University of Padova
Department of Medicine

PhD Course in Clinical and Experimental Sciences
Curriculum: Rheumatological and Laboratory Sciences
CYCLE: XXXIV

THE ROLE OF PENTRAXIN-3 AS PREDICTOR OF PREGNANCY COMPLICATIONS IN PATIENTS WITH SLE AND/OR ANTI-PHOSPHOLIPID SYNDROME AND APL CARRIERS

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1. ABSTRACT

Systemic Lupus Erythematosus (SLE) is an autoimmune disease affecting childbearing women. Nowadays, mainly due to preconception counselling, the overall prognosis of such pregnancies has consistently improved. Preconception visit is pivotal to assess anti-phospholipid (aPL) antibodies and/or anti-phospholipid syndrome (APS), drugs' safety and disease activity, in order to prevent adverse pregnancy outcomes (APOs) and maternal flares.

The general aim of the thesis was to improve the management of pregnant SLE and/or APS patients by assessing pregnancy outcomes and identifying predictors of APOs and flares.

Firstly, we focused on severe preeclampsia (PE) leading to preterm delivery<34 weeks, which is one of the classification criteria of APS. The sample included 40 APS patients with severe PE enrolled in 6 European centres. PE occurred very early in gestation (median 25.5 weeks) and with a high mortality during the offspring (65%). In the follow-up period (5 years), none of these patients experienced 3 consecutive miscarriages, whereas thromboses, intrauterine foetal deaths and HELLP syndromes were observed, suggesting that recurrent miscarriages APS criterion may have a different physiopathology compared to the other criteria.

Secondly, we tested a new score, the SLE-disease activity score (SLE-DAS) as predictor of flares and APOs in 2nd and 3rd trimester in SLE women enrolled at two referral centres (Italy and France). SLE activity was assessed at first trimester by SLE-pregnancy disease activity index (SLEPDAI) and SLE-DAS. In this cohort of 158 pregnant patients with a very stable lupus, a significant correlation between SLE-DAS and SLEPDAI was observed (Spearman's ρ=0.97). Both SLE-DAS and SLEPDAI

predicted flares (p=0.02 and p=0.01, respectively), and resulted associated with APOs (p=0.02). Thus, SLE-DAS seems a reliable instrument to measure SLE activity during pregnancy.

Planning pregnancy when SLE is quiescent is a cornerstone of the management of SLE patients. However, the impact of lupus low disease activity state (LLDAS), remission and damage accrual in early gestation has never been simultaneously studied. We evaluated SLE women in the prospective GR2 study with an ongoing singleton pregnancy at 12 weeks (one pregnancy/woman). Several sets of criteria were used to define remission, disease activity, and damage. First trimester maternal and SLE features were tested as predictors of flares and APOs. 238 women (98.3% on hydroxychloroquine) had 230 live births. Thirty-five (14.7%) patients had at least one flare; APOs occurred in 34 (14.3%) women. At logistic regression models, damage (SLICC-Damage Index) (odds ratio-OR- 1.8, 95% confidence intervals-CI-: 1.1-2.9 for model 1 and OR 1.7, 95% CI: 1.1-2.8 for model 2) and lupus anticoagulant (LAC, OR 4.2, 95% CI: 1.8-9.7 for model 1; OR 3.7, 95% CI: 1.6-8.7 for model 2) resulted significantly associated with APOs. Hence, damage should be considered in preconception counselling and early gestation along with LAC.

Pentraxin-3 (PTX3) has been studied as promising biomarker of pregnancy complications, although no data on SLE/APS pregnant women are available. Hence, we evaluated serum PTX-3 and anti-PTX3 antibodies as predictors of pregnancy outcomes in SLE and/or APS women at our Rheumatology Unit. The current analysis included pregnant SLE (SLICC 2012) and/or APS (Sydney, 2006) women. The control group included healthy patients/affected with other rheumatic diseases (nor SLE nor APS) referred to our clinic with a conception date <1st April 2021 (one

pregnancy/patient). Among 79 pregnancies, APS occurred in 7 (8.9%), SLE in 9 (11.4%) and SLE with APS in 3 women (3,8%). Overall, the control group included 66 (83.6%) patients. Serum IgG anti-PTX3 Abs were found in 11 (13.9%, 95% CI 7.2-23.5) women. Anti-PTX3 were slightly associated with gestational diabetes mellitus (GDM) (p=0.04) and resulted slightly associated with intra-uterine growth restriction (IUGR) (p=0.09).

2. INTRODUCTION

SLE is a chronic autoimmune disease which encompasses a spectrum of several clinical and serological manifestations [1]. The clinical spectrum varies from mild to severe features including skin rash, arthritis, serositis, vasculitis, but also renal, hematologic, neuropsychiatric, pulmonary, and cardiac manifestations. The involvement of kidney, central nervous system, lung, and heart is critical since it can worsen the patient prognosis and quality of life [2].

SLE mostly affects young women, thus, the management of pregnancy is essential for clinicians who take care of these patients. Indeed, the maternal immune system goes through some modifications in order to become tolerant to paternal antigens expressed in foetal cells/tissues. These modifications can potentially stimulate autoimmune response triggering SLE or fuelling the disease immunological burden. Conversely, an increase in SLE activity during gestation can lead to irreversible organ damage and to APOs [3].

During the last few decades, the outcome of pregnancy in SLE has consistently improved, possibly due to preconception counselling [4]. Risk stratification before conception is nowadays pivotal to achieve a favourable pregnancy outcome [4] and it should assess SLE remission, Abs profile, treatment and previous pregnancy morbidity [4]. Indeed, active/flaring SLE in the 6-12 months before conception is a major risk factor for flares during pregnancy and/or puerperium, as well as for foetal morbidity (i.e. intrauterine growth restriction-IUGR-, pregnancy loss and preterm delivery) [4].

Several Authors focused on the frequency of maternal complications in SLE patients [5–9]. However, due to the wide variability of APOs definitions, contrasting results emerged from different studies.

2.1 Maternal complications in SLE

2.1.1 Preeclampsia

PE and/or severe PE represent a major concern for SLE pregnant women since they occur in 2–35% of the cases, which means 10 times more common than in general population [10–12]. The “Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus” (PROMISSE) study, a large multicentre study carried out in US and Canada, reported PE in 15% of the SLE women, rising to 22% if they had positive aPL Abs [7]. Moroni et al. observed PE in 8.4% of the patients with lupus nephritis (LN) [13] which is keeping with data reported by Smyth et al. who observed PE in 7.6% of their LN cases [14]. A high frequency of PE (about 20%) was observed in Japanese patients with LN [15].

As aforementioned, it is still challenging to assess the frequency of PE , due to the differential diagnose between PE and SLE flares pregnant women. Indeed, some PE features overlap with those of LN as they both can be characterized by an increase in 24 hours (h) proteinuria, impairment of renal function, hypertension, and thrombocytopenia [16]. PE and LN can also occur in the same patient, which is critical since these manifestations require different, and sometimes opposite, therapeutic strategies [16].

Overall, women who have already experienced PE are exposed to the risk of developing PE in a subsequent pregnancy [17]; notably, patients who develop early-

onset PE (before 34th weeks of gestation-WG) have a four-fold increased risk of stillbirth in a subsequent pregnancy, whereas the risk is lower in women with late-onset PE [17,18].

Over the last decades, several predictive factors of PE have been identified. Some are the same of those reported in general population, others are specific of SLE. The first group encompasses diabetes mellitus (DM), hypertension, nulliparity, obesity and previous PE [16]; the latter include clinical SLE features and biomarkers. In particular, clinical risk factors associated with PE in SLE women are active disease at conception, LN in the 6 months before gestation or even a history of LN [14,19–24]. Other predictors are aPL Abs [10,13,16,25,26], thrombocytopenia and low complement serum levels [10]. A long disease duration at the time of conception was also associated with an increased risk of PE in a prospective Italian cohort of 71 pregnancies in LN patients [27]. In the same study, PE was also associated with arterial hypertension (with a relative risk of 39.2) [27]. Finally, a dosage of prednisone ≥10 mg/day [10,27] and ethnic background were also found to be risk factors of PE [10,20]. Recently, some Authors focused on novel predictive biomarkers of PE such as maternal pregnancy-associated plasma protein A (PAPPA), placental protein 13 (PP 13), inhibin A, activin A, soluble endoglin (sEng), soluble fms-tyrosine kinase molecule-1 (sFlt-1), placental growth factor (PlGF), pentraxin-3 (PTX-3), and p-selectin [28].

PTX3 seems to be implicated in placentation and fetus tolerance during pregnancy, being mostly secreted in response to inflammatory stimuli by dendritic cells, macrophages, fibroblasts and endothelial cells [29]. Notably, elevated levels of PTX3 between the 11th and the 13th WG have been shown to be associated with PE in the general population [30]. Since PTX3 is involved in tissue inflammation in SLE

patients [31] as well as in SLE mouse models [32], it is possible that PTX3 can predict PE even in SLE women.

Some therapeutic options have been analysed in order to prevent PE. Low dose aspirin (LDA) (administered at 60–100 mg/day) was reported to reduce the risk of PE and IUGR in healthy women by 10% and 20%, respectively [33]; however, the best time to introduce LDA during pregnancy in order to prevent PE remains a matter of debate [33]. A meta-analysis showed that the effect of LDA on PE and its complications is consistent, regardless of whether treatment is started before or after 16 WG [33]. It is recommended not to exceed the dose of 100 mg/day since a higher dose administered for a long period of time during pregnancy can increase the risk of bleeding in the brain of premature infants [33].

3.1.2 HELLP Syndrome

Haemolysis Elevated Liver Enzymes Low Platelets (HELLP) syndrome usually occur with other APOs [34]. In a recent study, women with PE and HELLP were shown to have an increased risk of blood transfusion, intensive care unit admission, and postpartum haemorrhage [34]. Furthermore, all patients who developed placental abruption had developed PE with severe features and HELLP [34]. Finally, women with PE and HELLP were more likely to deliver by caesarean section [34] and PE represents one of the most consistent predictors of HELLP syndrome. Higher rate of HELLP was observed in patients with SLE and secondary APS, compared to patients with only SLE ($p<0.01$) [26], suggesting that aPL can contribute to the development of HELLP and other thrombotic or micro thrombotic complications during gestation.

3.1.3 Gestational hypertension

It has been reported that SLE incidence is higher among patients who had developed hypertension during pregnancy compared to matched controls (incidence rate ratio=4.02) [35]. Although gestational hypertension is less severe than PE, patients are likely to develop superimposed PE [17].

2.1.4 SLE flares

SLE flares, can occur up to 65% of the cases [8]. However, the results of the studies focusing on the prevalence of SLE exacerbations during pregnancy are conflicting, probably due to different designs and inconsistent flare definitions.

Overall, the impact of flares on pregnancy outcome depends on their type and number [6]: cutaneous flares are not life-threatening, whereas haematological and LN can be severe and even lead to irreversible renal function impairment [36]. SLE flares can occur at any time during gestation and the risk of flare is quite high up to 1 year after delivery [10]. Indeed, during a normal pregnancy, there is a decrease in the balance of T helper (Th)1/Th2 and Th-17/T-regulatory (reg) cells [37]. SLE is a Th2 mediated disease characterized by a defect in Treg. Thus, in SLE patients Th2 polarization is not counterbalanced by a Treg expansion leading to an increased risk of flare during pregnancy [2]. In the study carried out by Kroese et al. [26], only 20% of the women experienced a flare during gestation and this low frequency was attributed to the low disease activity before conception [26]. A systematic review and meta-analysis of 2751 pregnancies [14] showed a 25.6% of the disease flares [14]. Notably, in the PROMISSE study, less than 10% of the women had mild or moderate flares, and only 3% of them developed severe flares during pregnancy. In the same study, 65% of the patients received hydroxychloroquine (HCQ), suggesting HCQ might have a role in protecting SLE patients from flares during pregnancy [7]. In the postpartum, flares

occurred in 15% of the cases in a cohort of 144 SLE pregnancies [26] and about two-thirds of them had developed a flare before or during pregnancy, suggesting that a tight control in the postpartum period is recommended even after delivery, especially in patients with active disease before or during gestation.

2.1.5 Maternal mortality

Death of SLE women during pregnancy has consistently decreased over the last few decades, as well as other APOs. However, death still occurs in some cases [5,10] and the risk of mortality is 20-fold higher in SLE than in the general population [10]. In a recent review, the Authors identified 13 studies, reporting a total of 17 deaths, all in the 6-week post-partum period, that were attributable to SLE and LN [38]. In all cases, death occurred in patients with active disease, and was attributed either to infection in 41.2% or disease activity in 29.4%; notably, two women died of pulmonary embolism [38].

2.1.6 Other maternal complications

Beside pregnancy morbidity, SLE patients with isolated aPL positivity and/or defined APS can experience thrombotic events during pregnancy and puerperium. A recent metanalysis systematically compared arterial/venous thrombosis in pregnant women with SLE, primary APS (PAPS) and secondary APS (SAPS) [39]. PAPS and SAPS were found to be associated with a significantly higher frequency of arterial/venous thrombosis in comparison to SLE [39]; however, no difference was observed between PAPS and SAPS. Another study comparing thrombosis in PAPS and SAPS with SLE showed that thrombotic events were more common in SLE with SAPS than in PAPS [40].

APOs and thrombotic events were also evaluated in a recent multicentric study carried out in 283 patients, with confirmed positive aPL, isolated aPL carriers or associated with an established PAPS [41]. In this study, 2.4% of the patients experienced a thrombotic event, which in the majority of cases occurred during puerperium [41], despite an adequate anti-thrombotic treatment. This finding is consistent with the well-known risk of postpartum thrombosis in the general obstetric population.

Infections can be observed in SLE patients during pregnancy particularly after delivery, at least in part related to immunosuppressive treatments. Indeed, a recent metanalysis analysed APOs (including infections) in SLE patients compared to the general population from 2001 to 2016 [42] showing that post-partum infections were significantly more common in the SLE subgroup (relative risks-RR-: 4.35, 95% CI: 2.69–7.03; P=0.00001) [42]. A population-based cohort study [43] evaluated the infection risk in women with and without SLE and in their infants [43], reporting that the former were 1.7 times more likely (95% CI 1.4, 2.0) to develop infections during birth hospitalization and more likely to receive antibiotics during labour (RR 1.3, 95% CI 1.1, 1.5) than the latter. Infants of women with SLE had an increased risk of infections during birth hospitalization (RR 2.2, 95% CI 1.3, 3.5), although the difference was small when adjusted for gestational age (RR 1.4, 95% CI 0.9, 2.1) [43]. Women with SLE and renal disease were at even greater risk of infections [43] and about half of this additional risk seem to be related to the high frequency of preterm birth; however, an increased risk of infections is still present when considering only term pregnancies [43]. Similarly, Clowse et al. found that the incidence of sepsis during the delivery hospitalization was 0.5% in women with SLE compared with 0.1% in general

population and the incidence of pneumonia was 1.7% in SLE patients vs. 0.2% in women without SLE (OR 4.3,95% CI 3.1, 5.9) [44].

2.2 Foetal complications in SLE pregnancies

Poor foetal outcomes include IUGR, small for gestational age (SGA), preterm delivery, foetal loss/stillbirth, caesarean section and neonatal SLE [6,24,45,46]. Overall, SLE activity in the 6 months before conception was found to be an independent predictor of prematurity, IUGR and foetal demise [47].

2.2.1 Intra-uterine growth restriction

IUGR is defined as the birth weight below the 10th percentile according to WG at delivery and foetal gender. This condition occurs in 10–30% of the pregnancies in SLE patients [22,23]. Several papers documented a high rate of IUGR in patients with active SLE, LN, and SAPS [48]. Indeed, in SLE patients with APS, microinfarcts in the placenta can impair foetal nutrition leading to growth retardation and –in severe cases– late pregnancy losses [10,14,48]. Thus, patients with active disease, LN, and secondary APS require a close monitoring during gestation [3,14].

2.2.2 Small for gestational age

SGA foetuses or new-borns are defined as a weight below the 10th percentile for gestational age [49]. This definition was developed by a World Health Organization (WHO) expert committee in 1995 and is based on a birthweight-for- gestational-age measure in relation to a gender-specific reference population [50]. A case-control study reported a significant higher frequency of this complication in SLE patients compared to healthy subjects (25% vs 4.5%, $p<0.05$) [51]. Moroni et al. observed SGA in 16.4% of the patients with LN [51]. Interestingly, the probability of having a baby with SGA decreased by 85% in patients who were treated with HCQ [51]. Similar

frequencies were reported by Zhan et al. [49], who observed SGA in 18.9% of the newborns; interestingly, SGA was predicted by abnormalities in third-trimester umbilical artery Doppler [49], suggesting the use of ultrasound scan in monitoring foetal growth in all women with SLE, especially in those at high risk of pregnancy complications [2]. Interestingly, a recent Japanese study showed that LDA was protective against SGA development [52].

