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Complement levels during the first trimester predict disease flare and adverse pregnancy outcomes in systemic lupus erythematosus: A network meta-analysis on 532 pregnancies

Massimo Radin [a,1], Irene Cecchi [a,1], Francesca Crisafulli [b,1], Evandro Mendes Klumb [c], Guilherme Ramires de Jesús [d], Marcela Ignacchiti Lacerda [d], Miguel Ángel Saavedra [e], Geraldine Vanessa Reyes-Navarro [f], Luca Iaccarino [g], Maddalena Larosa [g,h], Gabriella Moroni [i,j], Francesco Tamborini [k], Dario Roccatello [a], Laura Andreoli [b,2], Savino Sciascia [a,2,*], Cecilia Beatrice Chighizola [l,2]

[a] University Center of Excellence on Nephrologic, Rheumatologic and Rare Diseases (ERK-Net, ERN-Reconnect and RITA-ERN Member) with Nephrology and Dialysis Unit and Center of Immuno-Rheumatology and Rare Diseases (CMID), Coordinating Center of the Interregional Network for Rare Diseases of Piedmont and Aosta Valley, San Giovanni Bosco Hub Hospital, Department of Clinical and Biological Sciences, University of Turin, Turin, Italy
[b] Rheumatology and Clinical Immunology Unit, Department of Clinical and Experimental Sciences, ASST SpedaliCivili of Brescia, University of Brescia, Brescia, Italy
[c] Department of Rheumatology, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[d] Department of Obstetrics, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[e] RheumatologyDepartment, Hospital de Especialidades Dr. Antonio Fraga Mouret, Centro Médico Nacional La Raza, Instituto Mexicano del Seguro Social, Mexico City, Mexico
[f] School of Medicine Puebla Campus, Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
[g] Rheumatology Unit, Department of Medicine, University of Padova, Padova, Italy
[h] Division of Rheumatology, Department of Locomotor System, ASL3, Genoa, Italy
[i] Department of Biomedical Sciences, Humanitas University, 20089 Rozzano, Italy
[j] IRCCS Humanitas Research Hospital, 20089 Rozzano, Italy
[k] UO Nephrology and Dialysis, Ospedale Civile di Vigevano, ASST Pavia, Italy
[l] University of Milan, Pediatric Rheumatology Unit, ASST G. Pini - CTO, Milan, Italy

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ABSTRACT

Background: Complement levels have been proposed as candidate biomarkers of disease activity and obstetric risk in systemic lupus erythematosus (SLE) pregnancies, but their reliability has been questioned due to the physiologic fluctuations of complement during gestation. Thus, this network meta-analysis aimed at assessing the clinical significance of complement fluctuations in lupus pregnant women.

Methods: Corresponding authors of 19 studies meeting inclusion criteria were invited to contribute with additional data including C3 and C4 levels [before pregnancy, at conception, in every trimester (T) and 3 months after delivery]; data were pooled together in a network meta-analysis.

Results: A total of 532 lupus women from four studies were included in the analysis. In SLE women, C3 and C4 increased progressively during gestation: levels remained stable during T1 and peaked in T2 to decrease in T3. Patients with previous lupus nephritis (LN) and those who experienced flares during pregnancy had significantly lower mean levels of C3 and C4 at all timepoints. The lowest levels of complement were observed, particularly during T1, in patients with LN and gestational flare. Both reduction and the lack of increase of C3 and C4 levels at T1 *versus* conception were associated with gestational flares, particularly in LN patients. Pregnancies with flare had a statistically significant higher rate of maternal and fetal complications(60% versus 50.3%; $p = 0.03$).

* Corresponding author.
E-mail address: savino.sciascia@unito.it (S. Sciascia).
[1] These Authors contributed equally to this work and share the first authorship.
[2] These Authors contributed equally to this work and share the senior authorship.

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Conclusions: Low complement levels, particularly in T1, were associated with a higher frequency of gestational flare. Either reduction or smaller increase of C3 and/or C4 levels, even within normal range, might predict flares especially in early gestation.

1. Introduction

Systemic lupus erythematosus (SLE) is a prototypical immune complex-mediated disease, characterized by a wide spectrum of clinical phenotypes with heterogeneous courses and progression, varying from persistently low, relapsing-remitting, to persistently high disease activity [1]. The epidemiology of SLE, which mainly presents in young women of childbearing age [2], accounts for the fact that clinicians assist lupus patients very often in their journey towards motherhood. To explain such epidemiological female predominance, several hypotheses have been formulated: candidate risk genes for SLE map on the X chromosome, and estrogens favour autoimmunity by promoting B-cell maturation, antibody production, Th2 responses, and survival of autoreactive cells [3].

