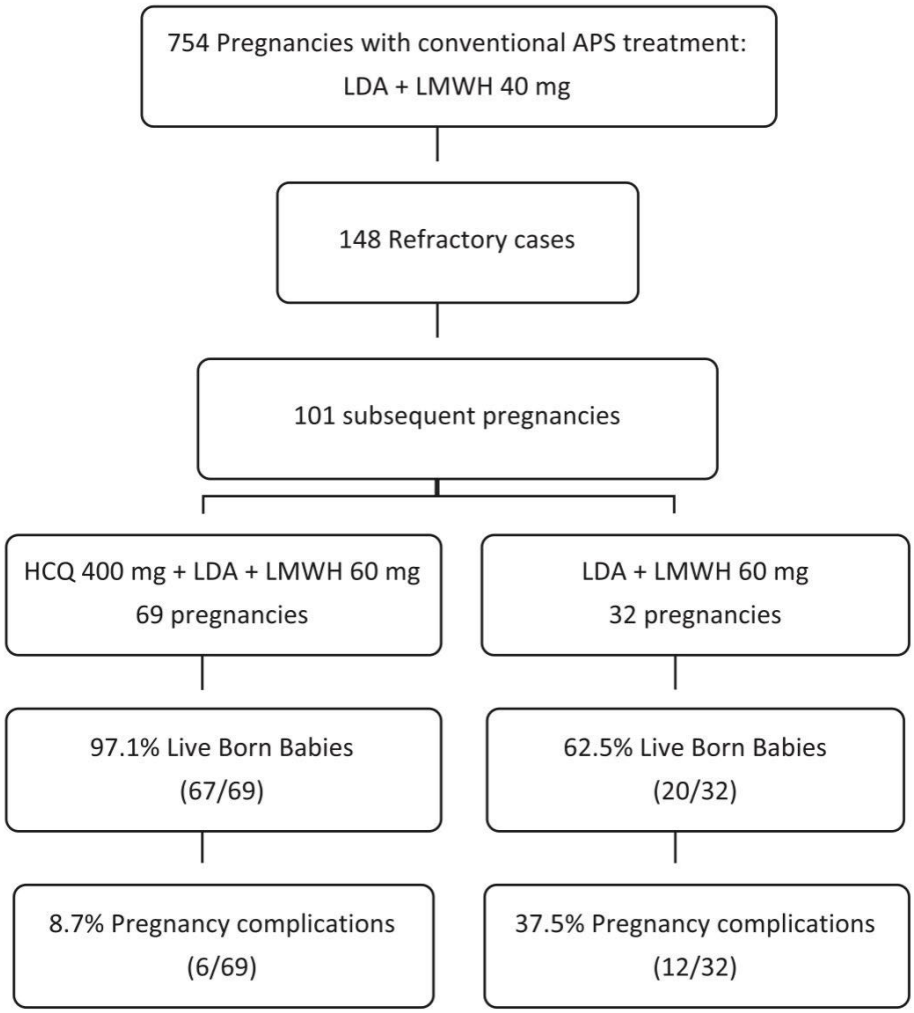

Fig. 1. Flowchart showing the distribution of treatments in subsequent pregnancies in patients with refractory primary obstetric antiphospholipid syndrome. APS: antiphospholipid syndrome; LDA: low-dose aspirin; LMWH: low molecular weight heparin; HCQ: hydroxychloroquine.

no thrombotic complications (deep venous thrombosis or pulmonary embolism) in either group. The median gestational age at delivery was 38 weeks in both groups, and no significant difference was observed in birth weights at delivery between the two groups (3191 g in Group A vs. 3262 g in Group B). Placental-mediated complications (PE and IUGR) occurred only in Group A (5.7%, 4/69), although this study was not powered to analyse individual complications.

In a secondary analysis, we compared placental-mediated complications in Group A before HCQ exposure (in previous pregnancies) and in the subsequent index pregnancies with HCQ. The addition of HCQ to classical treatment was associated with a significantly lower prevalence of placental-mediated complications (14.7 vs. 5.4%, $p < 0.05$) and a lower prevalence of preterm delivery before 34 weeks due to placental insufficiency (8.1 vs. 1.3%; $p = 0.045$).