2.2.3 Preterm delivery

Preterm delivery is the most common gestational complication in SLE women [2] and is defined as a delivery <37 WG [2]. It approximately occurs in 33% of the SLE pregnancies, which is higher than in the general population (12%) [10,53–55]. This high percentage is also due to early pregnancy termination as a consequence of APOs. The frequency of preterm delivery is even higher in patients with LN, where is reported in up to 50% of the cases [56,57]. In a multicentre cohort of LN patients [51], 28.2% of the pregnancies ended before 37 WG, after an average gestation of 33.9 ± 2.1 weeks; in this cohort preterm labor did not occur isolated, but in association with LN flares in nine cases, PE in five, and IUGR in two cases [51]. In another retrospective multicentre study, preterm birth occurred after 34 WG with favourable outcomes and no neonatal deaths, probably due to a family planning strategy and preconception stratification [9]. Other Authors also identified positive aPL, thyroid disease, uric acid, and ferritin levels [56,57], as risk factors for preterm delivery [16]. Preterm birth before 36 WG has been associated with some novel biomarkers [58]. Kim et al. [58] reported that Bb andC5b-9 fragments, markers of the alternative pathway of complement activation, are involved in the development of obstetrical complications in SLE women including preterm birth, fetal death and/or neonatal death [58]. Notably, Bb and sC5b-9 serum levels were

significantly higher in the early pregnancy (12-15th WG) in patients who developed APOs compared to those without APOs and the difference tended to increase through week 31 [58].

2.2.4 Pregnancy loss

The proportion of miscarriage, fetal loss, and stillbirth in SLE women were much higher in the past than nowadays [59]. Indeed, miscarriages (defined as a pregnancy loss <10th WG) rates declined from 43% in 1960–70 to 17% in 2000–03 [59] and live birth rate is currently reported to be 80–90% of the cases [46]. In 2008, a literature review showed a foetal death rate (miscarriage and stillbirth) of approximately 20%, with a very wide range between 4% and 43% [2,60]. The PROMISSE study showed that foetal loss (defined as death after 12 WG) rate was higher in Afro-American and Hispanic women, where the foetal loss was observed in 27.4% of the cases [7] in comparison with 5% in Caucasian patients [7]. Thus, racial and ethnic factors might influence SLE course, as well as SLE pregnancy outcome. Alternatively, socioeconomic status might contribute to APOs in women with SLE [84]. Other predictors of foetal loss were active disease [47], LN in the patient history [3], positive aPL and SAPS [6,16].

APL are a heterogeneous group of Abs which, despite their name, do not seem to bind phospholipids, but are directed to plasma proteins with affinity for anionic surfaces (i.e. phospholipids) [61]. Recently, anti-prothrombin (aPT) and anti-phosphatidylserine ant prothrombin (aPS/PT) Abs were investigated in a cross-sectional study [61]. Positive LAC and IgG and/or IgM aPS/PT were independent risk factors for foetal loss and thrombosis [61].

Notably, a new predictive model for the foetal loss was developed by Wu et al. [62]. In this study, 338 SLE pregnancies were retrospectively analysed in order to identify predictors of such complication leading to the development of a risk score. As result, the Authors found that unplanned pregnancies, hypocomplementemia, and 24h urinary protein level (0.3≤ protein<1.0g/24 h), were independent risk factors of foetal loss after adjusting for possible confounders [62]. Notably, this model is based on variables included in the clinical practice setting; hence, it may be useful for clinicians in identifying women with high-risk pregnancies.

2.2.5 Neonatal lupus

Neonatal lupus (NL) is a syndrome characterized by different clinical features, including cutaneous, haematological and/or hepatic manifestations, which are usually transient, and a severe condition named congenital atrioventricular block (CHB) which can be permanent. NL is due to a passive immunization of the foetus by maternal anti-SSA/Ro and anti-SSB/La Abs [2,16]. These are actively transported across placenta starting from 16th WG [2]. These Abs are specific for three different proteins (Ro52, Ro60, and La) and one or more non- coding RNAs, showed Y RNAs particles [63]. The majority of NL features tend to resolve within 6–9 months after birth, when maternal Abs are cleared from neonatal circulation [16]. According to the apoptosis-inflammation hypothesis, anti- SSA/Ro 60Kd are the main drivers of CHB, since Ro60, but not Ro52 is mainly expressed on the surface of apoptotic cardiomyocytes [63]. Tonello et al. [64] evaluated the predictive value of anti-SSA/Ro 52 kd, anti-SSA/Ro 60kd, and anti-p200. The p200 is a specific subunit of the 52 Kd antigen expressed on the cell surface of cardiomyocytes inducing a dysregulation of intracellular calcium intake leading to cellular apoptosis [64]. The Authors showed that the simultaneous presence of anti-

SSA/Ro 52kd, anti-p-200, and anti-SSA/Ro 60kd Abs, especially at high levels, increased the risk of CHB [64]. By contrast, low titre Abs and isolated anti-SSA/Ro 60 kd were associated with a favourable pregnancy outcome suggesting a less stringent foetal echocardiography follow up in these cases [64].

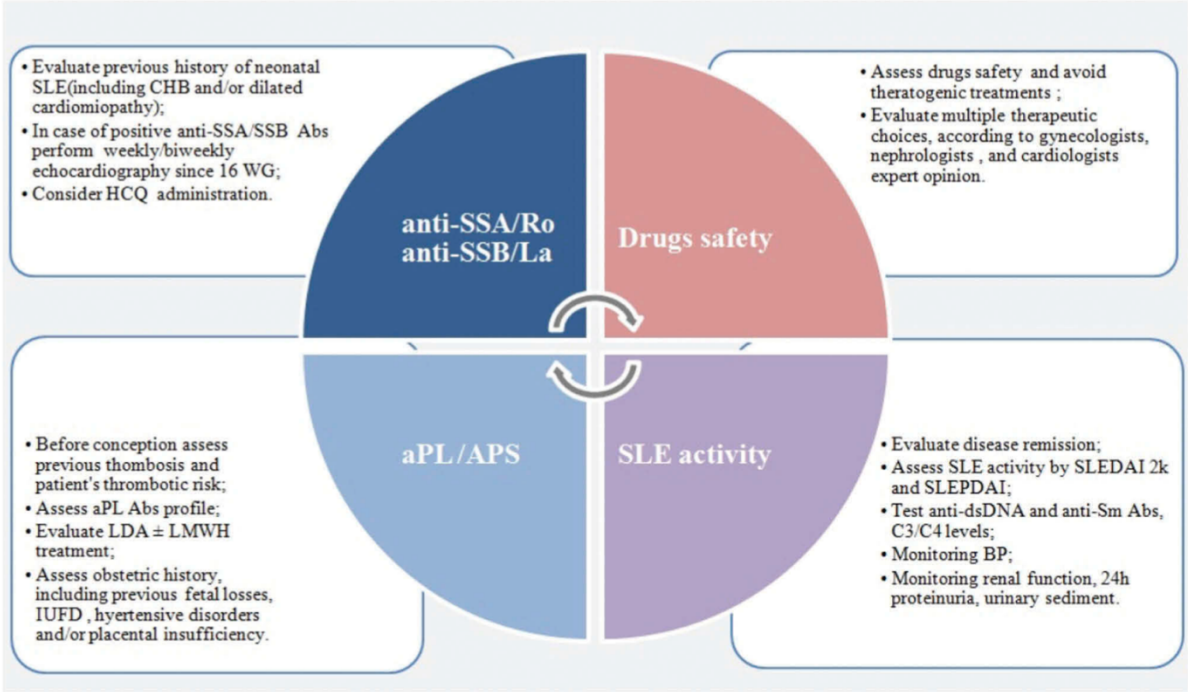
CHB is the most severe manifestation of NL and might result in permanent damage leading to a neonatal death in up to 20% of the cases [8], pacing in 70% [8], and late dilated cardiomyopathy in 5–19% [65]. Fortunately, CHB is a very rare condition, being observed in about 2% of the positive SSA primigravid women [16]. The risk of CHB tends to increase in a subsequent pregnancy, with a recurrence rate varying from 16% to 20% of the cases [17]. Different protective and therapeutic strategies have been tested in CHB [6], including plasmapheresis, intravenous (IV) Immunoglobulin (Ig), and fluorinated steroids [10]. Data from the French registry show that fluorinated steroids are unable to reverse CHB or increase survival [66]. In addition, no controlled randomized trials (RCTs) were performed to confirm the role of plasmapheresis despite its theoretical benefits and finally, IV immunoglobulin (Ig) was not shown to prevent CHB [5]. Thus, preventing strategies and treatment options in CHB are still under evaluation. A retrospective case–control study showed a decrease in the frequency of CHB in foetuses exposed to HCQ compared to non-exposed women (7.5% vs. 21%) [67]. Interestingly, in the PROMISSE cohort, about 65% of the women received HCQ and less than 1% of the pregnancies was complicated by CHB [12]. Thus, HCQ could be a potential option in preventing CHB recurrence, as recently published [67].

Other clinical manifestations of NL spectrum include cutaneous, hepatic and hematologic manifestations. Skin rash appears more frequently at 3–6 weeks of age

but can also occur at birth with erythematosus annular skin lesions that resemble those of subcutaneous lupus erythematosus (SCLE) [5]. Usually, skin manifestations resolve in about 15–17 weeks [68]. Some Authors suggested that anti-U1RNP is also involved in NL [69,70], but not in CHB development. Sheth et al. [69] observed cutaneous lesions in infants who had positive anti-U1RNP, without anti-SSA/Ro and anti-SSB/La Abs. However, these infants had negative anti-SSA/Ro and anti-SSB/La Abs tested by immunodiffusion and ELISA, and not by an immunoblotting essay which is a more sensitive technique.

Other manifestations of NL include hepatic disorders such as asymptomatic elevated liver enzymes, cholestasis, hepatitis, and mild hepatosplenomegaly. Hematologic manifestations encompass anaemia (rarely aplastic anaemia), neutropenia and also thrombocytopenia [5]. Neurological involvement in neonates has also been associated with the transplacental passage of anti-SSA/Ro and/or anti-SSB/La. However, a strong association between these Abs and neurological neonatal lupus is still unclear [5].

Figure 1. Factors to assess in the preconception visit in SLE women



Footnotes to Figure 1 (adapted from [1] : The "PREGNA-RING" consists of four main assessments: (l) anti-SSA/SSB Abs, (2) aPL profile, and/or APS; (3) SLE activity and (4) drugs safety. At least 6 months before conception, clinicians should evaluate all these variables, in keeping with the recent EULAR guidelines. In particular, disease activity should be assessed in terms of remission, by using SLE Disease Activity Index-2000 (SLEDAI-2k) (before conception) and SLEPDAI scores (during pregnancy), respectively. Antidouble-stranded (ds) DNA/anti-Sm should also be considered, along with C3/C4, being indirect signs of active disease. Regarding SLE flares, a tight monitoring of urinary sediment, 24h proteinuria, and BP should be performed, as some features can also overlap with other maternal complications related to SLE, i.e. preeclampsia and HELLP syndrome. Potential teratogenic drugs should be discontinued when pregnancy is planned. Conversely, safe drugs (including HCQ) should be taken throughout gestation, unless contraindications are present. LDA and/or low molecular weight heparin (LMWH) should be administered in case of APS

and adverse obstetric events. Due to the intrinsic thrombotic risk related to pregnancy, aPL profile should be assessed before pregnancy, both in patients affected with SLE and in those with SLE and secondary APS. Foetal echocardiography is recommended in case of positive anti-SSA/Ro and/or anti-SSB/La. However, the role of foetal ultrasound surveillance has not yet been clarified, since currently available therapeutic options for CHB do not seem to prevent or arrest this condition.

2.3 Challenging topics in SLE and/or APS pregnancies

Difficulties in assessing prevalence and predictors of APOs in SLE patients are due to lack of uniformity in the definitions and methods used in the different studies. In addition, some maternal and foetal complications are difficult to diagnose and to differentiate from each other (i.e. PE and LN flare) requiring clinicians‘ personal skills. Despite these limitations, our knowledge in this field has greatly improved in the last few decades.

Assessing patients‘ risk before conception is paramount in order to avoid APOs; thus, preconception counselling should be always performed and can definitely improve pregnancy outcome in SLE women [4].

As aforementioned, SLE pregnancy’s prognosis has widely improved in the last decades, due to family planning strategies and a better control of the disease. However, there are still some critical points which need to be discussed and further studied to further improve the management of SLE women in childbearing age.

First, the evaluation of the disease during gestation. Although it has never been validated, one of the most used tool in clinical practice is SLEPDAI [71]. This score encompasses 24 original items of the SELENA-SLEDAI score; among them, 15 have been modified, including complement levels [71], by taking into account some

physiological changes that occur during pregnancy. Recently, Jesus et al. [72] developed a new score to measure disease activity, named SLE-DAS [72]. This score encompasses 17 items and includes some continuous variables such as 24h-proteinuria and the joints count [72]. When comparing the performance of this instrument with the SLEDAI-2K [73], the Authors found that SLE-DAS has a higher accuracy in measuring SLE disease activity, a better sensitivity-to-change and a higher predictive value for damage accrual [74]. Other advantages are that SLE-DAS is a continuous measure of disease activity and includes two important features absent in the SLEDAI-2K, i.e. haemolytic anaemia and lupus enteritis [72]. Thus, testing SLE-DAS in pregnant SLE women was one of our research focus during the PhD course, in order to find out if this new score can be reliable even during pregnancy.

Second, although it is clear [4,75] that remission or alternatively LLDAS are recommended before getting pregnant, it is still not clear which of these states should be achieved prior to conception [75]. While several definitions of remission and LLDAS [76] have been validated out of pregnancy, those proposed by the DORIA/Zen [77] and DORIS [78] groups for remission and by Franklyn for LLDAS [79], have not been tested in pregnant women [80]. Hence, we tried to assess all these definitions,, along with damage in a prospective cohort of SLE pregnant women, in order to show which definition is the best to apply for a favourable outcome.

Remarkably, also the management of APS needs to be improved, and new preventive therapeutic strategies have to be pursued [81], in order to avoid PE, hypertensive disorders, foetal losses and recurrent miscarriages. In that, sense we might speculate that new APS classification criteria are needed, since the “recurrent miscarriage phenotype” seem to be different from the other 2 obstetric criteria [82].

In the next years, further studies are needed in order to discover new early biomarkers and predictors of APOs. Pro-angiogenic and anti-angiogenic factors (i.e. sFlt1, PlGF) and complement activation products (Bb and C5b-9) represent very promising biomarkers which were able to predict APOs [58]. Novel unconventional aPL have also been analysed in SLE patients including aPT and aPS/PT Abs although their predictive role for APOs still remains unclear [61]. Since we need consistent results which can be translated into clinical practice, it is pivotal to study well-defined cohort of patients using a widely accepted definition of maternal and foetal complications.

2.4 PTX3 in pregnancy

Pentraxins are essential components of the humoral arm of the innate immune response and act as soluble pattern recognition particle (PRRs) in response to pro-inflammatory signals and Toll-like receptors (TLRs) activation [29]. PTX3 is produced and released by a variety of cell types such as mononuclear cells, phagocytes, dendritic cells (DCs), fibroblasts, and endothelial cells and it is able to recognizes microbial products, opsonizes fungi, selected Gram-positive and Gram-negative bacteria, viruses, and activates complement [83]. It is considered an acute phase response protein, because its concentrations increase considerably and rapidly in plasma of patients with systemic inflammatory response syndrome, sepsis, or septic shock [29].

During embryo implantation, the maternal immune system needs to be modified, in order to tolerate the paternal antigens [84] and not to reject the fetus. Notably, this process is feasible due to a complex interaction between maternal and foetal factors by inducing an inflammatory systemic response, as proved by the complement and endothelial activations and high cytokines levels [84].

Recently, a PTX3 deficiency has been associated to a condition of infertility in mice models [85]. Larsson et al. [84] showed that PTX3 levels tend to progressively increase in a normal pregnancy, mostly after week 31 [84]. Garg et al. [86] also observed that PTX3 levels between the 11th and the 13th WG was predictor of later preeclampsia.

Finally, anti-PTX3 IgG Abs have been proved to protect from developing LN both in mice and human models, but no data on pregnant patients with SLE and /or APS are available to date.

3. AIMS

3.1 General Aim

The general aim of the thesis was to improve the management of pregnant patients affected with SLE and/or APS by assessing pregnancy outcome and identifying predictors of APOs and disease flares.

3.2 Specific Aims

1. to analyse severe PE in a large series of women with APS and to assess the prevalence of other classification criteria for APS;
2. to evaluate SLE-DAS in the first trimester as predictor of maternal flares and obstetrical complications in a cohort of prospective SLE pregnant patients;
3. to assess the frequency of SLE pregnancy outcomes (maternal flares and APOs) in multicentric, prospective large cohort of SLE pregnant women and test remission definitions and LLDAS as well as cumulative damage in the first trimester as predictors of poor outcome (flares and APOs) later in pregnancy;
4. to assess the role of serum PTX3 and anti-PTX3 Abs as predictors of pregnancy complications in SLE and/or APS.

4 PATIENTS AND METHODS

4.1 PAPER I: "Evaluation of the severe preeclampsia classification criterion for antiphospholipid syndrome in a study of 40 patients."

Patients. The first retrospective study took place in five French hospital internal medicine departments and one Italian hospital rheumatology unit, where women who met the inclusion criteria were analysed. These inclusion criteria were 1) at least one episode of severe PE [87] before 34 weeks' gestation, 2) in pregnancies observed between 2000 and 2017; 3) in women who met the revised classification criteria for APS [88].