As expected, pregnancy can impact SLE disease activity, and in turn SLE may affect obstetric outcomes. Pregnancy in women with SLE has always been regarded as at high risk; however, the significant advancements made in the overall disease management have led to a net improvement of both maternal and fetal outcomes [4,5]. Nevertheless, pregnancy still represents a challenge in women with SLE, especially in those with renal involvement, especially for those with proliferative glomerulonephritis, due to the hazard of disease flare, gestational diabetes and placenta-related disorders, including pre-eclampsia (PE), as well as fetal complications such as miscarriages, fetal loss, intrauterine growth restriction, prematurity, and neonatal lupus [6]. Reliable biomarkers to stratify the risk of a disease flare during pregnancy and to early detect adverse pregnancy outcomes (APO) in pregnant lupus women are still lacking. Complement levels have been proposed as candidate biomarkers of disease activity and of obstetric risk in lupus pregnancies, but their reliability has been questioned due to the physiologic fluctuation of complement levels during gestation [7,8]. Recently, one cohort study described a predictive role of low pre-pregnancy C4 levels towards disease flare during pregnancy [9], while another one found that low-pregnancy C3 levels were associated with preterm birth [10].

In order to optimize the interpretation of available data on the variation of complement levels during SLE pregnancy, we performed a network meta-analysis to assess the fluctuations of C3 and C4 levels from preconception period, throughout pregnancy, and up to 3 months after delivery and to evaluate the association of complement levels with the occurrence of disease flares and/or APO.

2. Patients methods

2.1. Systematic literature review

A detailed literature search strategy has been developed *a priori* to identify articles that reported findings from available prospective

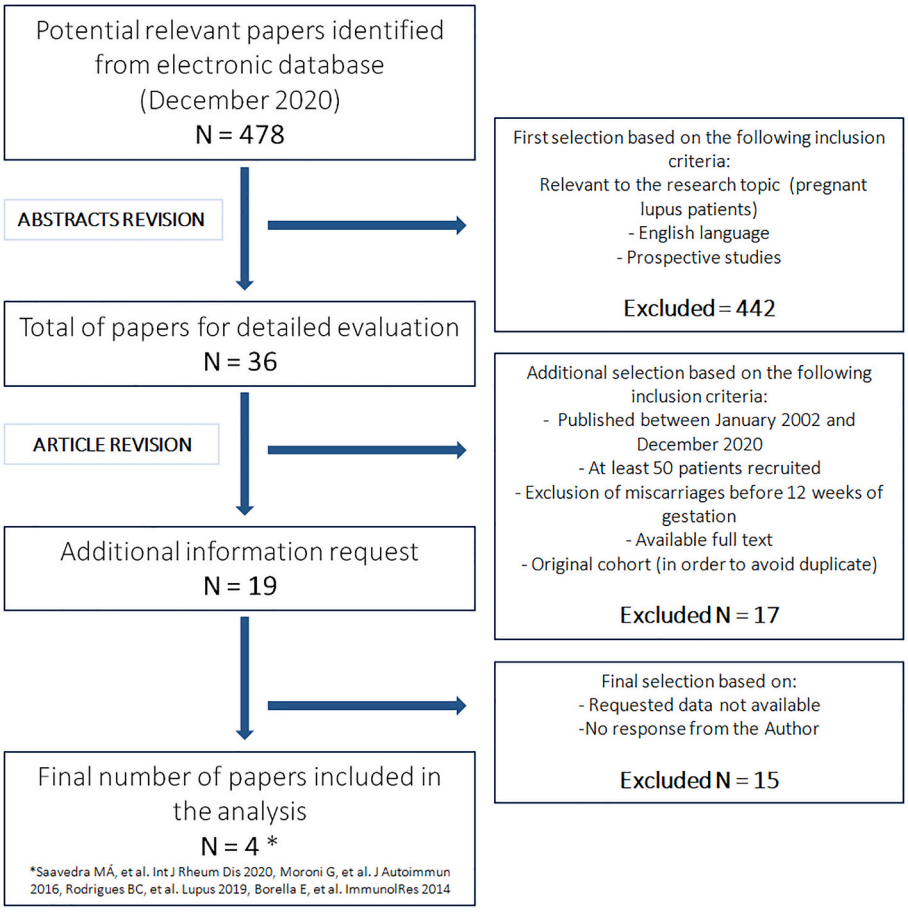

Fig. 1. Literature search strategy.