In the final logistic regression model, we included the age, lupus anticoagulant (LA), APS + inherited thrombophilia, complement deficiency and triple (+) status of individuals to adjust the impact of HCQ on the odds of live births. The model showed good calibration (Hosmer Lemeshow test $p = 0.43$) and discrimination (AUC 0.84). Since this was a retrospective cohort study with common outcomes, we used the adjusted OR obtained from the logistic regression to estimate the corresponding adjusted relative risks to facilitate the interpretability of the main results [52]. Hence, the **adjusted relative risk** (RR) for **live births** was 1.55 (95% confidence interval, 1.19–2.1; $p < 0.001$), and for **obstetric complications**, the RR was 0.22 (95% CI: 0.15–0.30; $p < 0.001$). Finally, using PS matching and IPWRA as alternative methods to adjust for confounders, the estimated adjusted treatment impact of HCQ showed an absolute increase in live births of 31% (95% CI: 14%–48%) and an absolute reduction in obstetric complications of −28.8% (95% CI: [−10.9%]–[−46.6%]).

Hydroxychloroquine was well tolerated during the treatment period. Two women had mild cutaneous reactions that appeared during the first month of HCQ treatment and discontinued the treatment. One baby was born with cleft lip in Group A. No severe bleeding was observed with 60 mg of enoxaparin.

4. Discussion

In our cohort, the addition of HCQ in refractory patients with primary obstetric APS was associated with a higher rate of live births and a lower frequency of pregnancy complications.

To the best of our knowledge, this is the first retrospective study performed exclusively with primary refractory obstetric APS comparing obstetric outcomes with and without the addition of HCQ to conventional treatment without associated steroids or immunoglobulin.

The most interesting finding was observed in the high rate of live births in the HCQ group (97.1% Group A vs. 62.5% Group B, $p < 0.001$). The number of pregnancy complications was also lower in the HCQ group (8.7 vs. 37.5% in Group B, $p < 0.001$), although Group A represented the highest-risk population with a history of the most severe

Table 1
Patient characteristics in Group A (treated with HCQ + LDA + LMWH 60 mg) and Group B (treated with LDA + LMWH 60 mg).

Patient characteristics	Group A (HCQ)	%	Group B (no HCQ)	%
Patients refractory to conventional treatment, n	56	100	31	100
Pregnancies, n	69		32	
Pregnancies per woman	1–3[a]		1–2[b]	
Caucasian	56	100	31	100
Age, years, mean (SD)	35.3 (3.7)		34.4 (4.5)	
Lupus Anticoagulant	52	92.8	21	67.7
Anticardiolipin antibodies IgG/IgM, n	11	19.6	9	29
Anticardiolipin antibodies IgG/IgM, median range (SD)	45.8 (20–166)/ 24.4 (20–45)		49.9 (21–100)/ 47.3 (21–80)	
Antiβ2-glycoprotein I IgG/IgM, n	8	14.2	2	6.4
Antiβ2-glycoprotein I IgG/IgM, median range (SD)	104 (20–202)/ 314 (24–189)		104 (47–161)/0	
Triple Antibodies (+)	7	12.5	1	3.2
Complement deficiency (C4 < 16)	8	14.2	0	0
Anti SSA-Ro (+)	2	0.03	0	0
Primary APS	56	100	31	100
APS+ inherited thrombophilia	4	7.1	1	3.2
Chronic hypertension	3	5.3	1	3.2
Diabetes mellitus	5	8.9	1	3.2
Smoking tobacco	1	1.7	0	0
History of thrombosis+obstetric event	1	1.7	1	3.2
Weight, kg (SD)	65 (48–93)		68 (50–96)	

LDA: low-dose aspirin; LMWH: low molecular weight heparin; HCQ: hydroxychloroquine; SD: standard deviation; Ig: immunoglobulin; APS: antiphospholipid syndrome.
[a] Group A: 9 women with 2 pregnancies, 2 women with 3 pregnancies.
[b] Group B: 1 woman with 2 pregnancies.

Table 2
History of pregnancy complications in the **previous pregnancy** under **classical treatment** (both groups had received LDA + LMWH at 40 mg).