PE was defined as the onset of hypertension (≥140/90 mmHg) with proteinuria ≥ 0.3 g/24 hours, after 20 weeks' gestation [87], and was defined as severe when accompanied by one or more of hypertension ≥160/110 mmHg, proteinuria ≥5 g/24 hours, oliguria <500 ml/24 hours, impaired liver function, epigastric or right-upper quadrant pain, cerebral or visual disturbances, pulmonary oedema or cyanosis, and foetal growth restriction with thrombocytopenia [87]. Neonatal death was defined as death of a neonate within 28 days after birth. When a patient had more than one episode of severe preeclampsia, we analysed the first episode that met the inclusion criteria. We included both primary APS and APS associated with SLE (defined according to the SLICC classification criteria) [89]. Thirteen women had also been included in our study of intrauterine foetal death (IUFD) in APS patients, although for 7 of them we considered a different pregnancy [90].

Methods. We retrospectively collected from the patients' medical charts demographic, clinical, laboratory, and ultrasound data as well as treatments received

during pregnancy. We considered that women had been treated during pregnancy if they were on LDA and/or LMWH before the diagnosis of severe preeclampsia.

The antibody profile at the time of the APS diagnosis was recorded, including LAC, IgG and IgM anti cardiolipin (aCL), and IgG and IgM anti-beta2 Glycoprotein I (β2GPI). As recommended by the International Society of Thrombosis and Hemostasis, the presence of LAC was explored by activated partial thromboplastin time (APTT) and dilute Russell's viper venom time (dRVVT) in most patients and occasionally with the dilute prothrombin time and kaolin clotting time [91]. Results of aCL were considered positive when they exceeded the 99th percentile of the laboratory's control values or if the laboratory had none, when they exceeded 40 IgG or IgM phospholipid units (GPL or MPL). The upper reference limits supplied by the laboratory performing the test were used for the anti-ß$_2$GPI antibodies. In accordance with the classification criteria [88], women were included only when they had at least two positive laboratory tests 12 weeks apart or more and within 5 years of the qualifying event. Because this is a retrospective study, antibodies could not be tested in a centralised laboratory.

Statistical analysis. Categorical data are expressed by proportions. All continuous variables are presented as means and standard deviations (SD) if their distributions are parametric, and otherwise as medians and interquartile ranges (IQR). Significance for the univariate analysis was set at 0.05. Statistical analyses used STATA v.13.1 software.

4.2 PAPER II: "SLE-DAS in the first trimester of gestation predicts maternal lupus flares later in pregnancy."

Patients. This study was carried out at two referral centres for rare systemic and autoimmune diseases: Rheumatology Unit, University of Padova, Italy (from 2002 to July 2019), and Internal Medicine Department, Cochin Hospital, Paris, France (from 2014 to July 2019, by using all pregnancies from the prospective GR2 study clinicaltrial.gov NCT02450396).

Inclusion criteria were 1) women ≥ 18 years; 2) affected with SLE (SLICC 2012 criteria) (15); 3) with an ongoing singleton pregnancy at 12 weeks (only one pregnancy per patient). Patients underwent at least one visit in the first trimester, and were followed up according to current clinical practice until the end of pregnancy. This project adheres to the principles of the Declaration of Helsinki and was approved by the local ethics committees.

Methods. Demographic, clinical and laboratory findings were collected at first trimester by assessing the following variables: age, disease duration (years), skin colour, multiparity, associated APS and SLE manifestations.

Serological and other laboratory data were assessed at first trimester according to standard tests including anti-dsDNA, C3 and/or C4 serum levels, and 24h proteinuria. APL Abs were also tested including aCL IgM and IgG, anti-ß2GP1 IgM and IgG, and LAC according to standard definitions [88] and recommendations [92].

Women were considered on therapy when they were taking at least one of the following drugs: HCQ, prednisone, immunosuppressants (IS), LDA, and LMWH.

Definition of disease activity. Disease activity was assessed at first trimester by applying SLEPDAI [71] and by retrospectively applying SLE-DAS using the online free calculator available at http://sle-das.eu/ [72].

Definition of maternal flares. Maternal flares in the 2nd and 3rd trimester of gestation were assessed according to SELENA-SLEDAI flare index (SFI) [93]; flares were subdivided into mild/moderate and severe.

Definition of APOs. APOs were defined with a binary composite score obtained when any of the following event occurred: an otherwise unexplained IUFD >12 weeks; a neonatal death within the first 7 days after birth; placental insufficiency (IUGR, PE/E-, HELLP, and/or placental abruption) leading to a premature delivery before 37 weeks; SGA: birth weight ≤3rd percentile according the AUDIPOG curve [94]. Definitions of PE and HELLP are summarised in Supplementary Material.

Statistical analysis. We described patients' characteristics applying mean ± SD and median with IQR for parametric and non-parametric continuous variables, respectively. APO's and maternal flares' incidences was reported by CI set at 95%. The following variables were assessed at first trimester by univariate analysis:

- continuous: age at pregnancy (years), disease duration (years), SLEPDAI and SLE-DAS scores, prednisone dosage (mg/day);

- categorical/binary: skin colour, associated APS, previous renal involvement, serological features including anti-dsDNA, hypocomplementemia (low C3 and/or C4), aPL profile (LAC, IgG/IgM anti-aCL, IgG/IgM anti-beta2GPI, triple positive aPL), active 24h proteinuria (>0.5 g/day); concomitant treatment including HCQ, prednisone, IS, LDA, and/or LMWH.

Comparison of continuous variables with a parametric and non-parametric distribution was performed using T-test and Wilcoxon's rank-sum test, respectively. Pearson's chi-square (or Fisher's exact test when appropriate) was used to evaluate bivariate associations between categorical variables at univariate analyses. A correlation between SLEPDAI and SLE-DAS in the first trimester was assessed according to Spearman's correlation test, considering the non-parametric distribution of these scores. Multivariate analysis was performed according to a logistic regression model. The choice of independent variables was based on current knowledge and significant variables at univariate analysis ($p<0.1$). Two separate multivariate analyses were performed for the outcomes of maternal flares and APOs, respectively. Significance at logistic regression analysis was set at 5%. Models' performance was evaluated using the Hosmer-Lemeshow (HL) goodness-of-fit test and area under the ROC curve (AUC). All statistics were conducted using R software version package 1.3.1073.

4.3 PAPER III: "Evaluation of lupus anticoagulant, damage, and remission as predictors of pregnancy complications in lupus women: the French GR2 study."

We report data from the GR2 ("Groupe de recherche sur la Grossesse et les Maladies Rares") study, a French multicentre prospective observational study of pregnant women with rare and/or rheumatological diseases, including SLE and APS, conducted since October 2014 in 63 active centres (not all recruiting patients with SLE as the cohort is intended to study several rare and rheumatological diseases). Pregnant women are included by their clinicians (internists, rheumatologists, and nephrologists) and are followed up to 12 months postpartum. The treating physicians made all treatment decisions.

The GR2 study is part of the European network of pregnancy registers in Rheumatology (EuNeP) supported by FOREUM (Foundation for Research in Rheumatology) [95] and follow EULAR recommendations regarding core data sets for pregnancy registers in rheumatology [96].

Inclusion criteria. Criteria for the current analysis required inclusion in the GR2 before 13 weeks, SLE classified according to the SLICC 2012 criteria [89], and conception before July 15, 2019 (to have complete data at delivery), with an ongoing singleton pregnancy that reached 12 weeks. Only the first singleton pregnancy per woman was analysed.

Data collected. At first-trimester consultations, we assessed demographic, clinical, serological, and treatment features. APL status included aCL, anti-β2GPI, and LAC. In France, all laboratories are regularly audited and certified by a central agency. More details on the variety and types of assays are reported in supplementary text 1. Triple positive aPL status was defined by positive aCL, anti-β2GPI, and LAC.

All data were prospectively collected in electronic case report forms at each consultation. Because all women received standard treatment, written informed consent was not required by French law. This project adheres to the principles of the Declaration of Helsinki and was approved by the Local Ethics Committee.

Definitions of remission, LLDAS, disease activity, and damage. Disease activity was scored by the SLEDAI-2K [73] adapted to pregnancy (SLEPDAI) [71] and we considered the first SLEPDAI available during the first trimester. Remission status was assessed by the DORIA/Zen [77] and DORIS [78] criteria and by clinical SLEPDAI=0 [80] Damage was scored by the SLICC-Damage Index [97] (see definitions in Supplementary text 2).

Definition of outcomes. Maternal flares were defined according to the SELENA-SLEDAI Flare Index, SFI) [93] This score divides flares into mild/moderate and severe flares and notably captures any increase in the Physician Global Assessment (PGA) or in the steroid dose, any introduction of an immunosuppressive drug, and any hospitalization.

To make our results comparable to those of the PROMISSE study [7], we defined APOs by a composite binary variable (the occurrence of at least one of the following events versus the non-occurrence of any of them): an otherwise unexplained IUFD $\geq$12 weeks, a neonatal death (within 28 days after birth), placental insufficiency (foetal growth restriction, i.e., FGR, preeclampsia/eclampsia, HELLP syndrome, and/or placental abruption, see supplementary text 3) leading to preterm delivery <37 weeks, SGA (birth weight below the third percentile according to the French AUDIPOG curve) [94].

Statistical analyses. Continuous parametric and non-parametric variables were expressed by their means ± SD and medians with IQR. Incidence and 95% CIs were assessed for both maternal flares and APOs. To identify their predictors, we tested the following variables during the first trimester: 1) continuous: maternal age, disease duration, SLEPDAI, PGA, SLICC-Damage Index scores; 2) categorical: skin colour, overweight, tobacco and alcohol consumption, associated APS, nulliparity, previous thrombosis, IUFD, or renal involvement, low platelet count, positive anti-dsDNA, hypocomplementemia, positive aPL, 24h proteinuria, concomitant treatment, remission, and LLDAS.

Pearson's chi square test (or Fisher's exact test when appropriate) was used to evaluate univariate associations between categorical variables. Student's t-test and Wilcoxon's rank-sum test were used to compare the parametric and non-parametric continuous variables, respectively. The choice of independent variables added to the logistic regression model in the multivariate analysis was based on current knowledge and the variables significant at the univariate analysis (P<0.1). Significance for the logistic regression analyses was set at 5%. When the univariate analysis found significant associations between variables with high collinearity, separate multivariate models were tested.

All analyses were conducted with STATA v.16.1.

4.4 PAPER IV: "Role of pentraxin-3 (PTX3) as predictor of maternal and foetal complications in patients affected with SLE and/or APS."

Study design. We carried out a longitudinal (both retrospective and prospective), observational study of pregnancies in women with rare and/or rheumatic diseases, including SLE and APS, conducted since January 2015 at the University of Padova,

Department of Rheumatology, in the multidisciplinary Out-patient Clinic shared by Obstetrics and Gynaecologists and Rheumatologists. Pregnant women with any of the specified diseases were included and followed up by rheumatologists during pregnancy until 6 months after the end of pregnancy. Each visit was performed according to physicians' clinical practice. Patients were prospectively visited by monthly visits and at least once during postpartum period (up to 12 months).

Inclusion criteria. Criteria for the current analysis included all pregnant women affected with SLE (SLICC 2012) [89] and/or APS (according to Miyakis et al, 2006) [88]; the matched control group included pregnancies of patients affected with other rheumatic diseases rather than APS and/or SLE.

All these pregnancies were followed up and recruited at our multidisciplinary out-patient clinic with a conception date before the 1st April 2021. Overall, only the first pregnancy per woman meeting the inclusion criteria was analysed.

Methods. At first-trimester consultation, the following characteristics were assessed: demographics, clinical, and serological variables, as well as treatment.
We considered that women had been treated during pregnancy if they were on LDA and/or LMWH, antimalarials (hydroxychloroquine or chloroquine), prednisone (or prednisone equivalent), immunosuppressive drugs.

Biomarkers. We collected patients sera at least once during early gestation (1st trimester), by analysing serum levels of PTX3, anti-PTX3 Abs, IgG/IgM anti-aCL, IgG/IgM anti-beta2GPI, and LAC. Other Abs including anti-dsDNA, anti-extractable nuclear antigens (ENA) were determined using routine methods.

ELISA test for human IgG anti-PTX3 Abs. Serum levels of anti-PTX3 Abs were determined by home-made ELISA. Briefly, Maxisorp immunoplates (Nalgene

Nunc, New York, USA) were coated with 50 µl/well of recombinant human PTX3 diluted in phosphate-buffered saline (PBS) at a concentration of 5 µg/ml and incubated overnight at 4°C. The wells were blocked with 3% bovine serum albumin (BSA)/PBS and incubated at room temperature for 2 h. After washing, the serum samples were added in duplicate diluted 1:100 in 1% BSA/PBS and incubated at room temperature for 4h. After washing, alkaline phosphatase-conjugated goat anti-human IgG (Sigma, St Louis, Missouri, USA) diluted 1:5000 in 1% BSA/ PBS was added and incubated for 1h at 37°C. After washing, p-nitrophenyl phosphate (Sigma) was added. Serum IgG anti-PTX3 antibody levels are expressed as Optical Density (OD) values, measured at 405 nm by microplate spectrometer. Cut-off of positivity corresponds to 0.234 OD.

ELISA test for PTX3 levels. PTX3 levels (ng/ml) were detected by performing a commercially available sandwich ELISA, (Alexis, UK), according to the manufacturer's instruction.

ELISA test for aPL including LAC. As recommended by the International Society of Thrombosis and Hemostasis, the presence of LAC was explored by APTT and dRVVT in most patients and occasionally with the dilute prothrombin time and kaolin clotting time [92]. Results of aCL were considered positive when they exceeded the 99th percentile of the laboratory's control values or if the laboratory had none, when they exceeded 40 IgG or IgM phospholipid units (GPL or MPL). The upper reference limits supplied by the laboratory performing the test were used for the anti-ß$_2$GPI Abs. In accordance with the classification criteria [88], women were included only when they had at least two positive laboratory tests 12 weeks apart or more and within 5 years of the qualifying event.

Triple positive aPL status was defined by positivity for aCL, anti-β2GPI, and LAC. All data were prospectively collected in electronic case report forms at each consultation. IgG/IgM aβ2GPI and IgG/IgM aCL were tested by home-made ELISA [98].

Ethics. Patients who fulfilled the inclusion requirements were contacted and asked to sign informed consent forms. Their medical records were then retrieved and reviewed.

Definition of pregnancy outcomes.

Maternal Complications. They included:

-SLE disease flare during pregnancy or in the post-partum period, defined as an increase of ≥1 point in the SLEPDAI or SLEDAI-2K in patients affected with SLE;

-preeclampsia (PE), assessed according the last ACOG guidelines [87];

-eclampsia (E): assessed according the last ACOG guidelines [87];

-HELLP syndrome [99];

-pregnancy induced hypertension [100];

-maternal death;

-gestational diabetes mellitus (GDM).

All these definitions are summarised in Supplementary Material.

Foetal complications. They included:

-Spontaneous abortion (<10 WG);

-IUFD (≥10 WG);

-preterm delivery (<37 WG);

-IUGR [100];

-foetal death during delivery or in the post datum period (within 28 days).

Statistical analyses. Continuous parametric and non-parametric variables were reported by their means ± SD and medians with IQR, respectively. Incidence and 95% CI were assessed to report proportions of categorical variables.

The following variables were assessed at 1st trimester: 1) age at pregnancy (in years), nulliparity, aPL positive tests (LAC, anti-aCL, anti-β2GPI, triple positive aPL test), concomitant treatment including antimalarials, prednisone (or prednisone equivalent), immunosuppressive drugs, LDA, LMWH.

Pearson's chi square test (or Fisher's exact test when appropriate) was used to assess univariate associations between categorical variables. Student's t-test and Wilcoxon's rank-sum test were used to compare the continuous variables with parametric and non-parametric distributions, respectively. Statistical significance was set at 5%. All analyses were conducted with STATA software version 16.1.

5 SUMMARY OF RESULTS

5.1 PAPER I

Patient's characteristics. The study included 40 women. Their demographic, clinical, and serological variables are summarized in Table 1**.** Their mean age at the index preeclampsia episode was 30.5 years ± 4.6 SD, and it occurred during their first pregnancy in 21 women (52.5%). Overall, 14 women (35%) had at least one thrombotic event before the index episode, with a median time of 7.5 years (IQR 3-12) between the thrombotic and obstetric events. Nine women (22.5%) had had obstetric manifestations of APS before the index episode.

APS was diagnosed before the index episode of preeclampsia in 12 women (30%), with a median follow up of 5 years (IQR 3-12) between APS diagnosis and this episode, while the remaining 28 (70%) were diagnosed with APS when the preeclampsia occurred. LAC was positive in 30 women (82.5%), and aPL antibodies triple positive in 21 (52.5%).

Previous pregnancies. Before the index episode of preeclampsia, 19 women (47.5%) had had a total of 45 pregnancies that resulted in 11 live births (24.4%, including two premature births with IUGR associated with HELLP syndrome in one and non-severe preeclampsia in another), 13 miscarriages (28.9%), 11 IUFD (24.4%), and 10 elective abortions (22.2%). Finally, three women (15.8%) had two consecutive miscarriages before the index preeclampsia episode, but none had a history of three consecutive miscarriages.

Description of the index pregnancy with severe preeclampsia. For various reasons (known APS, positive aPL, previous obstetric complications), 23 (57.5%) women were receiving a treatment during the index preeclampsia episode: 4 by LDA,

4 by LMWH and 15 by both LDA and LMWH. Seven patients were also under treatment with HCQ, eight with glucocorticoids and three with immunosuppressive drugs. The other 17 women (42.5%) were not receiving any treatment at the onset of the preeclampsia episode.