studies investigating pregnancies in patients with SLE from January 2002 to December 2020. Key words and subject terms included: ((“longitudinal studies”[MeSH Terms] OR (“longitudinal”[All Fields] AND “studies”[All Fields]) OR “longitudinal studies”[All Fields] OR “prospective”[All Fields] OR “prospectively”[All Fields]) AND (“lupus vulgaris”[MeSH Terms] OR (“lupus”[All Fields] AND “vulgaris”[All Fields]) OR “lupus vulgaris”[All Fields] OR “lupus”[All Fields] OR “lupus erythematosus, systemic”[MeSH Terms] OR (“lupus”[All Fields] AND “erythematosus”[All Fields] AND “systemic”[All Fields]) OR “systemic lupus erythematosus”[All Fields]) AND (“pregnancy”[MeSH Terms] OR “pregnancy”[All Fields] OR “pregnancies”[All Fields] OR “pregnancy s”[All Fields])) AND (1000/1/1:2021/6/15[pdat]).

The search strategy was applied to Ovid MEDLINE, In-Process and Other Non-Indexed Citation from January 2002 to December 2020. Fig. 1 resumes the search strategy.

Retrieved papers were further screened upon additional inclusion criteria in order to refine the search strategy. Inclusion criteria included: a) prospective design, b) a sample size of at least 50 lupus patients, c) exclusion of miscarriages before 12 weeks of gestation as obstetric outcome.

Given the nature of this study, ethics committee approval was not required.

2.2. Data collection and analysis

Two review Authors (M.R. and I.C.) independently assessed studies for inclusion. One review Author completed data extraction, which was checked by a second review Author. A total of 19 studies were finally selected for data request. Each corresponding Author of the selected manuscripts was invited to contribute with additional data of individual pregnancies that were not presented in the published manuscript, including complement levels, C3 and C4 separately, at 6 months before pregnancy, at conception, during the first trimester (T1), during the second trimester (T2), during the third trimester (T3), and 3 months after delivery (post-partum, PP). Further details on the number of pregnancies, patients’ classification, diagnosis at conception, treatment during pregnancy, occurrence of flares during gestation, as well as maternal and fetal outcomes were also recorded. We performed a network meta-analysis within a Bayesian framework as previously described [11].

2.3. Statistical analysis

Categorical variables are presented as numbers (%) and continuous variables are expressed as mean ± standard deviation (SD). The significance of baseline differences was determined by the chi-squared test, Fisher’s exact test or the unpaired *t*-test, as appropriate. Correlation analysis, linear regression, and Odds Ratio (OR) were also performed. Missing data were approached with mean substitution system. A two-sided p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM, Armonk, NY, USA).

2.4. Study variables definitions

SLE, lupus nephritis (LN), and antiphospholipid syndrome (APS) diagnosis and classification were based upon each study definition [12–14]. SLE flare was defined by the need of new immunosuppressive therapy or increase in the dosage of prednisone $\geq$10 mg/day.

APO were defined as follows:

a) fetal death after 12 weeks’ gestation in the absence of chromosomal abnormalities, anatomic malformations, or congenital infections;
b) neonatal death before hospital discharge due to complications related to prematurity or placental insufficiency (*e.g.*, abnormal fetal surveillance test results, abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, or oligohydramnios, or both);
c) preterm delivery or pregnancy loss at $<$36 weeks due to gestational hypertension, PE, or placental insufficiency;
d) small for gestational-age neonate, defined as one with a birth weight below the 5th percentile without anatomical or chromosomal abnormalities.

The fluctuation of C3 and C4 levels between T1 and conception was defined as $\Delta C3_{T1\text{-conception}}$ and $\Delta C4_{T1\text{-conception}}$. When the decrease in C3 levels between T1 and conception was below 2 mg/dl or the increase in C3 at T1 *versus* conception was below 4 mg/dl (defined using two standard deviations from mean, as per Westgard rules), $\Delta C3_{T1\text{–conception}}$ was considered as clinically not relevant.