Previous failure with LDA + 40 mg LMWH	Group A 56 patients	%	Group B 31 patients	%
Thrombotic complications, n	0	0	0	0
Obstetric complications, n	56	100	31	100
Pregnancy loss	51	91	31	100
Early pregnancy losses < 10 weeks	38	67.8	25	80.6
Pregnancy losses > 10 weeks	13[a]	23.2	6	19.3
Preterm delivery < 34 weeks due to PE or IUGR	5	8.9	0	0
Placental mediated complications: (PE, IUGR)	9	16	0	0

LDA: low-dose aspirin; LMWH: low molecular weight heparin; PE: preeclampsia; IUGR: intrauterine growth restriction.
[a] n: 7 stillbirths.

pregnancy complications under conventional treatment in previous pregnancies. When analysed individually, the most significant difference was found in the lower rate of pregnancy losses before 10 weeks in Group A. Our results could be explained by the fact that hydroxychloroquine may improve pregnancy outcomes, especially in women with recurrent pregnancy losses refractory to conventional treatment, as suggested by Hunt [53].

Our findings agree with three clinical studies that reported better live birth rates and pregnancy outcomes in women treated with the addition of HCQ. Mekinian et al. [41] reported that pregnancy losses decreased

Table 3
Pregnancy outcomes in the **index pregnancy** with (HCQ + LDA + LMWH 60 mg) or (LDA + LMWH 60 mg).

Pregnancy outcomes with (LDA + 60 mg LMWH) ± HCQ	Group A (HCQ) 69 pregnancies	%	Group B (no HCQ) 32 pregnancies	%	*p* value
Live born babies, n	67	97.1	20	62.5	**<0.001**
Any obstetric complication, n	6	8.7	12	37.5	**<0.001**
Pregnancy losses, n	2	2.8	12	37.5	**<0.001**
Early pregnancy losses <10 weeks	2	2.8	11	34.3	**<0.001**
Pregnancy losses >10 weeks	0	0	1	3.1	
Gestational age at delivery, weeks	38.1 (1.4)		38.4 (1.1)		
Weight at delivery mean, g (SD)	3191 (314.7)		3262 (293.8)		
Placental mediated complications (PE, IUGR), n	4[a]	5.7	0	0	

LDA: low-dose aspirin; LMWH: low molecular weight heparin; HCQ: hydroxychloroquine; SD: standard deviation; PE: preeclampsia; IUGR: intrauterine growth restriction.
[a] Preeclampsia n = 1; Intrauterine Growth Restriction n = 3.

from 81 to 19% ($p < 0.05$) with the addition of HCQ. Sciascia et al. [42] showed that the use of HCQ in women with persistent aPL was associated with an increased rate of live births (67 vs. 57%, $p = 0.05$) and a lower rate of pregnancy complications (47 vs. 63%, $p = 0.004$). Ruffatti et al. [43] reported that HCQ 400 mg/daily beginning preconceptionally was more efficacious than other oral treatments in improving live birth outcomes in refractory obstetric APS. In these previous studies, HCQ was used alone or combined with steroids or immunoglobulins. Some included patients had thrombotic APS or SLE or represented a high-risk APS population without any previous failure with classical treatment.

The main strength of our study is that it was performed in a homogeneous population of entirely primary refractory obstetric APS women. We included purely obstetric APS cases because, according to recent understanding, they may represent a different subset than thrombotic APS cases, with different pathogenic mechanisms and risk factors, as suggested by Alijotas-Reig et al. [35].

The other important fact is that we compared only two strict pharmacological approaches: an increased enoxaparin dose + LDA vs. the addition of HCQ to this scheme, with no other concomitant drugs or different enoxaparin dosages that could bias results. Moreover, our cohort was diagnosed and treated at a single centre using only one core laboratory according to current international guidelines, reducing laboratory tests and management bias. Furthermore, we evaluated the association between hydroxychloroquine and obstetric outcomes in a very high-risk refractory primary obstetric APS population, including a history of severe pregnancy complications under classical treatment.