The median gestational age at the index episode was 25.5 weeks' gestation (IQR 23-29). Maternal complications occurred in 21 women (52.5%), including HELLP syndrome in 18 (45%), eclampsia in 6 (15%), placental abruption in 3 (7.5%), and/or catastrophic APS (CAPS) in 3 (7.5%). Foetal complications were observed in all index pregnancies and included 11 IUFD (27.5%) and 29 preterm live births, 15 of whom (born at a median term of 24 weeks) died before day 28. The 14 premature surviving children were born at a median term of 31 weeks (IQR 27-33), 2 (14.3%) with IUGR.

Doppler ultrasound examinations at or after 22 weeks' gestation were available for 38 women and reported as abnormal in 17 (44.7%). Abnormalities included bilateral uterine artery proto-diastolic notches (n=10) or a unilateral notch (n=5) and/or reverse or absent end-diastolic umbilical flow (n=2).

Subsequent pregnancies. After a median follow-up of 3.5 years (IQR 2-6) after the index preeclampsia episode, 26 (65%) women had at least one new pregnancy, with a total of 37 new pregnancies. Treatment was LDA in 37 pregnancies (100%), LMWH in 33 (89.2%), and HCQ in 16 (43.2%). The overall outcomes were live births in 33 pregnancies (89.2%), IUFD in 3 (8.1%), and one miscarriage (2.7%). No woman had 3 consecutive miscarriages. Of the 33 live births, 20 (60.6%) were uncomplicated, while 13 had at least one complication including preeclampsia (n=8), IUGR (n=5), HELLP syndrome (n=4) and/or preterm delivery (n=4). No eclampsia, CAPS, or placental abruptions were observed.

Thrombotic events. By the end of the follow-up after the index preeclampsia episode (median 3.5 years, IQR 2-6), 16 women (40%) had had at least one thrombotic event. The first thrombosis occurred before or at the time of the index episode, except in two who had their first thrombosis, one after delivery and the other 12 years afterwards. The sites of thromboses were venous in 12 women, arterial in 6, and micro thrombotic in 4. Four women experienced CAPS, 3 simultaneously with preeclampsia and one before the index case.

Associated autoimmune diseases. At the end of follow-up after the index preeclampsia episode (median 3.5 years, IQR 2-6), 12 women had been diagnosed with SLE (30%), 9 (22.5%) before the index episode, 2 (5%) during that episode, and 1 (2.5%) 4 years after it. The live birth rate did not differ between women with SLE and APS and those with primary APS at the time of the index pregnancy (*P*=0.60).

Table 1: Characteristics of the index pregnancies complicated by severe	
Patient's characteristics	**N=40 (%)**
Previous thrombotic events	14 (35.0)
Previous obstetric manifestations of APS	9 (22.5)
Known APS	12 (30.0)
Associated SLE*	11 (27.5)
Primiparous	21 (52.5)
Mean age at index pregnancy ± SD	30.5 ± 4.6
Antibody profile	
IgG/IgM anti-cardiolipin antibodies	34 (85.0)
IgG/IgM anti-β2GPI antibodies	25 (62.5)
LAC	33 (82.5)
Triple positive aPL antibody tests	21 (52.5)
Treatment during index pregnancy, before onset of	
Only LDA	4 (10.0)
Only LMWH	4 (10.0)
LDA+LMWH	15 (37.5)
Hydroxychloroquine	7 (17.5)
Glucocorticoids	8 (20.0)
Immunosuppressants	3 (7.5)
None	17 (42.5)
Features of severe preeclampsia	
Term at onset, weeks of gestation (median, IQR)	**25.5 (23-29)**
Foetal outcome	
IUFD	11 (27.5)
Neonatal death	15 (37.5)
Survival at 28 days after birth	14 (35.0)
Gestational age at birth of surviving children (n=14)	31 (27-33)
Preterm delivery (<37 weeks) among surviving children (n=14)	14 (100.0)
IUGR among surviving children	2 (14.3)
Associated maternal complications	
HELLP syndrome	18 (45.0)
Eclampsia	6 (15.0)
Placental abruption	3 (7.5)
Catastrophic APS	3 (7.5)

Legend to Table 1: APS: antiphospholipid syndrome; IQR: interquartile range; SLE: systemic lupus erythematosus; *: Nine women had SLE at index pregnancy, whereas

2 patient developed SLE during this pregnancy. Abs: antibodies; aPL: antiphospholipid; LAC: lupus anticoagulant; LDA: low dose aspirin; LMWH: Low Molecular Weight Heparin; HELLP: Haemolysis, elevated liver enzymes, low platelet; IUGR: intrauterine growth restriction; IUFD: intrauterine foetal death; N: number; SD: standard deviation.

5.2 PAPER II

General features of SLE pregnancies at first trimester. A total of 158 pregnancies in 158 patients affected with SLE were collected (107 enrolled at Internal Medicine Department, Cochin Hospital, and 51 at the Rheumatology Department, University of Padova). Mean ± SD age was 31.9 ± 4.6 years, and 89 (56.3%) women were multiparous.

Concerning skin colour, the majority was White (n=124, 78.5%), followed by Black (n=21, 13.3%), Asian (n=11, 6.9%) and other origin (n=2, 1.3%).

Previous SLE manifestations before pregnancy occurred as following: articular involvement in 125 (79.1%) women, mucocutaneous in 108 (68.4%), renal and haematological in 59 (37.3%) respectively, serositis in 36 (22.8%) and neuropsychiatric (NPSLE) in 7 (4.4%).

Median SLEPDAI (IQR) was 2 (0-4) and median SLE-DAS (IQR) was 1.32 (0.37-2.08).

General pregnancy outcome. We observed 153 (96.8%) live births: their mean birth weight ± SD was 2946 ± 583 g (2 missing data), and the median delivery term (IQR) was at 38 WG (36-39) (3 missing data). Four (2.5%) IUFD occurred and a medical termination of pregnancy was performed in 1 (0.6%) case.

Maternal flares. At least one flare occurred in 25 (15.8%, 95% CI 9.9-20.9) patients during the 2nd and 3rd trimester: 23 (14.3%) women experienced mild/moderate flares and 2 (1.3%) severe renal flares. Overall, we observed that 10 (6.3%) patients had at least one articular flare, 7 (4.4%) mucocutaneous, 5 (3.2%) renal, 3 (1.8%) haematological and serositis flares, respectively. No NPSLE flares occurred in this study **(Table 1).**

APOs. Nineteen (12.0%; 95% CI: 7.8-18.0) pregnancies were complicated by obstetrical events **(Table 2).** Four patients (2.5%) had an IUFD and 13 (8.2%) preterm deliveries due to placental insufficiency. In addition, 3 pregnancies (2.0%, 7 missing data) ended with SGA births. No neonatal deaths occurred.

Univariate analyses. At univariate analysis, both SLE-DAS and SLEPDAI scores in the first trimester were associated with maternal flares in 2nd and 3rd trimester of gestation (p=0.01 for both). In addition, anti-dsDNA (p=0.02) and active 24h-proteinuria (p=0.02) were also found to be associated with maternal flares. Regarding treatment, the use of prednisone, prednisone dosage and immunosuppressants were associated with disease flares (p=0.04, p=0.02 and p=0.05, respectively**) (Table 2).** There was a high and significant correlation between SLEPDAI and SLE-DAS (rho=0.97, p<0.01) in the first trimester.

At univariate analysis, SLE-DAS (p=0.02), SLEPDAI (p=0.02) and anti-dsDNA (p=0.01) were associated with APOs **(Table 3).**

Multivariate analyses. Regarding maternal flares, since both anti-dsDNA and active 24h-proteinuria are components of SLEPDAI and SLE-DAS, we did not include these two variables into our logistic regression models in order to avoid collinearity issues. As we found a high correlation between SLEPDAI and SLE-DAS in the first trimester, two different logistic regression models were performed using SLEPDAI and SLE-DAS as explanatory variables **(Table 4).**

SLE-DAS in the first trimester was predictor of maternal flares in the 2nd and 3rd trimester (adjusted-adj-OR:1.2; 95% CI:1.0-1.3; p=0.02). Also, SLEPDAI resulted predictor of maternal flares in pregnancy (adjOR=1.3; 95% CI:1.1-1.6; p=0.01).

We did not perform a multivariate analysis for APOs as only anti-dsDNA, SLEPDAI and SLE-DAS resulted statistically associated at univariate analysis; therefore, due to collinearity issues a multivariate analysis was not assessed.

Table 1: Maternal flares (2nd and 3rd trimester) and APOs	
Patients with at least one flare, n (%)	**25 (15.8)**
Articular, n (%)	10 (6.3)
Mucocutaneous, n (%)	7 (4.4)
Renal, n (%)	5 (3.2)
Hematological, n (%)	3 (1.8)
Serositis, n (%)	3 (1.8)
NPSLE, n (%)	0 (0.0)
Patients with at least one APOs, n (%)	**19 (12.0)**
IUFD, n (%)	4 (2.5)
Preterm delivery due to placental insufficiency, n (%)	13 (8.2)
SGA, n (%)	3 (2.0)
Neonatal deaths, n (%)	0 (0.0)

Legend to Table 1: SLE: Systemic Lupus Erythematosus; NSPLE: Neuropsychiatric SLE; APOs: adverse obstetrical outcome; IUFD: intra-uterine foetal death; SGA: small for gestational age.

Figure 1. Correlation between SLEPDAI and SLE-DAS at first trimester

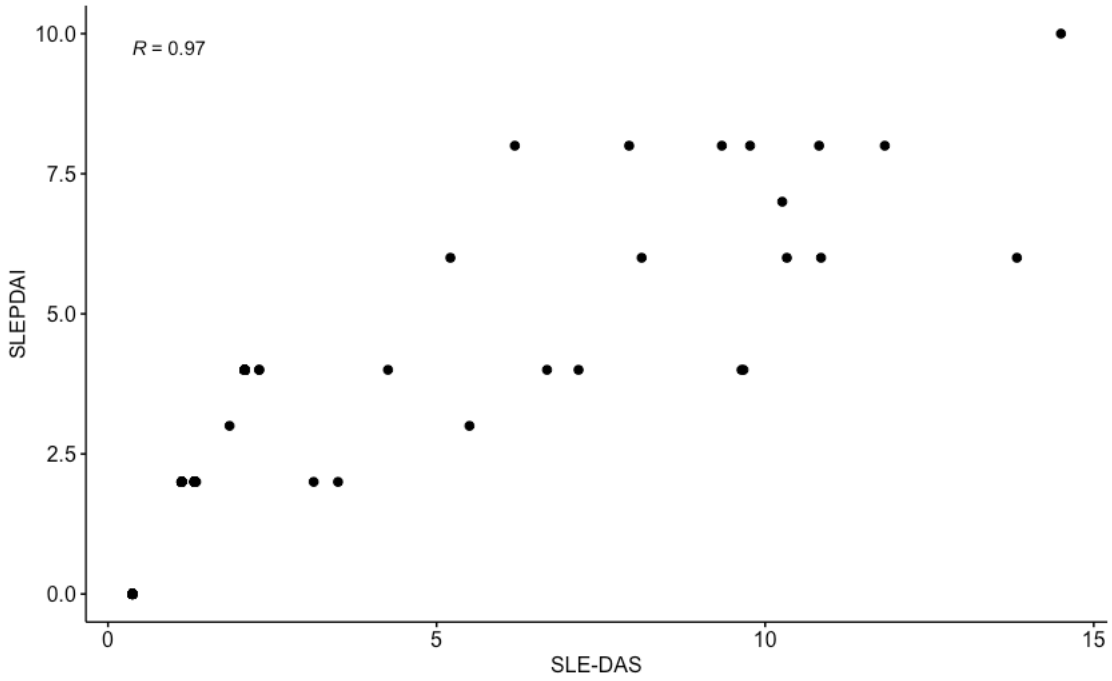


Table 2. Univariate analysis: Maternal flares in the 2nd and 3rd trimester				
	Overall (N=158)	**Flare (N=25)**	**Non flare (N=133)**	**P value**
Age at pregnancy, mean± SD (years)	31.9 ± 4.6	31.4 ± 4.6	32.0 ± 4.6	0.50
SLE characteristics				
Associated APS, n (%)	18 (11.4)	1 (4.0)	17 (12.8)	0.31
Disease duration, median (IQR)	9 (5-13)	10 (2-13)	9 (5-13)	0.66
Previous renal manifestation, n (%)	59 (37.3)	9 (36.0)	50 (37.6)	0.88
Laboratory features in the 1st trimester				
Positive anti-dsDNA, n (%)	80 (50.6)	18 (72.0)	62 (46.6)	**0.02**
Low C3/C4, n (%)	54 (34.2)	9 (36.0)	45 (33.8)	0.83
24h-proteinuria > 0.5 g/day, n (%)	12 (7.6)	5 (20.0)	7 (5.3)	**0.02**
IgG/IgM anti-cardiolipin, n (%)	22 (13.9)	3 (12.0)	19 (14.3)	1.00
IgG/IgM anti-beta2GPI, n (%)	12 (7.6)	1 (4.0)	11 (8.3)	0.69
LAC, n (%)	24 (15.2)	2 (8.0)	22 (16.5)	0.37
Triple positive aPL, n (%)	7 (4.4)	0 (0.0)	7 (5.3)	0.60
Treatment in the 1st trimester				
Prednisone, n (%)	72 (45.6)	16 (64.0)	56 (42.1)	**0.04**
Prednisone dose, median (IQR), mg/day	5 (5.0-7.5)	5 (0.0-7.0)	0 (0.0-5.0)	**0.02**
Immunosuppressants, n (%)	44 (27.8)	11 (44.0)	33 (24.8)	**0.05**
Hydroxychloroquine, n (%)	149 (94.3)	23 (92.0)	126 (94.7)	0.63
Low dose aspirin, n (%)	88 (55.7)	13 (52.0)	75 (56.4)	0.68
Low molecular weight heparin, n (%)	35 (22.6)	3 (12.0)	32 (24.1)	0.29
Disease activity in the 1st trimester				
SLEPDAI, median (IQR)	2 (0-4)	3 (2-6)	2 (0-4)	**0.01**
SLE-DAS, median (IQR)	1.32 (0.37-2.08)	1.85 (1.32-6.19)	1.32 (0.37-2.08)	**0.01**

Legend to Table 2. SD: standard deviation; IQR: interquartile range; SLE: Systemic Lupus Erythematosus; APS: antiphospholipid syndrome; anti-dsDNA: anti-double stranded DNA; 24h: 24 hours; LAC: Lupus anticoagulant; aPL: anti-phospholipid antibodies; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index; SLE-DAS: Systemic Lupus Erythematous disease activity score.

Table 3. Univariate analysis: Adverse pregnancy outcome (APOs)				
	Overall (N=158)	**APOs (N=19)**	**Non-APOs (N=139)**	**P value**
Age at pregnancy, mean± SD (years)	31.9 ± 4.6	31.4 ± 5.2	32.0 ± 4.5	0.57
SLE characteristics				
Associated APS, n (%)	18 (11.4)	3 (15.8)	15 (10.8)	0.46
Disease duration, median (IQR)	9 (5-13)	12 (5.5-17)	9 (4.5-13)	0.12
Previous renal manifestation, n (%)	59 (37.3)	9 (47.4)	50 (36.0)	0.33
Laboratory features in the 1st trimester				
Positive anti-dsDNA, n (%)	80 (50.6)	15 (78.9)	65 (46.8)	**0.01**
Low C3/C4, n (%)	54 (34.2)	8 (42.1)	46 (33.1)	0.44
24h-proteinuria > 0.5 g/day, n (%)	12 (7.6)	2 (10.5)	10 (7.2)	0.63
IgG/IgM anti-cardiolipin, n (%)	22 (13.9)	3 (15.8)	19 (13.7)	0.73
IgG/IgM anti-beta2GPI, n (%)	12 (7.6)	1 (5.3)	11 (7.9)	1.00
LAC, n (%)	24 (15.2)	5 (26.3)	19 (13.7)	0.17
Triple positive aPL, n (%)	7 (4.4)	1 (5.3)	6 (4.3)	1.00
Treatment in the 1st trimester				
Prednisone, n (%)	72 (45.6)	11 (57.9)	61 (43.9)	0.25
Prednisone dose, median (IQR), mg/day	5 (5.0-7.5)	5 (0.0-6.8)	0 (0.0-5.0)	0.16
Immunosuppressants, n (%)	44 (27.8)	8 (42.1)	36 (25.9)	0.14
Hydroxychloroquine, n (%)	149 (94.3)	19 (100.0)	130 (93.5)	0.60
Low dose aspirin, n (%)	88 (55.7)	13 (68.4)	75 (53.9)	0.23
Low molecular weight heparin, n (%)	35 (22.6)	6 (31.6)	29 (20.9)	0.38
Disease activity in the 1st trimester				
SLEPDAI, median (IQR)	2 (0-4)	2 (2-4)	2 (0-4)	**0.02**
SLE-DAS, median (IQR)	1.32 (0.37-2.08)	1.32 (1.32-2.08)	1.32 (0.37-2.08)	**0.02**

Legend to Table 3: APOs: adverse pregnancy outcome; SD: standard deviation; IQR: interquartile range; SLE: Systemic Lupus Erythematosus; APS: antiphospholipid syndrome; anti-dsDNA: anti-double stranded DNA; 24h: 24 hours; LAC: Lupus anticoagulant; aPL: anti-phospholipid antibodies; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index; SLE-DAS: Systemic Lupus Erythematous disease activity score.

Table 4. Multivariate logistic regression for maternal flares			
		Model 1	**Model 2**
	Crude OR (95% CI)	**adjOR (95% CI)**	**adjOR (95% CI)**
SLE-DAS in the first trimester (increase per 1 unit)	1.2 (1.1-1.4)	**1.2 (1.0-1.3)**	-
SLEPDAI in the first trimester (increase per 1 unit)	1.4 (1.1-1.7)	-	**1.3 (1.1-1.6)**
Prednisone in the first trimester	2.4 (1.0-5.9)	0.9 (0.2-4.4)	0.8 (0.2-3.7)
Prednisone dose (increase per 1 mg/day)	1.1 (1.0-1.3)	1.1 (0.9-1.3)	1.1 (0.9-1.3)
Immunosuppressants in the first trimester	2.4 (1.0-5.8)	1.5 (0.6-4.2)	1.6 (0.6-4.2)
H-L Goodness-of-fit*		0.90	0.88
AUC curve**		0.70	0.69

Legend to Table 4: OR: Odds ratio; adjOR: adjusted OR; CI: Confidence intervals; SLE-DAS: Systemic Lupus Erythematosus Disease Activity Score; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index; PDN: prednisone; mg: milligrams; H-L: Hosmer-Lemeshow; AUC: Area under the Curve; *=:refers to p value; **: refers to AUC.

5.3 PAPER III

Patient characteristics at enrolment. This study includes 238 women with SLE from 34 centres (see supplementary tables S1 and S2 and figure S1). Their mean age was 31.6 ± 4.5 years, 88 (37.0%) were nulliparous, and 34 (14.3%) had an associated APS.

Previous LN was reported in 67 women (28.2%) and was biopsy-proven in 62 (92.5%): 1 had class I, 1 class II, 12 class III, 19 class IV, 16 class V, 1 class VI, 5 class III+V, and 7 class IV+V. Nine women had positive 24-hour proteinuria (>0.5 g/g or 0.5 g/day), attributed to active renal disease in only three.

All but four women (98.3%) took hydroxychloroquine, 119 (50%) prednisone, 56 (23.5%) immunosuppressive drugs, and 165 (69.3%) low-dose aspirin. Finally, five women (2.1%) received antihypertensive drugs.

The median (IQR) SLEPDAI was 2 (0-3). Remission was achieved by 200 women (86.6%) with the clinical SLEPDAI=0, by 154 women (64.7%) with the DORIA/Zen definition, and by 147 (61.8%) with the DORIS definition. LLDAS was achieved by 157 patients (71.7%).

Irreversible chronic damage was reported in 30 women (12.7%, missing data for 2). All had been treated with prednisone, 7 (23.3%) also had APS, and 16 (53.3%) had a history of renal involvement. Details of SLICC-Damage Index domains are reported in supplementary table S3.

Maternal flares. Thirty-five women (14.7%, 95% CI: 10.7-19.8) had at least one flare during the second or third trimesters; most of them were articular (n=18, 7.6%) and/or cutaneous (n=15, 6.3%). Eight (3.4%) women had other types of flares: serositis in 5 (2.1%), renal in 3 (1.3%), and/or haematological in 2 (0.8%).

A severe flare occurred in only three women during the second trimester: two renal flares and one pericarditis associated with cutaneous rash. All three women required the addition of an immunosuppressive drug to the background treatment and had liveborn children, with an early delivery at 28 weeks for early preeclampsia in the woman with pericarditis and a rash.

At univariate analysis, only first-trimester hypocomplementemia was associated with flares (*P*=0.02) (Table 1). Since hypocomplementemia is included in the SLEPDAI, no multivariate analysis could be performed for flares.

Finally, we found no association between maternal flare and APOs (*P*>0.99). Neither the percentage of live births nor their median gestational age at delivery differed between patients with and without flares (97.1 vs 96.5% and 37.4 vs 37.7 weeks, respectively).

Obstetric and adverse pregnancy outcomes. Almost the entire cohort (230, 96.6%) had a live birth (median gestational age 37.7 ± 2.6 weeks). For the remaining eight women, one had a termination of pregnancy because of chromosomal abnormalities, and seven had an IUFD.

At least one APOs occurred in 34 women (14.3%, 95%CI: 10.4-19.4) (Table 2), including 22 (9.2%) preterm births due to placental insufficiency at a median gestational age of 33 weeks, 7 (2.9%) IUFDs, 5 (2.1%, 5 missing data for the weight) SGA infants, and one (0.4%) neonatal death. Among patients with placental insufficiency leading to preterm delivery, 8 had FGR, 6 HELLP syndrome, 14 preeclampsia/eclampsia, and/or one placental abruption.

At univariate analysis, women with at least one APOs were more likely to have LAC (*P*<0.001), at least one positive aPL (*P*<0.001), an associated APS (*P*=0.01), or

prior thrombotic event (*P*=0.04) (Table 2). They were also more likely to have positive anti-dsDNA (*P*=0.01) and, accordingly, a higher SLEPDAI (*P*=0.01). APOs were also associated with damage accrual (SLICC-Damage Index) (*P*=0.01), immunosuppressive drug use (*P*=0.03), low-dose aspirin (*P*=0.03), and low molecular weight heparin (*P*=0.01). Finally, APOs were not associated with antihypertensive drugs (*P*=0.15), a low platelet count (*P*>0.99), or skin colour (*P*=0.40) (Table 2).

To minimize collinearity, two different logistic regression models were tested for DORIA/Zen remission and LLDAS (Tables 3, 4). Predictors of APOs in both analyses were SLICC-Damage Index (per 1 unit increase) and positive LAC in the first trimester (adjusted (a)ORs of 1.8 and 4.2 in Model 1 and 1.7 and 3.7 in Model 2, respectively) (Tables 3,4). Neither DORIA/Zen remission nor LLDAS predicted APO. Multicollinearity was ruled out in both models (VIF<2).

Analysis of the PROMISSE predictors of APOs. Among the 121 White women who were concomitantly antihypertensive-free, LAC-negative, and had a PGA ≤ 1 in the first trimester and a platelet count > 100 $\times 10^9$/l, only 8 (6.6%) had an APOs at any time; one of these foetuses died in utero and another after birth. By contrast, among the combined group of non-White women treated with antihypertensive drugs (n=2) or women with positive LAC (n=41), 15 (34.9%) had an APOs at any time: two of these foetuses died in utero but no neonatal deaths occurred.

Table 1. Baseline patient characteristics associated with flares in the second and third trimesters

Maternal characteristics	Total (N=238)	Flare (N=35)	No flare (N=203)	*P* value
Age at pregnancy, mean ± SD	31.6 ± 4.5	30.9 ± 4.9	31.7 ± 4.4	0.37
Nulliparity	88 (37.0)	15 (42.9)	73 (36.0)	0.44
Skin colour (N=235)				
-White	166 (70.6)	25 (71.4)	141 (70.5)	
-Black	29 (12.3)	4 (11.4)	25 (12.5)	
-From Asia	17 (7.2)	2 (5.7)	15 (7.5)	0.99
-Other	23 (9.8)	4 (11.4)	19 (9.5)	
Overweight (BMI≥25 kg/m^2) (N=234)	71 (30.3)	7 (20.0)	64 (32.2)	0.17
Active smokers (N=233)	21 (9.0)	3 (8.6)	18 (9.1)	>0.99
Alcohol consumption (N=226)[§]	6 (2.7)	1 (2.9)	5 (2.6)	>0.99
Previous IUFD (N=237)	16 (6.8)	2 (5.9)	14 (6.9)	1.00
Previous thrombosis	41 (17.2)	5 (14.3)	36 (17.7)	0.81
Associated APS	34 (14.3)	5 (14.3)	29 (14.3)	>0.99
SLE duration, years, median (IQR)	7.2 (3.6-12.4)	7.7 (3.3-12.9)	7.2 (3.6-12.4)	0.92
Previous renal involvement	67 (28.2)	12 (34.3)	55 (27.1)	0.38
Laboratory characteristics				
Low platelets (<100×10^9/l)	3 (1.3)	1 (2.9)	2 (1.0)	0.38
24 h proteinuria>0.5 g/d (or >0.5 g/g)	9 (3.8)	2 (5.7)	7 (3.5)	0.62
Positive anti-dsDNA (N=222)	104 (46.9)	19 (55.9)	85 (45.2)	0.25
Hypocomplementemia (N=216)	57 (26.4)	15 (42.9)	42 (23.2)	**0.02**
At least one positive aPL (N=232)	61 (26.3)	9 (26.5)	52 (26.3)	>0.99
IgG/IgM anti-β2GPI (N=232)	26 (11.2)	4 (11.8)	22 (11.1)	>0.99
IgG/IgM aCL (N=232)	37 (16.0)	4 (11.8)	33 (16.7)	0.62
LAC (N=232)	41 (17.7)	6 (17.7)	35 (17.7)	>0.99
Triple positive aPL (N=232)	17 (7.3)	2 (5.9)	15 (7.6)	>0.99
SLE activity and damage				
PGA, median (IQR) (N=235)	0.1 (0-0.2)	0.1 (0-0.9)	0.1 (0-0.2)	0.65
SLEPDAI, median (IQR) (N=212)	2 (0-3)	2 (0-4)	2 (0-2)	0.06
SLICC-Damage Index, median (IQR) (N=236)	0 (0-0)	0 (0-0)	0 (0-0)	0.87
Clinical SLEPDAI=0	206 (86.6)	28 (80.0)	178 (87.7)	0.28
Remission (DORIA/Zen definition)	154 (64.7)	21 (60.0)	133 (65.5)	0.53
Remission (DORIS definition)	147 (61.8)	20 (57.1)	127 (62.6)	0.54
LLDAS (N=219)	157 (71.7)	25 (71.4)	132 (71.7)	0.97
Current treatment				
Prednisone	119 (50.0)	21 (60.0)	98 (48.3)	0.20
Prednisone mg/d, median (IQR) (N=119)	7 (5-10)	7 (5-10)	7 (5-10)	0.71
Immunosuppressive drugs*	56 (23.5)	12 (34.3)	44 (21.7)	0.10
Hydroxychloroquine**	234 (98.3)	34 (97.1)	200 (98.5)	0.47
Low-dose aspirin***	165 (69.3)	24 (68.6)	141 (69.5)	0.92
Low molecular weight heparin	61 (25.6)	8 (22.9)	53 (26.1)	0.68
Anti-hypertensive agents	5 (2.1)	1 (2.9)	4 (2.0)	0.55

Legends to Table 1: SD: standard deviation; BMI: body mass index; IUFD: intrauterine foetal death (>10 weeks); APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; IQR: interquartile range; g/d: grams per day; anti-dsDNA: anti-double stranded DNA; aPL: antiphospholipid; aCL: anti-

cardiolipin; anti-β2GPI: anti-beta2 Glycoprotein I; LAC: lupus anticoagulant; PGA: physician global assessment; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics; LLDAS: lupus low disease activity state.

§: at least 10 units per week.

***:** Immunosuppressive drugs: azathioprine (n=53, 22.3%) and tacrolimus (n=5, 2.1%); two women received both.

****:** All but four women (98.3%) took hydroxychloroquine; among those four, intolerance accounted for the lack of hydroxychloroquine treatment for two, retinopathy for one, and non-adherence for the fourth.

*****:** Low-dose aspirin was given to 165 women (69.3%). In particular, 52 of 67 patients (71.6%) with previous renal involvement and 56 of 61 patients (91.8%) with at least one positive aPL during pregnancy were treated with low-dose aspirin.

More details on skin colours are available in supplemental table S4.

Table 2. Baseline patient characteristics associated with APOs in the second and third trimesters

Maternal characteristics	Total (N=238)	APOs (N=34)	No APOs (N=204)	*P* value
Age at pregnancy, mean ± SD	31.6 ± 4.5	30.7 ± 4.8	31.7 ± 4.4	0.22
Nulliparity	88 (37.0)	11 (32.4)	77 (37.8)	0.55
Skin colour (N=235)				
- White	166 (70.6)	21 (61.8)	145 (72.1)	
- Black	29 (12.3)	7 (20.6)	22 (11.0)	
- From Asia	17 (7.2)	2 (5.9)	15 (7.5)	0.40
- Other	23 (9.8)	4 (11.8)	19 (9.5)	
Overweight (BMI≥25 kg/m^2) (N=234)	71 (30.3)	14 (41.2)	57 (28.5)	0.14
Active smokers (N=233)	21 (9.0)	5 (15.2)	16 (8.0)	0.19
Alcohol consumption (N=226)[§]	6 (2.7)	2 (6.3)	4 (2.1)	0.20
Previous IUFD (N=237)	16 (6.8)	5 (14.7)	11 (5.4)	0.06
Previous thrombosis	41 (17.2)	10 (29.4)	31 (15.2)	**0.04**
Associated APS	34 (14.3)	10 (29.4)	24 (11.8)	**0.01**
SLE duration, years, median (IQR)	7.2 (3.6-12.4)	10.0 (3.7-15.3)	7.0 (3.5-11.9)	0.13
Previous renal involvement	67 (28.2)	13 (38.2)	54 (26.5)	0.16
Laboratory characteristics				
Low platelets (<100 ×10^9/l)	3 (1.3)	0 (0.0)	3 (1.5)	>0.99
24 h proteinuria>0.5 g/d (or >0.5 g/g)	9 (3.8)	3 (8.8)	6 (2.9)	0.12
Positive anti-dsDNA (N=222)	104 (46.9)	21 (67.7)	83 (43.5)	**0.01**
Hypocomplementemia (N=216)	57 (26.4)	13 (40.6)	44 (23.9)	0.05
At least one positive aPL (N=232)	61 (26.3)	18 (52.9)	43 (21.7)	**<0.001**
IgG/IgM aCL (N=232)	37 (16.0)	9 (26.5)	28 (14.1)	0.08
IgG/IgM anti-β2GPI (N=232)	26 (11.2)	6 (17.7)	20 (10.1)	0.24
LAC (N=232)	41 (17.7)	15 (44.1)	26 (13.1)	**<0.001**
Triple positive aPL (N=232)	17 (7.3)	5 (14.7)	12 (6.1)	0.08
Disease activity and damage				
PGA, median (IQR) (N=235)	0.1 (0-0.2)	0.1 (0.0-0.4)	0.1 (0.0-0.2)	0.06
SLEPDAI, median (IQR) (N=212)	2 (0-3)	2 (2-4)	2 (0-2)	**0.01**
SLICC-Damage Index, median (IQR) (N=236)	0 (0-0)	0 (0-1)	0 (0-0)	**0.01**
Clinical SLEPDAI=0	206 (86.6)	28 (82.4)	178 (87.3)	0.42
Remission (DORIA/Zen definition)	154 (64.7)	17 (50.0)	137 (67.2)	0.05
Remission (DORIS definition)	147 (61.8)	17 (50.0)	130 (63.7)	0.13
LLDAS (N=219)	157 (71.7)	19 (57.6)	138 (74.2)	0.05
Current treatment				
Prednisone	119 (50.0)	23 (67.7)	96 (47.1)	**0.03**
Prednisone mg/d, median (IQR) (N=119)	7 (5-10)	7.5 (5-10)	7 (5-10)	0.13
Immunosuppressive drugs*	56 (23.5)	13 (38.2)	43 (21.1)	**0.03**
Hydroxychloroquine**	234 (98.3)	34 (100.0)	200 (98.0)	>0.99
Low-dose aspirin***	165 (69.3)	29 (85.3)	136 (66.7)	**0.03**
Low molecular weight heparin	61 (25.6)	15 (44.1)	46 (22.6)	**0.01**
Antihypertensive agents	5 (2.1)	2 (5.9)	3 (1.5)	0.15

Legends to Table 2: APOs: adverse pregnancy outcome; SD: standard deviation; BMI: body mass index; IUFD: intrauterine foetal death (>10 weeks); APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; IQR: interquartile range; g/d: grams per day; anti-dsDNA: anti-double stranded DNA; aPL: antiphospholipid; aCL: anti-cardiolipin; anti-β2GPI: anti-beta2 Glycoprotein I; LAC: lupus anticoagulant; PGA: physician global assessment; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics; LLDAS: lupus low disease activity state.

§: at least 10 units per week.

***:** Immunosuppressive drugs: azathioprine (n=53, 22.3%) and tacrolimus (n=5, 2.1%); two women received both.

****:** All but four women (98.3%) took hydroxychloroquine; among those four, intolerance accounted for the lack of hydroxychloroquine treatment for two, retinopathy for one, and non-adherence for the fourth.

*****:** Low-dose aspirin was given to 165 women (69.3%). In particular, 52 of 67 patients (71.6%) with previous renal involvement and 56 of 61 patients (91.8%) with at least one positive aPL during pregnancy were treated with low-dose aspirin.

More details on skin colours are available in supplemental table S4.

Table 3. Risk factors for APOs: multivariate analysis

Variables	Model 1§		
	Crude OR (95%CI)	**aOR (95%CI)**	***P* value**
Age at pregnancy	0.9 (0.9-1.0)	1.0 (0.9-1.1)	0.45
DORIA/Zen remission	0.5 (0.2-1.1)	0.5 (0.2-1.2)	0.11
SLICC-Damage Index (per 1-unit increase)	1.9 (1.2-3.0)	1.8 (1.1-2.9)	**0.02**
Positive LAC in the 1st trimester	5.2 (2.4-11.5)	4.2 (1.8-9.7)	**0.001**

Legends: *§: multivariate analysis performed on complete cases for the tested variables: N=230.* OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; LAC: lupus anticoagulant; SLICC: Systemic Lupus International Collaborating Clinics. Age at pregnancy was also forced into the analysis, as a known risk factor for APOs. We included LAC, and because of high collinearity, we excluded associated APS, at least one positive aPL test, and treatments such as low-dose aspirin and LMWH (as most women with APS or carrying aPL were treated with these drugs) from our regression models. Finally, we excluded prednisone dose, immunosuppressants, SLEPDAI, hypocomplementemia, and anti-dsDNA from both models, since both the DORIA/Zen definition of remission and LLDAS are composite scores that already include these factors.