We acknowledge that one of the study's limitations is its retrospective nature. Second, the prevalence of lupus anticoagulant, triple (+), complement deficiency and worse pregnancy complications under classical treatment was higher in the HCQ group. We are aware that this might represent a bias when data are analysed. This was a retrospective study in which the indication of HCQ was for the highest risk population. However, since the exposure of interest was an intervention not randomly assigned, the association of HCQ with better pregnancy outcomes was confirmed after multivariate analysis, supporting the strength of our results.

In conclusion, the addition of HCQ was associated with a significantly higher rate of live births and a lower rate of pregnancy complications, although patients receiving HCQ represented the highest-risk group.

Although these preliminary results should be interpreted with

caution, adding hydroxychloroquine to classical treatment was associated with a beneficial impact on pregnancy outcomes in women with obstetric APS. HCQ seems to be a promising approach in refractory primary OAPS that needs to be confirmed with prospective studies.

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Declaration of competing interest

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

[1] S. Miyakis, M.D. Lockshin, T. Atsumi, et al., International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J. Thromb. Haemost. 4 (2006) 295–306.
[2] M. Empson, M. Lassere, J.C. Craig, et al., Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials, Obstet. Gynecol. 99 (2002) 135–144.
[3] R. Rai, L. Regan, Antiphospholipid syndrome in pregnancy: a randomized controlled trial of treatment, Obstet. Gynecol. 100 (2002) 1354.
[4] P.D. Ziakas, M. Pavlou, M. Voulgarelis, Heparin treatment in antiphospholipid syndrome with recurrent pregnancy loss: a systematic review and meta-analysis, Obstet. Gynecol. 115 (2010) 1256–1262.
[5] A. Mak, M.W. Cheung, A.A. Cheak, R.C. Ho, Combination of heparin and aspirin is superior to aspirin alone in enhancing live births in patients with recurrent pregnancy loss and positive antiphospholipid antibodies: a meta-analysis of randomized controlled trials and meta-regression, Rheumatology (Oxford) 49 (2010) 281–288.
[6] K. Bramham, B.J. Hunt, S. Germain, et al., Pregnancy outcome in different clinical phenotypes of antiphospholipid syndrome, Lupus 19 (2010) 58–64.
[7] S. Bouvier, E. Cochery-Nouvellon, G. Lavigne-Lissalde, et al., Comparative incidence of pregnancy outcomes in treated obstetric antiphospholipid syndrome: the NOH-APS observational study, Blood 123 (2014) 404–413.
[8] S. De Carolis, S. Tabacco, F. Rizzo, et al., Antiphospholipid syndrome: an update on risk factors for pregnancy outcome, Autoimmun. Rev. 17 (2018) 956–966.
[9] A. Ruffatti, M. Tonello, M.S. Visentin, et al., Risk factors for pregnancy failure in patients with anti-phospholipid syndrome treated with conventional therapies: a multicentre, case-control study, Rheumatology (Oxford) 50 (2011) 1684–1689.
[10] M.D. Lockshin, M. Kim, C.A. Laskin, et al., Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies, Arthritis Rheum. 64 (2012) 2311–2318.
[11] F. Pregnolato, M. Gerosa, M. Raimondo, et al., EUREKA algorithm predicts obstetric risk and response to treatment in women with different subsets of anti-phospholipid antibodies, Rheumatology (Oxford) 60 (2021) 1114–1124.
[12] J. Latino, S. Udry, F. Aranda, et al., Risk factors for early severe preeclampsia in obstetric antiphospholipid syndrome with conventional treatment, The impact of Hydroxychloroquine. Lupus 29 (2020) 1736–1742.
[13] S.S. Pierangeli, D. Erkan, Antiphospholipid syndrome treatment beyond anticoagulation: are we there yet? Lupus 19 (2010) 475–485.