Table 4: Risk factors for APOs: multivariate analysis

Variables	Model 2§		
	Crude OR (95%CI)	**aOR (95%CI)**	***P* value**
Age at pregnancy	0.9 (0.9-1.0)	1.0 (0.9-1.1)	0.45
LLDAS	0.5 (0.2-1.1)	0.5 (0.2-1.1)	0.07
SLICC-Damage Index (per 1-unit increase)	1.9 (1.2-3.0)	1.7 (1.1-2.8)	**0.03**
Positive LAC in the 1st trimester	5.2 (2.4-11.5)	3.7 (1.6-8.7)	**0.002**

Legends: *§: multivariate analysis performed on complete cases for tested variables: N=212.* OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; LLDAS: lupus low disease activity state; LAC: lupus anticoagulant; SLICC: Systemic Lupus International Collaborating Clinics. Age at pregnancy was also forced into the analysis, as a known risk factor for APOs. We included LAC, and because of high collinearity, we excluded associated APS, at least one positive aPL test, and treatments such as low-dose aspirin and LMWH (as most women with APS or carrying aPL were treated with these drugs) from our regression models. Finally, we excluded prednisone dose, immunosuppressants, SLEPDAI, hypocomplementemia, and anti-dsDNA from both models, since both the DORIA/Zen definition of remission and LLDAS are composite scores that already include these factors.

Table 5. Major differences between GR2 and PROMISSE [7] studies

	GR2	PROMISSE
Time frame	2014-2019	2003-2012
Exclusion criteria	Twin pregnancy	Twin pregnancy UPCR >1000 mg/g Creatinine level > 1.2 mg/dl Prednisone > 20 mg/d
Ethnicity (Black)	12.3%	20.3%
History of thrombosis	17.2%	8.1%
Positive LAC	17.7%	8.8%
At least one positive aPL	26.3%	12.5%
Previous renal involvement	28.2%	20.5%
Hydroxychloroquine exposure	98.3%	64.7%
Mean SLEPDAI at 1st trimester	1.96	2.79

Legends: UPCR: urinary protein creatinine ratio; LAC: lupus anticoagulant; aPL: anti-phospholipid; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index.

5.4 PAPER IV

Patients' baseline features. The study included 79 pregnancies occurred in 79 women. Their demographic, clinical, and serological features are summarized in **Table 1.** Twin pregnancy occurred only in one patient.

We found an APS diagnosed before the index pregnancy in 7 women (8.9%), whereas SLE occurred in 9 (11.4%); 3 women (3,8%) were affected with both SLE and APS. Overall, the matched control group included 66 (83.6%) women (detailed in Supplementary Material).

Positive LAC test at first trimester occurred in 11 (13.9%) women, whereas triple positive aPL in 6 (7.6%).

Regarding patients' treatment, LDA was received by 44 of women (55.7%), LMWH by 15 (19.0%) and prednisone treatment by 9 (11.4%) at a median dose of 5 mg/day (interquartile range-IQR 2.5-7.5 mg/day). Other concomitant treatment included immunosuppressants, and intravenous (iv) immunoglobulins (Ig), and biologics in 3, 3 and 2 patients, respectively (3.8% and 2.5%). Finally antimalarials were administered in 21 patients (26.6%).

Pregnancy outcome. A total of 69 (90.7%, 3 missing data) live births occurred; rarely, 6 (7.9%) miscarriages and 1 IUFD during the only twin pregnancy (1.3%) were observed (Table 2). No foetal and/or maternal deaths were observed. As for maternal complications, GDM was found in 12 women (15.2%), IUGR in 4 (5.1%), PPROM in 2 (2.7%, 4 missing data), and preterm delivery in 12 women (15.4%, 1 missing data). Finally SLE flares occurred in 3 patients (27.3% of SLE patients). Gestational hypertension occurred in 3 women (3.8%) and no Preeclampsia or HELLP syndrome

occurred. Notably, no statistical differences were found between SLE/APS and the control group concerning pregnancy outcome (Table 3).

PTX and anti-PTX Abs. Serum IgG anti-PTX3 Abs were found in 11 (13.9%, 95% CI 7.2-23.5) women. At univariate analysis the frequency of these Abs was slightly different between SLE and/or APS patients and controls (p=0.08, 4 SLE/APS women vs. 7 controls), whereas PTX3 serum levels did not statistically differ between SLE/APS and controls (p=0.63, 0.37 $\pm$ 0.26 ng/mL in SLE/APS women vs. 0.33 $\pm$ 0.24 ng/mL controls). In addition, anti-PTX3 were slightly associated with GDM (p=0.06, Table 4) even when stratifying with age at conception (p=0.042); finally anti-PTX3 were also associated with IUGR (p=0.09,Table 4); conversely, no associations were found with the other maternal/foetal complications (Table 4). Notably, PTX3 serum levels were found lower in women who developed GDM (0.3 $\pm$ 0.2 in GDM compared to 0.4 $\pm$ 0.2 in non-GDM women) , although no statistical association was found. Finally PTX3 levels (ng/mL) were not different among women who developed IUGR and without IUGR, although the mean PTX3 $\pm$ SD was found lower in the IUGR group (0.25 $\pm$ 0.2 in women with IUGR vs 0.35 $\pm$ 0.2 in women without IUGR).

In a sub-analysis on 32 patients only, by analysing those women with at least 2 different PTX3 serum determination in at least 2 different trimesters. As results, we observed an increasing trend in 22 (68.8%) patients, a decreasing in 7 (21.9%), and no variations in 3 (9.4%) women **(Table 5).**

Table 1. Clinical and serological characteristics of out cohort (N=79)

	Patients (N, %)
Age at pregnancy, years (mean ± SD)	35.1 ± 3.9
SLE and/or APS	13 (16.5)
SLE	9 (11.4)
APS	8 (10.1)
Control Group*	66 (83.5)
Serological features	
LAC	11 (13.9)
IgG/IgM anti-cardiolipin	20 (25.3)
IgG/IgM antibeta2-Glycoprotein I	25 (31.6)
Triple positive aPL tests	6 (7.6)
Primiparous	23 (29.5)
IgG anti-PTX3 Abs	11 (13.9)
IgG anti-PTX3 level (cut-off 0,234 OD), (median, IQR)	0.2 (0.1-0.2)
PTX3 ng/mL (mean ± SD)	0.3 ± 0.2
Concomitant treatment	
Heparin	15 (19.0)
Aspirin	44 (55.7)
Immunosuppressants	3 (3.8)
Hydroxychloroquine	21 (26.6)
Prednisone	9 (11.4)
Prednisone dosage (mg/day) (median, IQR)	5 (2.5-7.5)

Legend to Table 1: SD: standard deviation; SLE: Systemic Lupus Erythematosus; APS: antiphospholipid syndrome; LAC: Lupus anticoagulant; aPL: antiphospholipid antibodies; PTX: Pentraxin-3; Abs: antibodies; OD: optical density; IQR: interquartile range.

Table 2. Pregnancy outcomes of our cohort (N=79)	
	N (%)
Live Births (N=76)	69 (90.7)
Maternal complications	
SLE flares	3 (27.3)
PE/HELLP	0 (0.0)
Gestational diabetes	10 (14.1)
Gestational hypertension	3 (3.8)
Death	0.(0.0)
Foetal complications	
Miscarriage	6 (7.9)
IUFD	1 (1.3)
IUGR	4 (5.1)
Preterm delivery (N=78)	12 (15.4)
P-PROM (N=75)	2 (2.7)
Death	0 (0.0)

Legend to Table 2: SLE: Systemic lupus erythematosus, PE: preeclampsia; HELLP :Haemolysis, elevated Liver Enzymes, Low Platelets syndrome; IUFD: intrauterine fetal death; IUGR: intrauterine growth restriction; P-PROM: premature rupture of membranes before 37 weeks.

Table 3. Pregnancy outcome: Differences between SLE and/or APS and controls			
	SLE and/or APS	**Control group***	***P value***
	N=13	**N=66**	
Live births (N=76)	12 (92.3)	57 (86.4)	>0.99
Gestational Hypertension	1 (7.7)	2 (3.0)	0.42
Gestational Diabetes	1 (7.7)	11 (16.7)	0.68
Miscarriage	1 (7.7)	5 (7.6)	>0.99
IUFD	0 (0.0)	1 (1.5)	>0.99
Pre-term delivery (N=68)	3 (25.0)	9 (16.1)	0.43
IUGR (N=78)	1 (7.7)	3 (10.8)	0.53
P-PROM (N=75)	0 (0.0)	2 (3.2)	>0.99

Legend to Table 3: *: healthy subjects and aPL negative patients affected with other rheumatic diseases (nor SLE and/or APS); SLE: Systemic Lupus Erythematosus; APS: anti-phospholipid syndrome; aPL: anti-phospholipid antibodies; IUFD: Intrauterine foetal death; IUGR: intrauterine growth restriction, P-PROM: premature rupture of membranes.

Table 4. Univariate analysis: Association between anti-PTX3 and maternal/foetal complications			
	Positive anti-PTX3	**Negative anti-PTX3**	***P value***
	N=11	**N=68**	
Live births (N=76)	10 (90.9)	58 (89.2)	>0.99
Gestational Hypertension	0 (0.0)	3 (4.4)	>0.99
Gestational Diabetes	4 (36.4)	8 (11.8)	***0.06***
Miscarriage	1 (9.1)	5 (7.4)	>0.99
IUFD	0 (0.0)	1 (1.5)	>0.99
Pre-term delivery (N=68)	2 (22.2)	10 (16.9)	0.65
IUGR (N=78)	2 (18.2)	2 (3.0)	***0.09***
P-PROM (N=75)	1 (10.0)	1 (1.5)	0.25

Legend to Table 4: PTX3: Pentraxin-3; IUFD: Intra-uterine foetal death; IUGR: intrauterine growth restriction, P-PROM: premature rupture of membranes before 37 weeks.

Table 5. Variations of PTX3 in 32 (40.5%) women during pregnancy	
	Patients (N, %)
Increasing trend	22 (68.8)
Increasing trend (≥ 50% of basal value)	8 (25.0)
Increasing trend (< 50% of basal value)	14 (43.8)
Decreasing trend	7 (21.9)
Unchanged trend	3 (9.4)

6 DISCUSSION OF RESULTS

6.1 PAPER I

We describe the largest series of 40 women with severe PE and confirmed APS. The median time of onset of the severe preeclampsia was 25.5 weeks of gestation, which explains the poor prognosis of these pregnancies: 11 IUFDs, 15 neonatal deaths, and only 14 surviving premature children, born at a median term of 31 weeks. Maternal complications were also frequent: 18 HELLP syndrome, 6 eclampsia, 3 placental abruption, and 3 CAPS. No maternal death was observed.

Severe PE led to the diagnosis of APS in 70% of cases and occurred during their first pregnancy in half the women; this timing explains why the majority did not receive the recommended treatment for APS before the index episode [4] The prognosis for subsequent pregnancies was much better, even though maternal and foetal complications still occurred. All women received LDA then, and 89.2% of patients received therapeutic or prophylactic doses of LMWH, as recommended by the EULAR recommendations [4,81].

Tests for aPL Abs showed that most women had positive LAC (82.5%) and more than half had triple positive aPL tests (52.5%). These results are very similar to other reports including our previous study of 65 APS patients with IUFD (72% were LAC-positive, and 35% triple-positive) [90]. In the PROMISSE study of 385 SLE patients, LAC was associated with adverse pregnancy outcomes (OR 8.32, 95% CI 3.95-19.26) [7] and it is not surprising that we found a high rate of LAC compared to that observed in unselected APS patients [101]. At the end of the follow-up period, 30% of the women had been diagnosed with SLE, a rate quite similar to other APS series [90,102] where SLE occurred in 29% to 36% of cases.

Because the obstetric APS classification criteria have not been studied in detail, we examined the overlap between the different criteria. Our finding that over the entire follow-up (median 5 years, IQR 2-8), 17 women (42.5%) had at least one IUFD suggests a substantial overlap between severe preeclampsia and IUFD. Other features of placental insufficiency were also observed in 19 women (47.5%), who had at least one episode of HELLP syndrome, while IUGR was reported in 21. Moreover, 16 patients (40%) experienced at least one thrombotic event, most before the index pregnancy and 4 women experienced CAPS, concurrent with preeclampsia in 3 cases.

By contrast, the APS criterion of “three consecutive miscarriages” was not observed in any of our patients, results similar to those reported in our retrospective series of IUFD: among 65 women with APS and IUFD, only one had 3 consecutive miscarriages [90]. These results might be due to the various immunological pathways and phenotypes of APS, as Bramham et al. [103] proposed, suggesting that patients be subdivided into three groups: recurrent miscarriage, late foetal loss, or early delivery due to placental dysfunction before 32 weeks' gestation and thrombotic APS [103]. While thrombosis and foetal loss seem to overlap, the apparent difference of the “recurrent miscarriage” criterion calls into question the relevance of this specific form of APS.

Our study has limitations, first and foremost, its retrospective nature. A potential consequence of this is the possibility that not all obstetrics departments in our centres looked for APS in all patients with early preeclampsia, so that this diagnosis might have been missed in some women. In addition, some immunological features were not assessed and the obstetric care of these women with APS was not standardized, as shown by the fact that 4 further pregnancies in 3 patients were not treated with LMWH.

Finally, we cannot exclude the possibility that patients might have failed to observe early miscarriages and thus not reported them. In any case, to our knowledge, despite its small number of patients, it is nonetheless the largest cohort focusing on severe PE in patients with APS, and it provides valuable information on APS phenotype in such patients.

6.2 PAPER II

In this study of 158 pregnant women affected with SLE we analysed disease activity in the 1st trimester assessed by SLEPDAI and SLE-DAS. Although 59 (37.3%) patients had had a previous renal involvement, SLE activity during the1st trimester was mild: median (IQR) SLEPDAI of 2 (0-4) and median (IQR) SLE-DAS of 1.32 (0.37-2.08). Even though remission/LLDAS state cut-off definitions in pregnant patients have not been validated yet, the low scores observed in disease activity indexes in our study, suggest that most patients were not active in the first trimester.

Twenty-five (15.8%) women experienced at least one flare in the 2nd and 3rd trimester. Among them, the majority of our patients had mild/moderate flares (n=23, 14.3%), and only 2 women (1.3%) developed severe flares. This is in line with the PROMISSE study which reported mild/moderate flares occurred in 22.3% and severe flares in 5.5% of SLE patients during 385 prospective pregnancies [7].

APOs occurred in 12% of patients, which is also in keeping with the PROMISSE study where these complications occurred in 19% of cases [7]. Finally, a high rate of live births was found (96.8%), probably due to the the high rate of LDAS/remission state observed in the first trimester of pregnancy in our study. Hence, our results strengthen that remission or LDAS in the early pregnancy is important to avoid foetal complications during pregnancies [4].

A high correlation between SLE-DAS and SLEPDAI in the 1st trimester was observed in our cohort (ρ=0.97, p<0.001). This is in line with previous findings [72] who reported a high correlation between the SLE-DAS and SLEDAI-2K. SLEPDAI [71] is a non-validated score adapted to pregnancy that includes the 24 items from the SELENA-SLEDAI with modifications of 15 of them, to avoid wrongly attribution of some

physiological or pathological pregnancy changes to SLE [7,104]. Even if SLEDAI-2K and SELENA-SLEDAI are slightly different, correlation between SLE-DAS and both scores was therefore expected.

In multivariate analyses, both SLE-DAS and SLEPDAI at first trimester of pregnancy were independent predictors of SLE flares in the 2nd and 3rd trimester (adjOR, 95% CI=1.2 (1.0-1.3); adjOR 95% CI =1.3 (1.1-1.6). In addition, performance of both scores was assessed by AUC and Goodness-of-fit analysis which proved that SLE-DAS model performs slightly better than SLEPDAI model (Table 4). Thus, the high correlation between SLE-DAS and SLEPDAI, the predictive value of SLE-DAS for lupus flares, as well as the aforementioned performance models' tests prove that SLE-DAS is an appropriate instrument for monitoring SLE disease activity during pregnancy.

Both SLE-DAS and SLEPDAI were associated with APOs at univariate analysis (p=0.02), but since anti-dsDNA is already included in both scores, no multivariate analysis could be done. Although our patients had mainly inactive mild SLE, our results suggest that active SLE may still be associated with poor obstetrical prognosis. Such association between disease activity and APOs has been already observed in elderly cohorts in which patients had a more active SLE [105]. These findings has led to current EULAR recommendations which pinpoint the importance of a stable mild/inactive disease when pregnancy is planned [4].

Our study has some limitations. First, we applied SLE-DAS retrospectively. This was unavoidable since SLE-DAS was validated in 2019 [72]. Second, in keeping with current recommendations [4], most pregnancies occurred in patients in clinical remission or LDAS, therefore limiting the possibility to demonstrate a stronger

association between high disease activity and APOs. The strengths of this study also need to be remarked: first, this a quite large multicentric cohort from 2 referral centers in SLE; second, we acknowledged the role of SLE-DAS as a very simple tool to define disease activity in pregnant patients.

6.3 PAPER III

After the large North American PROMISSE study, where 385 women with SLE were prospectively included between 2003 and 2012, we report the second largest prospective study carried out on 238 pregnant women with SLE included between 2014 and 2019. Overall, we found that flares, especially severe ones, were uncommon and did not influence pregnancy outcomes. APOs were also rare (14.3%) and mainly associated with positive LAC and damage accrual.

In contrast to the PROMISSE study [7], which aimed to identify risk factors for and mechanisms of APOs specifically attributable to SLE and/or aPL, and because we wanted a sample closer to real-life practice, we did not apply any of the following exclusion criteria: prednisone>20 mg/day, urinary protein-creatinine ratio>1000 mg/g, erythrocyte casts on urine analysis, serum creatinine level>1.2 mg/dl, diabetes mellitus, or hypertension [7]. Apart from inclusion/exclusion criteria, several aspects distinguish the populations of the two studies: their genetic background, with 12.3% of Blacks in our study vs 20.3% in PROMISSE, and the rate of overweight women (30.3% vs 39.7%, respectively). The frequency of several baseline characteristics, which are well-known risk factors for APOs, was similar or slightly higher in our cohort than in PROMISSE: previous biopsy-proven LN (26.1% vs 20.5%), positive LAC (17.7% vs 8.8%), at least one positive aPL test (26.3% vs 12.5%), and a history of thrombosis (17.2% vs 8.1%) (the number of patients with APS in the PROMISSE study is not available for comparison). However, SLE was probably better controlled in our study: fewer patients had hypocomplementemia (26.4% vs 34.0%) and their disease activity was lower (mean SLEPDAI=1.96 vs 2.79). This latter difference may be due to the higher percentage of our patients on hydroxychloroquine (98.3% vs 64.7%) as well as

to the routine monitoring of hydroxychloroquine levels in France, which leads to a better treatment adherence [106]. Finally, besides the difference in hydroxychloroquine exposure, we had more patients on low-dose aspirin (69.3% vs 35.1%). The publication of the PROMISSE study in 2015 before the current recommendations [4,75] may explain this difference (Table 5).

Importantly, 71.6% of our patients with previous renal involvement and 91.8% of those with at least one positive aPL received low-dose aspirin. This finding might explain the lower rate of APOs in our cohort, and confirms the good application of current guidelines [4,75].

Severe maternal flares occurred in three women (1.2%) in our study: among them only one woman gave birth preterm, due to placental insufficiency at 28 weeks (with preeclampsia/eclampsia and HELLP syndrome); by contrast, both women with severe renal flares gave birth to healthy children at term. In the multicentre PROMISSE study [7], 5.5% patients had severe flares, even though patients with severe disease at conception were excluded. As we did not exclude such women in our study, a higher rate of flares (mild/moderate and severe) might theoretically be expected. Nevertheless, more than 60% of our patients were in remission/LLDAS in the first trimester, possibly because nearly all of our patients were on hydroxychloroquine, as recently recommended [4,107]. Antimalarials have been widely demonstrated to mitigate the risk of flares both during pregnancy and in the postpartum period [4,108]. A recent retrospective study of 398 pregnancies in 304 patients reported a higher flare rate during pregnancy (HR: 1.59; 95%CI, 1.27–1.96), but this was no longer true for patients on hydroxychloroquine: the HR for flares during pregnancy compared with non-pregnant/non-postpartum periods was 1.83 (95%CI: 1.34–2.45) in patients not

treated with hydroxychloroquine vs 1.26 (95% CI: 0.88–1.69) in those who were on hydroxychloroquine [108].

Maternal flares were associated with hypocomplementemia (P=0.02), consistently with previous reports [7,109]. Notably, flares during the second and third trimesters were not associated with APOs (P>0.99), in contrast to older cohorts and the PROMISSE study [7,20]. The discrepancies between our study and prior cohorts are probably due to the low rate of patients with severely active SLE in our study, which likely prevented us from finding an association between disease activity and APOs. This difference may be due also to the improvement in the management of SLE; both physicians and patients now understand the importance of achieving remission/LLDAS before conception as well as of maintaining hydroxychloroquine during pregnancy.

We evaluated three definitions of remission and found no substantial differences between them in terms of association with maternal flares or APOs. This could be due to the high frequency of patients on remission in the first trimester and consequently, to a lack of power. It may also be explained by the fact that the definitions of remission that were assessed are indeed relatively close and partially use the same variables. Hence, analyses of wider cohorts are needed to test each remission sub-class during pregnancy, including those with serologically active but clinical quiescent disease.

Overall, 230 (96.6%) women had liveborn infants who survived to discharge. APOs were observed in 14.3% of women, whereas they occurred in 19% of patients in the PROMISSE study. This difference might be due to the different definition of SGA (below the third percentile in our cohort vs. the fifth in PROMISSE) and the high proportion of patients treated with aspirin (69.3% vs 35.1%).

In our study, LAC and damage accrual predicted APOs. The PROMISSE study [7] had previously shown that LAC is a predictor of APOs, pinpointing that the risk of pregnancy complication in women with SLE is due to aPL antibodies more than SLE itself. In addition, we demonstrated for the first time that damage accrual is associated with APOs. Analysis of patients with damage (n=30, supplementary table S3) showed diverse irreversible damage, driven both by disease activity and glucocorticoid treatment, but also aPL status and/or associated APS. This finding suggests that damage should be considered in preconception counselling and in early pregnancy. It also reinforces the importance of achieving remission/LLDAS to prevent the accrual of additional damage [110–112].

In contrast to PROMISSE [7], we did not find any significant association between APOs and active disease or ethnicity. This findings may be due to different health-care systems and socioeconomic status of patients included in both cohorts [7].

Our study has some limitations. First, the assessment of aPL/anti-dsDNA antibodies was not centralized as in the PROMISSE study due to the real-life design of our study and the large number of centres. This limitation is at least partially offset by the fact that all laboratories in France require regular accreditation. The exact impact of disease activity in the first trimester could not be assessed since patients had to have an ongoing pregnancy at 12 weeks to be included, and it could be hypothesized that some active patients were excluded because their pregnancies ended spontaneously during the first trimester. This limitation also applies to the PROMISSE study as we chose to have a similar design to enable comparison.

In conclusion, we confirmed that positive LAC predicts APOs and observed for the first time that chronic irreversible damage in the first trimester also predicts APOs.

Neither remission nor LLDAS appeared to influence APOs in this cohort of women with stable, well-controlled SLE treated with hydroxychloroquine. These results should be helpful to physicians caring for pregnant women with SLE.

6.4 **PAPER IV**

In this study, we aimed to assess anti-PTX3 and PTX3 levels as predictors of maternal/foetal complications in a cohort of pregnant SLE and/or APS patients. Overall, the frequency of anti-PTX Abs was quite low (13.9%). Notably, these Abs slightly differed between SLE/APS and the control group (p=0.08), as the majority of anti-PTX3 occurred in controls (7 vs 4 SLE/APS women).

This result is quite interesting from different points of view, as observations on anti-PTX3 Abs in SLE and/or APS patients are scanty to date [113].

Recent studies assessed anti-PTX3 Abs both in mice and human models: Gatto et al. [32] reported that these may delay lupus-like nephritis and prolong survival of New Zealand Black/White (NZB/NZW F1) mice, by dampening complement activation via their Fc fragment, likely hindering renal inflammation [32]; similar results were also found in human models [114], where Bassi et al. [99] showed that anti-PTX3 Abs to be significantly prevalent in SLE patients compared to controls affected with other autoimmune rheumatic diseases. Notably, the same Authors found that these Abs may also exert a protective role from renal involvement [100]. For the first time we assessed anti-PTX3 Abs during pregnancy, finding a weak association between SLE/APS women, probably due to the small sample size of this cohort.

At univariate analysis, anti-PTX3 Abs resulted associated with GDM (p=0.06, Table 4) even when adjusting with age at conception (p=0.042).

GDM refers to abnormal glucose metabolism which can occur during pregnancy. This condition can increase the risk of type 2 diabetes mellitus and cardiovascular diseases in pregnant women [87]. Notably, there is an acute phase inflammatory response in women with hyperglycaemia and PTX3 levels, but not anti-PTX3 Abs, may provide a

useful approach for early prediction of GDM [99]. However, contrasting evidence on such topic has been published to date.

Indeed, PTX3 levels were lower in the GDM than the non-GDM group, which is in line with a recent study conducted by Lekva et al. [77]. In this cohort, PTX3 levels resulted lower early in pregnancy and at 5-year follow-up in GDM women compared to non-GDM women; moreover, PTX3 was consistently negatively correlated with BMI during pregnancy, and it was associated with multiple metabolic risk factors for cardiovascular disease at 5-year follow-up [78].

Conversely, other evidences were unlikely to confirm these data [79]: serum PTX3 in early pregnancy predicted GDM later in pregnancy [116] and PTX3 levels along with hypersensitive C reactive protein (CRP) were shown to increase in the second trimester in GDM group, compared to non-GDM [115]. However, no data on PTX3 and anti-PTX3 simultaneously are reported to date, and more importantly, no SLE/APS patients were enrolled in such studies [115–117].

In our cohort, a slightly association between anti-PTX3 abs and IUGR was also found ($p=0.09$). As it occurred for GDM, PTX3 levels were found lower in patients with IUGR compared to patients who did not develop any IUGR, although a statistical difference was not shown.

PTX3 is recognized to be implicated in endothelial disfunction; thus, several Authors tried to assess the role of PTX3 levels in patients with PE and IUGR [99]. Indeed, Cetin et al. [87] observed higher levels of PTX3 in PE (median values 13.8 versus 2.2 ng/mL; $P<0.001$), compared with normal pregnancies; in addition IUGR pregnancies showed intermediate levels between normal and preeclamptic patients, but this difference was not significant, compared with normal pregnancies (median

values 3.9 versus 2.2 ng/mL). In another study [120], PE and IUGR pregnancies were confirmed to have higher maternal PTX3 levels compared to normal pregnancies, with IUGR significantly lower than PE. Besides, IUGR fetuses had higher PTX3 values than controls and the increase was related to IUGR severity, likely reflecting the hypoxic environment [119]. These findings are very interesting, but again, need to be confirmed along with anti-PTX3 Abs, especially in SLE/APS patients. Of note, no women in this cohort developed any PE or HELLP; therefore we could not derive any conclusion on PTX3/anti-PTX3 and such hypertensive disorders in pregnant women.

As for PTX3 levels measured in two different trimesters, we found an increasing trend in the majority of our patients (68.8%), whereas 21.9% patients showed a decreasing trend, and no variations were found in 9.4% of cases. These results are in line with previous literature reports [84] who showed a continuous increase of serum PTX3 as pregnancy progressed. Notably, the increase was most evident after week 31 with the highest levels just before delivery [84].

Our study has different limitations. Firstly, the monocentric nature of this cohort. Secondly, due to the available data, we did not adjust or analyses by BMI and obesity, which can influence GDM and other pregnancy complications.

Importantly, our cohort has also several strengths. Indeed, this is the first effort assessing anti-PTX3 Abs in a cohort of pregnant women, where, remarkably, we chose to include APS and/or SLE patients which have an increased risk of developing hypertensive disorders and pregnancy complications; finally, we also assessed PTX3 variations per trimesters; nonetheless, due to our limited sample size, these findings have to be proved in wider research settings.

In conclusion, anti-PTX3 Abs at first trimester of pregnancy occurred in 13.9% of patients. Notably, the frequency of such Abs did not differ between controls and patients affected with SLE/APS. As expected, in a sub-analysis of PTX3 variations during pregnancy the majority of pregnancies had an increasing trend during gestation of PTX3 serum levels.

7. CONCLUSIONS, IMPLICATIONS FOR CLINICAL PRACTICE AND RECOMMENDATIONS FOR FUTURE RESEARCH

This PhD thesis evaluated the predictive role of anti-PTX3 Abs and PTX3 serum levels during pregnancy in SLE/APS and aPL women (these latter enrolled in the control group). Before analysing this topic, we firstly focused on important clinical challenging factors in SLE and APS pregnancies.

Firstly, we analysed severe PE in a multicentric cohort of APS women. Our study showed that this complication occurred very early in gestation and with a high offspring mortality (65%). In addition, among the 40 APS patients who experienced severe PE, almost half also underwent at least one episode of thrombosis, HELLP and/or IUFD. However, no woman met the “three consecutive miscarriages” APS classification criterion, suggesting that the underlying physiopathology of these two obstetric phenotypes might be different.

By evaluating other maternal complications (i.e. SLE flares) we tested SLE-DAS as a potential tool for measuring disease activity. As mentioned before, measuring SLE activity in pregnancy is still challenging, mainly due to physiological changes during gestation. Altough several disease scores have been already proposed to evaluate SLE activity in pregnancy, very few of them were validated. In our study, SLE-DAS resulted a simple tool to assess SLE in pregnancy, and consequently, it may be a useful instrument to predict maternal flares in the 2nd and 3rd trimester.

By analysing clinical predictors of APOs and maternal flares in SLE women, we confirmed that positive LAC at first trimester predicts APOs, in line with previous studies [99]. Notably, for the first time we also found that chronic irreversible damage related to SLE also predicts APOs, suggesting that clinicians should take

it into account in preconception counselling or early gestation, even in patients with a very stable, well controlled SLE.

Finally, when assessing PTX3 and anti-PXT3 Abs as predictors of pregnancy complications, we found that anti-PTX3 did not differ between SLE/APS and the control group (including aPL carriers). However, these Abs resulted slightly associated with GDM and IUGR. Besides, PTX3 tends to increase during pregnancy, as already describe in current scientific literature.

Future research should confirm whether anti-PTX3 are associated with GDM and IUGR in larger cohorts; secondly further studies are needed to evaluate new predictive biomarkers of pregnancy complications in SLE/APS women,

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9. SUPPLEMENTARY MATERIAL

9.1 PAPER I

- Hemolysis, elevated liver enzymes, Low Platelets (HELLP) syndrome was defined as aspartate amino transferase>2 fold the normal, platelet count <100.000/μL, and lactate dehydrogenase >600 U/L [100];
- Premature birth was defined as a live birth before 37 WG;
- Intra-uterine growth retardation (IUGR) was defined as an estimated fetal weight less than the 10^{th} percentile for gestational age [100],
- Neonatal death was defined as a death within 28 days from birth.

9.2 PAPER II

Definition of associated maternal complications:

- Preeclampsia (PE) was defined by the presence of systolic blood pressure (BP) ≥ 140 mmHg or a diastolic BP ≥ 90 mmHg, after 20 weeks in a previously normotensive patient with a proteinuria of 0.3 g or higher in a 24-hours urine specimen [87];
- Haemolysis Elevated Liver Enzymes Low Platelets (HELLP) syndrome was defined as aspartate amino transferase>2 fold the normal, platelet count <100.000/μL, and lactate dehydrogenase >600 U/L [99].

9.3 PAPER III

Supplementary Text S1

Type of assays used to test for antibodies:

- Anti-dsDNA antibodies (abs) were tested by enzyme-linked immunoassays (ELISA), *Crithidia luciliae* immunofluorescence tests and/or FARR-RIA test.
- Anti-cardiolipin (aCL) antibodies IgG/IgM were tested by ELISA and considered positive when they exceeded the 99th percentile of the laboratory's control values or when they exceeded 40 IgG or IgM phospholipid units (GPL or MPL).
- Anti-ß$_2$GPI antibodies were tested by ELISA and we used the upper reference limits supplied by the laboratory performing the test
- The Lupus anticoagulant (LAC) was measured according to the international recommendations of the International Society of Thrombosis and Hemostasis (required to obtain the COFRAC accreditation): in most patients by activated partial thromboplastin time (APTT) and dilute Russell's viper venom time (dRVVT) and occasionally with the dilute prothrombin time and kaolin clotting time.

Supplementary Text S2

Definition of remission/LLDAS

- DORIA/Zen definition: patients were considered in remission if they met the criteria for clinical remission (SLEDAI-2K=0), with a prednisone dosage ≤ 5 mg/day; antimalarial and immunosuppressive drugs were allowed [77];
- DORIS definition: patients were considered in remission if they met the criteria on clinical remission (SLEDAI-2K=0), with a prednisone dosage ≤ 5 mg/day; PGA < 0.5; maintenance immunosuppressive drugs and/or biological agents were allowed [78] ;
- LLDAS: SLEDAI-2K ≤ 4, prednisone dosage ≤ 7.5 mg/day; PGA ≤ 1; no new features of lupus disease activity compared with previous visit; well-tolerated standard maintenance doses of immunosuppressive drugs and approved biological agents, excluding investigational drugs [79].