[14] D. Erkan, C. Aguiar, D. Andrade, et al., 14th international congress on antiphospholipid antibodies: task force report on antiphospholipid syndrome treatment trends, Autoimmun. Rev. 13 (2015) 685–696.
[15] G. De Jesus, A. Benson, C. Chighizola, et al., 16th international congress on antiphospholipid antibodies task force report on obstetric antiphospholipid syndrome, Lupus 29 (2020) 1601–1615.
[16] J. Alijotas-Reig, R. Ferrer-Oliveras, A. Ruffatti, et al., The european registry on obstetric antiphospholipid syndrome (EUROAPS): a survey of 247 consecutive cases, Autoimmun. Rev. 14 (2015) 387–395.
[17] J. Alijotas-Reig, Treatment of refractory obstetric antiphospholipid syndrome: the state of the art and new trends in the therapeutic management, Lupus 22 (2013) 6–17.
[18] K. Bramham, M. Thomas, C. Nelson-Piercy, et al., First-trimester low-dose prednisolone in refractory antiphospholipid antibody-related pregnancy loss, Blood 117 (2011) 6948–6951.
[19] A. Tincani, W. Branch, R.A. Levy, et al., Treatment of pregnant patients with antiphospholipid syndrome, Lupus 12 (2003) 524–529.
[20] E. Lefkou, A. Mamopoulos, T. Dagklis, et al., Pravastatin improves pregnancy outcomes in obstetric antiphospholipid syndrome refractory to antithrombotic therapy, J. Clin. Invest. 126 (2016) 2933–2940.
[21] Z. Yang, X. Shen, C. Zhou, et al., Prevention of recurrent miscarriage in women with antiphospholipid syndrome: a systematic review and network meta-analysis, Lupus 30 (2021) 70–79.
[22] A. Ruffatti, M. Tonello, M. Favaro, et al., The efficacy and safety of second-line treatments of refractory and/or high risk pregnant antiphospholipid syndrome patients. A systemic literature review analyzing 313 pregnancies, Semin. Arthritis Rheum. 51 (2021) 28–35.
[23] G. Ruiz-Irastorza, M. Ramos-Casals, P. Brito-Zeron, et al., Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review, Ann. Rheum. Dis. 69 (2010) 20–28.
[24] S. Sciascia, D.W. Branch, R.A. Levy, et al., The efficacy of hydroxy- chloroquine in altering pregnancy outcome in women with antiphospholipid antibodies. Evidence and clinical judgment, Thromb. Haemost. 115 (2016) 285–290.
[25] G. Bertsias, J.P. Ioannidis, J. Boletis, et al., EULAR recommendations for the management of systemic lupus erythematosus: report of a task force of the EULAR standing Committee for International Clinical Studies Including Therapeutics, Ann. Rheum. Dis. 67 (2008) 195–205.
[26] M. Talabi, M. Clowse, Antirheumatic medications in pregnancy and breastfeeding, Curr. Opin. Rheumatol. 32 (2020) 238–246.
[27] G. Ruiz-Irastorza, M. Ramos Casals, P. Brito-Zeron, et al., Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systemic review, Ann. Rheum. Dis. 69 (2010) 20–28.
[28] N. Costedoat-Chalumeau, J.S. Hulot, Z. Amoura, et al., Heart conduction disorders related to antimalarials toxicity: an analysis of electrocardiograms in 85 patients treated with hydroxychloroquine for connective tissue diseases, Rheumatology (Oxford) 46 (2007) 808–810.
[29] N. Costedoat-Chalumeau, Z. Amoura, P. Duhaut, et al., Safety of hydroxychloroquine in pregnant patients with connective tissue diseases: a study of one hundred thirty-three cases compared with a control group, Arthritis Rheum. 48 (2003) 3207–3211.
[30] EMA, Orphan designation (EU/3/16/1820). https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3161820, 2018.
[31] M. Khamashta, M. Taraborelli, A. Tincani, et al., Antiphospholipid syndrome, Best Pract. Res. Clin. Rheumatol. 30 (2016) 133–148.
[32] N. Di Simone, P.L. Meroni, N. de Papa, et al., Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered beta2-glycoprotein I, Arthritis Rheum. 43 (2000) 140–150.
[33] J.H. Rand, X.-X. Wu, A.S. Quinn, D.J. Taatjes, The annexinA5-mediated pathogenic mechanism in the antiphospholipid syndrome: role in pregnancy losses and thrombosis, Lupus 19 (2010) 460–469.
[34] C.M. Salafia, F.S. Cowchock, Placental pathology and antiphospholipid antibodies: a descriptive study, Am. J. Perinatol. 14 (1997) 435–441.
[35] J. Alijotas-Reig, M. Vardell-Tarres, et al., Is obstetric antiphospholipid syndrome a primary nonthrombotic, proinflammatory, complement-mediated disorder related to antiphospholipid antibodies? Obstet. Gynecol. Surv. 65 (2010) 39–45.
[36] M. Mulla, J. Brosens, L. Chamley, et al., Antiphospholipid antibodies induce a proinflammatory response in first trimester trophoblast via the TLR4/MyD88 pathway, Am. J. Reprod. Immunol. 62 (2009) 96–111.
[37] M.L. Bertolaccini, G. Contento, R. Lennen, et al., Complement inhibition by hydroxychloroquine prevents placental and fetal brain abnormalities in antiphospholipid syndrome, J. Autoimmun. 75 (2016) 30–38.
[38] C. Albert, W. Schlesinger, C. Viall, et al., Effect of hydroxychloroquine on anti-phospholipid antibody-induced changes in first trimester trophoblast function, Am. J. Reprod. Immunol. 71 (2014) 154–164.
[39] T. Marchetti, A. Ruffatti, C. Wuillemin, et al., Hydroxychloroquine restores trophoblast fusion affected by anti- phospholipid antibodies, J. Thromb. Haemost. 12 (2014) 910–920.
[40] X. Wu, S. Guller, J. Rand, Hydroxychloroquine reduces binding of antiphospholipid antibodies to syncytiotrophoblasts and restores annexin A5 expression, Am. J. Obstet. Gynecol. 205 (576) (2011) e7–e14.
[41] A. Mekinian, M.G. Lazzaroni, A. Kuzenko, et al., Obstetrical APS: is there a place for hydroxychloroquine to improve the pregnancy outcome? Autoimmun. Rev. 14 (2015) 23–29.
[42] S. Sciascia, B. Hunt, E. Talaver-Garcia, et al., The impact of hydroxychloroquine treatment on pregnancy outcome in women with antiphospholipid antibodies, Am. J. Obstet. Gynecol. 214 (273) (2016) e1–e8.
[43] A. Ruffatti, M. Tonello, A. Hoxha, et al., Effect of additional treatments combined with conventional therapies in pregnant patients with high-risk antiphospholipid syndrome: a multicenter study, Thromb. Haemost. 118 (2018) 639–646.
[44] K. Schreiber, K. Breen, H. Cohen, et al., 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, Semin. Thromb. Hemost. 43 (2017) 562–571.
[45] C. Belizna, F. Pregnolato, S. Abad, et al., HIBISCUS: hydroxychloroquine for the secondary prevention of thrombotic and obstetrical events in primary antiphospholipid syndrome, Autoimmun. Rev. 17 (2018) 1153–1568.
[46] A. Mekinian, J. Alijotas-Reig, F. Carrat, et al., Refractory obstetrical antiphospholipid syndrome: features, treatment and outcome in a European multicenter retrospective study, Autoimmun. Rev. 16 (2017) 730–734.
[47] V. Pengo, A. Tripodi, G. Reber, et al., Update of the guidelines for lupus anticoagulant detection. subcommittee on lupus Anticoagulant/Antiphospholipid

antibody of the scientific and standardization Committee of the International Society on thrombosis and haemostasis, J. Thromb. Haemost. 7 (2009) 1737.
[48] D. Rolnik, D. Whright, L.C. Poon, et al., Aspirin vs placebo in pregnancies at high risk for preterm preeclampsia, N. Engl. J. Med. 377 (2017) 613–622.
[49] R.B. D'Agostino Jr., Propensity score methods for bias reduction in the comparison of a treatment to a nonrandomized control group, Stat. Med. 17 (1998) 2265–2281.
[50] P. Rosenbaum, D. Rubin, Reducing bias in observational studies using subclassification on the propensity score, J Am Stat Ass 79 (1984) 516–524.
[51] P.R.D. Rosenbaum, The central role of the propensity score in observational studies for causal effects, Biometrika 70 (1983) 41–55.
[52] J. Zhang, K.F. Yu, What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes, JAMA 280 (1998) 1690–1691.
[53] B. Hunt, A novel antiphospholipid antibody agent? Blood 115 (2010) 2124–2125.