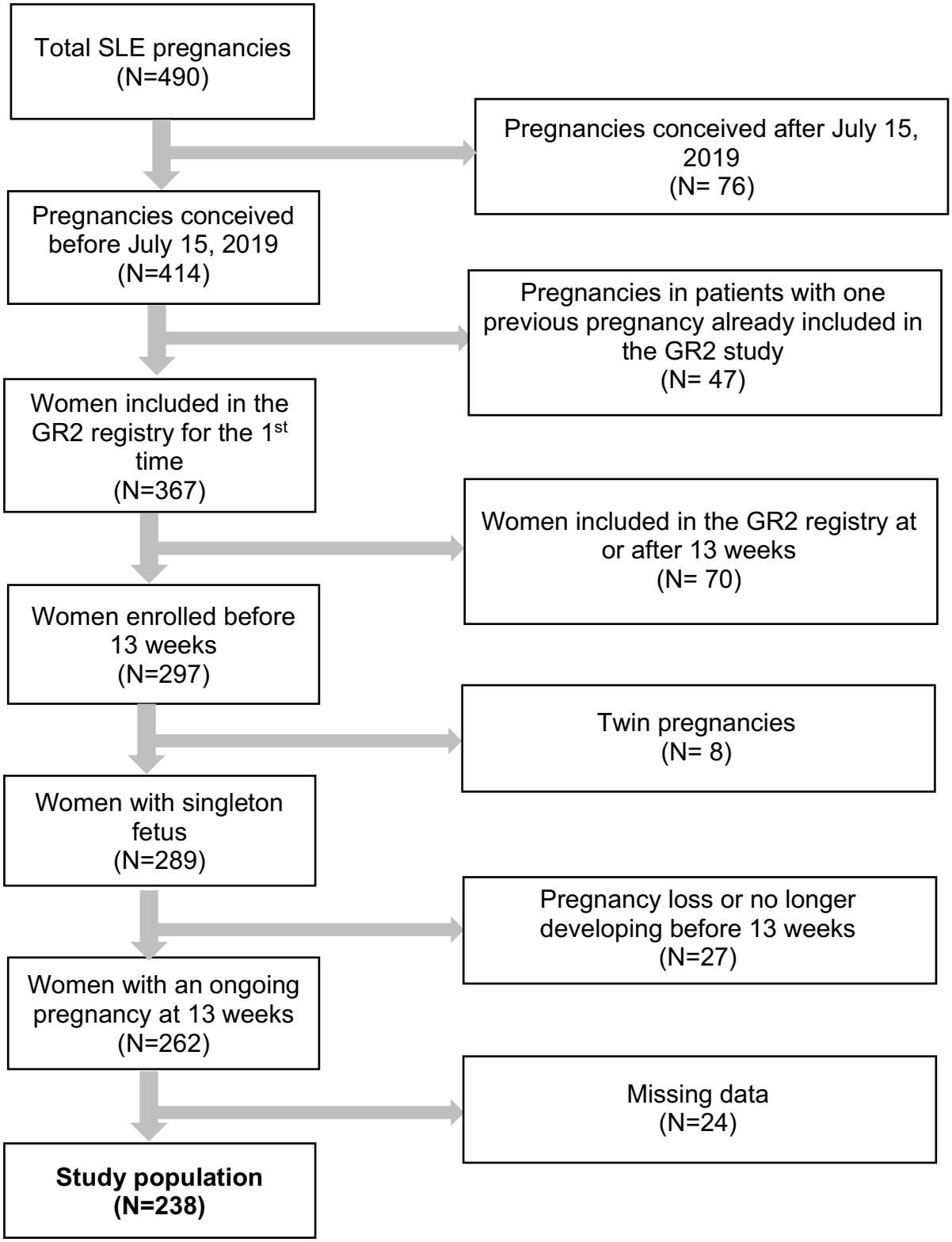
Supplementary Text S3

Definition of maternal complications

- HELLP syndrome was defined by the presence of all three of the following criteria: peripheral blood smear and serum lactate dehydrogenase levels ≥600 U/L), serum aspartate aminotransferase levels ≥70 U/l, and platelet count <100×10^9/l [99];
- Preeclampsia was defined by hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) and proteinuria ≥0.3 g/24 h [87];

- Fetal growth restriction and preeclampsia or eclampsia were defined according to the last guidelines issued by the International Federation of Gynaecology and Obstetrics (FIGO) [120].

Supplementary Figure S1: Study flow chart



Supplementary Table S1. List of 34 centres with patients included in this study

Name of Centre	N	%
Angers-CHU d'Angers - Hôtel Dieu	3	1.26
Bois-Guillaume-CHU de Rouen - Bois Guillaume	5	2.10
Bordeaux-CHU de Bordeaux - Pellegrin	1	0.42
Caen-CHU de Caen - Côte de Nacre	1	0.42
Chambray-les-Tours-CHRU de Tours - Trousseau	1	0.42
Clermont-Ferrand-CHU de Clermont-Ferrand - Gabriel Montpied	1	0.42
Cochin - Médecine Interne	**109**	**45.80**
Créteil-APHP - Henri Mondor	7	2.94
Dijon-CHRU de Dijon - Bocage	2	0.84
Grenoble Michallon - Médecine Interne	7	2.94
Le Mans-CH du Mans	1	0.42
Lille-CHRU de Lille - Claude Huriez	4	1.68
Lyon-CH Saint Joseph - Saint Luc	3	1.26
Marseille-APHM - Conception	3	1.26
Marseille-Hôpital Saint Joseph	1	0.42
Metz-Hôpital Sainte Blandine	4	1.68
Montivilliers-CH du Havre - Jacques Monod	1	0.42
Nice-CHU de Nice - Archet 1	6	2.52
Paris-APHP - Bichat	5	2.10
Paris-APHP - Saint Antoine	10	4.20
Paris-APHP - Saint Louis	1	0.42
Perpignan-Hôpital Saint Jean	1	0.42
Pessac-CHU de Bordeaux - Haut Lévéque	13	5.46
Pierre-Bénite-HC de Lyon - Sud	4	1.68
Poitiers-CHU de Poitiers - Milétrie	6	2.52
Pringy-CHR d'Annecy	9	3.78
Reims-CHU de Reims - Robert Debré	8	3.36
Rennes-CHU de Rennes - Sud	2	0.84
Saint Paul -Hôpital Gabriel Martin	1	0.42
Saint-Denis-de-la-Réunion-CHD Felix Guyon	2	0.84

Saint-Priest-en-Jarez-CHU de Saint Etienne - Nord	1	0.42
Strasbourg-CHRU de Strasbourg - Civil	1	0.42
Toulouse-CHRU de Toulouse - Purpan	6	2.52
Tours-CHRU de Tours - Bretonneau	8	3.36
Total	**238**	**100.00**

Since the patients were homogeneously distributed among the centres except for Cochin Hospital, which was the largest centre by far, including 109 patients, we provide a supplementary table S2 comparing characteristics of the patients included in this centre versus other centres.

Supplementary Table S2. Baseline characteristics of patients at Cochin (n=109) and those at other centres (n=129)

Maternal characteristics	Total (N=238)	Cochin cohort (N=109)	Other centres (N=129)	*P* value
Age at pregnancy, mean ± SD	31.6 ± 4.5	31.9 ± 4.4	31.3 ± 4.5	0.26
Nulliparity	88 (37.0)	42 (38.5)	46 (35.7)	0.65
Skin colour (N=235)				
-White	166 (70.6)	76 (69.7)	90 (71.4)	
-Black	29 (12.3)	20 (18.4)	9 (7.1)	
-From Asia	17 (7.2)	11 (10.1)	6 (4.8)	**<0.001**
-Other	23 (9.8)	2 (1.8)	21 (16.7)	
Overweight (BMI≥25 kg/m^2) (N=234)	71 (30.3)	30 (28.0)	41 (32.3)	0.48
Active smokers (N=233)	21 (9.0)	7 (6.4)	14 (11.3)	0.25
Alcohol consumption (N=226)[§]	6 (2.7)	2 (1.8)	4 (3.4)	0.68
Previous IUFD (N=237)	16 (6.8)	5 (4.6)	11 (8.6)	0.30
Previous thrombosis	41 (17.2)	18 (16.5)	23 (18.3)	0.79
Associated APS	34 (14.3)	14 (12.8)	20 (15.5)	0.56
SLE duration, years, median (IQR)	7.2 (3.6-12.4)	8.5 (4.5-13.4)	6.7 (3.5-10.9)	0.07
Previous renal involvement	67 (28.2)	13 (38.2)	54 (26.5)	0.16
Laboratory characteristics				
Low platelets (<100×10^9/l)	3 (1.3)	1 (0.9)	2 (1.6)	>0.99
24 h proteinuria>0.5 g/d (or >0.5 g/g)	9 (3.8)	4 (3.7)	5 (3.9)	>0.99
Positive anti-dsDNA (N=222)	104 (46.9)	57 (52.8)	47 (41.2)	0.09
Hypocomplementemia (N=216)	57 (26.4)	26 (24.3)	31 (28.4)	0.49
At least one positive aPL (N=232)	61 (26.3)	26 (23.9)	35 (28.5)	0.43
IgG/IgM anti-β2GPI (N=232)	26 (11.2)	6 (5.5)	20 (16.3)	**0.01**
IgG/IgM aCL (N=232)	37 (16.0)	17 (15.6)	20 (16.3)	0.89
LAC (N=232)	41 (17.7)	16 (14.7)	25 (20.3)	0.26
Triple positive aPL (N=232)	17 (7.3)	5 (4.6)	12 (9.8)	0.21
SLE activity and damage				
PGA, median (IQR) (N=235)	0.1 (0-0.2)	0.1 (0-0.2)	0.0 (0-0.2)	0.21
SLEPDAI, median (IQR) (N=212)	2 (0-3)	2 (0-3)	2 (0-3)	0.61
SLICC-Damage Index, median (IQR) (N=236)	0 (0-0)	0 (0-0)	0 (0-0)	0.24
Clinical SLEPDAI=0	206 (86.6)	95 (87.2)	111 (86.1)	0.80
Remission (DORIA/Zen definition)	154 (64.7)	72 (66.1)	82 (63.6)	0.69
Remission (DORIS definition)	147 (61.8)	72 (66.1)	75 (58.1)	0.21
LLDAS (N=219)	157 (71.7)	84 (78.5)	73 (65.2)	**0.03**
Current treatment				
Prednisone	119 (50.0)	48 (44.0)	71 (55.0)	0.09
Prednisone mg/d, median (IQR) (N=119)	7 (5-10)	7 (5-10)	7 (5-10))	0.38
Immunosuppressive drugs	56 (23.5)	24 (22.0)	32 (24.8)	0.61
Hydroxychloroquine	234 (98.3)	106 (97.3)	128 (99.2)	0.34
Low-dose aspirin	165 (69.3)	64 (58.7)	101 (78.3)	**0.001**
Low molecular weight heparin	61 (25.6)	29 (26.6)	32 (24.8)	0.75
Antihypertensive agents	5 (2.1)	4 (3.7)	1 (0.8)	0.18
Pregnancy outcome				

APOs	34 (14.3)	14 (12.8)	20 (15.5)	0.56
Maternal flares	35 (14.7)	17 (15.6)	18 (14.0)	0.72

Legend to Supplementary Table S3: SD: standard deviation; BMI: body mass index; IUFD: Intrauterine fetal death (>10 weeks); APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; IQR: interquartile range; g/d: grams per day; anti-dsDNA: anti-double stranded DNA; aPL: antiphospholipid; aCL: anti-cardiolipin; anti-β2GPI: anti-beta2 Glycoprotein I; LAC: lupus anticoagulant; PGA: physician global assessment; SLEPDAI: Systemic Lupus Erythematosus Pregnancy Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics; LLDAS: lupus low disease activity state; APOs. Adverse pregnancy outcome.

§: at least 10 units per week.

Interpretation tables S1 and S2: The patients were homogeneously distributed among the centres except for Cochin Hospital, which was the largest centre by far, including 109 patients.

As shown in Table S2, neither the characteristics of the patients included in this centre nor their pregnancy outcomes were statistically different from those of the other centres except for skin colour and aspirin use (see Supplementary Table S2). Specifically, Cochin, located in Paris, had more Black and Asian patients, due to local demography. Of note, the other centres had more "other" patients probably because they might have just classified non-white as "other" especially among North African patients.

Aspirin use at Cochin is not as systematic as in some other centres because it is not prescribed there systematically in the absence of antiphospholipid antibodies or past renal manifestations. This remains in keeping with international recommendations, as the interest of aspirin in such patients is largely unproven. The treatment was otherwise very similar and is therefore unlikely to have impacted the conclusions of our stud

Supplementary Table S3. SLICC-Damage Index domains in patients with SLICC-Damage Index>0

	Patients (%)
Total	**30 (12.7)**
Musculoskeletal	10 (4.2)
Renal	8 (3.4)
Neuropsychiatric	5 (2.1)
Ocular	4 (1.7)
Cardiovascular	4 (1.7)
Skin	2 (0.8)
Peripheral vascular	1 (0.4)
Pulmonary	1 (0.4)
Malignancy	1 (0.4)
Gastrointestinal	0 (0.0)
Premature gonad failure	0 (0.0)
Diabetes	0 (0.0)

Legends: Data were available for 236 patients. Domains are extracted from the SLICC-Damage Index score [15]. SLICC: Systemic Lupus International Collaborating Clinics.

Supplementary Table S4. Country of birth of the 23 patients with "Other skin colour"

Other	N	%
Haiti	1	4.3
Sri Lanka	1	4.3
North Africa	14	60.9
Syria	1	4.3
Peru	1	4.3
Madagascar	1	4.3
Not available	4	17.4
Total	**23**	**100**

Comments on the terms used: Currently, the French legislation does not recognise the term "race", a social construction that was banned by the French constitution in 2018. The term "ethnicity" is also highly sensitive and debatable, and research on ethnic backgrounds is complicated to perform in France. Since this concept is widely used in the international literature on SLE, we have tried to overcome this difficulty by using skin colour as a biological and relatively objective variable, and this was allowed by our regulatory authorities (CNIL).

9.4 PAPER IV

Definition of maternal complications

- HELLP syndrome was defined by the presence of all three of the following criteria: peripheral blood smear and serum lactate dehydrogenase levels ≥600 U/L), serum aspartate aminotransferase levels ≥70 U/l, and platelet count $<100\times10^9$/l [99];
- Preeclampsia was defined by hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) and proteinuria ≥0.3 g/24 h [87];
- Intra uterine growth restriction (IUGR): an estimated fetal weight less than the 10^{th} percentile for gestational age [100];
- Pregnancy induced hypertension: systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥ 90 mmHg without senza 24 proteinuria, developed after the 20_{th} WG in a woman with a previous normal blood pressure (BP) [100].

10. AKNOWLEDGMENTS

Contributors of the GR2 group: Béatrice Banneville, Antoine Baudet, Constance Beaudouin-Bazire, Cristina Belizna, Rakiba Belkhir, Ygal Benhamou, Alice Berezne, Sabine Berthier, Emilie Berthoux, Holy Bezanahary, Lisa Biale, Boris Bienvenu, Claire Blanchard-Delaunay, Anne Calas, Pascal Cathebras, Claire Cazalets, Benjamin Chaigne, Olivia Chandesris, Jérémy Chatelais, Emmanuel Chatelus, Elodie Chauvet, Fleur Cohen, Pascal Coquerelle, Marion Couderc, Mathilde de Menthon, Claire de Moreuil, Juliette Delforge, Azeddine Dellal, Catherine Deneux-Tharaux, Amélie Denis, Emmanuelle Dernis, Alban Deroux, Sandra Desouches, Guillaume Direz, Maxime Dougados, Marine Driessen, Aurélie Du Thanh, Laetitia Dunogeant, Cécile Durant, Isabelle Durieu, Elisabeth Elefant, Marc Fabre, Olivier Fain, Nicole Ferreira-Maldent, René-Marc Flipo, Aline Frazier, Antoine Froissart, Sophie Georgin-Lavialle, Elisabeth Gervais, Bertrand Godeau, François Goffinet, Anne Gompel, Laure Gossec, Phillipe Goupille, Claire Grange, Constance Guillaud-Danis, Eric Hachulla, Aurélie Hummel, Moez Jallouli, Patrick Jego, Stéphane Jobard, Laurence Josselin-Mahr, Noémie Jourde-Chiche, Anne-Sophie Korganow, Marc Lambert, Vincent Langlois, Delphine Lariviere, Claire Larroche, Céline Lartigau-Roussin, Augustin Latourte, Christian Lavigne, Thomas Le Gallou, Gaëlle Leroux, Hervé Levesque, Frédéric Lioté, Laurence Loeuillet, Jonathan London, Valentine Loustau, Emmanuel Maheu, Matthieu Mahevas, Hélène Maillard, Xavier Mariette, Hubert Marotte, Nicolas Martin-Silva, Nihal Martis, Agathe Masseau, François Maurier, Arsène Mekinian, Sara Melboucy-Belkhir, Martin Michaud, Marc Michel, Jacques Morel, Guillaume Moulis, Jérémy Ora, Elisabeth Pasquier, Jean-Loup Pennaforte, Antoinette Perlat, Hélène Petit Bauer, Laurent Perard, Evangeline Pillebout, Jean-Maxime Piot, Geneviève Plu-Bureau, Vincent

Poindron, Agnès Portier, Gregory Pugnet, Loïc Raffray, Alexis Regent, Christophe Richez, Mélanie Roriz, Gaëtan Sauvetre, Léa Savey, Nicolas Schleinitz, Jeremy Sellam, Raphaele Seror, Vincent Sobanski, Christelle Sordet, Martin Soubrier, Katia Stankovic-Stojanovic, Nathalie Tieulé, Thierry Thomas, Marie-Agnès Timsit, Vassilis Tsatsaris, Maria Letizia Urban, Geoffrey Urbanski, Cécile Yelnik.

We thank all the investigators of the GR2 group. We also thank Miss Ada Clarke for her assistance, the patients for agreeing to participate, and the patient associations for their strong support. Finally, we acknowledge the French Society of Internal Medicine (SNFMI), the French Society of Rheumatology (SFR), and the FAI2R (filière de santé des maladies auto-immunes et auto-inflammatoires rares) for their scientific, technical and financial support.