3. Results

3.1. C3 and C4 levels progressively increased during gestation in women with SLE

A total of 532 SLE women from 4 studies were included in the analysis [15–18]. APS had been diagnosed in 68 women (12.8%), while 82 patients (15.4%) were positive for antiphospholipid antibodies (aPL) without overt clinical manifestations of APS (referred as “aPL carriers”). As detailed in Table 1 and visually presented in Fig. 2A, both C3 and C4 levels increased progressively in women with SLE during gestation. In particular, C3 and C4 levels remained stable during T1 and peaked at T2, then decreased during T3. At 3 months after delivery, a different behavior was noted for C3 and C4: C3 continued to decrease whereas C4 levels in the PP period were higher than those registered in T3.

3.2. Patients with flares during pregnancy displayed significantly lower levels of complement compared to patients without gestational flare

A flare during pregnancy was observed in 170 patients (32%). Levels of both C3 and C4 were lower at all timepoints in subjects who experienced flares during pregnancy (C3 at T1 78.3 ± 22.8 *versus* 100.5 ± 20.7, $p < 0.001$; C3 at T2 94.2 ± 13.4 *versus* 115.7 ± 12.3, $p < 0.001$; C3 at T3 99 ± 18.6 *versus* 111.4 ± 16, $p < 0.001$; C3 at PP 92.4 ± 15.7 *versus* 102.6 ± 13.4, $p < 0.001$; Table 1 and Fig. 2B).

The physiological increase in complement levels throughout gestation was rather marked among patients who did not experience a disease flare while pregnant. Complete data on complement levels fluctuation at all time-points in patients experiencing a gestational flare *versus* those who did not present a disease flare while pregnant are listed in Table 1 and illustrated in Fig. 2B.

3.3. Patients with LN displayed significantly lower levels of complement compared to patients without renal involvement

LN had been diagnosed in 237 women (44.5%). Patients with LN had significantly lower levels of complement when compared to patients without renal involvement (C3 at T1 84.6 ± 32.2 *versus* 98.4 ± 14.1, $p < 0.001$; C3 at PP 93.4 ± 12 *versus* 103.1 ± 15.4, $p < 0.001$; C4 at T1 15 ± 7.8 *versus* 16.3 ± 2.8, $p < 0.001$; C4 at PP 16.2 ± 4.3 *versus* 19.8 ± 6.9, $p < 0.001$, Table 1and Fig. 2C).

3.4. Patients with previous LN and flare during pregnancy displayed the lowest complement levels

A flare during pregnancy was observed in 73 women with a previous diagnosis of LN. The lowest levels of complement, both for C3 and C4, were observed in patients with a previous diagnosis of LN who experienced a flare during pregnancy. Complete data are listed in Table 1 and visually represented in Fig. 2C.

Table 1
Complement levels at the six different timepoints (values expressed as mean ± SD), according to diagnosis of lupus nephritis (LN) or presence of a disease flare during pregnancy.

	All SLE patients (*N* = 532)	Patients with LN (*N* = 237)	Patients without LN (*N* = 295)	Patients with flare (*N* = 170)	Patients without flare (*N* = 362)	Patients with LN and flare (*N* = 73)	Patients with LN and without flare (*N* = 164)
C3 6 months before pregnancy	92.3 ± 22.9	90.7 ± 18.6	94.1 ± 25.2	**85.6 ± 19.1**	**95.6 ± 23.3**	**75 ± 17.9**	**99.1 ± 12.5**
C3 at conception	92.4 ± 14.4	**96.1 ± 13.9**	**91.1 ± 13**	**95.3 ± 19.5**	**91.8 ± 9.1**	97 ± 21.6	95.6 ± 7.1
C3 T1	92.9 ± 23.8	**84.6 ± 32.2**	**98.4 ± 14.1**	**78.3 ± 22.8**	**100.5 ± 20.7**	**56.8 ± 19.9**	**97.2 ± 28.7**
C3 T2	107.8 ± 16.9	108.5 ± 21	108.3 ± 12.2	**94.16 ± 13.4**	**115.7 ± 12.3**	**87.5 ± 10.9**	**118.6 ± 16.8**
C3 T3	106.9 ± 18.1	105.5 ± 15.7	108.2 ± 19.1	**98.97 ± 18.6**	**111.4 ± 16**	**98.1 ± 12.6**	**109.1 ± 15.8**
C3 3 months PP	99.1 ± 14.9	**93.4 ± 12**	**103.1 ± 15.4**	**92.4 ± 15.7**	**102.6 ± 13.4**	**90.5 ± 10.8**	**94.8 ± 12.3**
C4 6 months before pregnancy	14.7 ± 4.2	**15.7 ± 5.5**	**14.1 ± 2.8**	**11.8 ± 3.9**	**16.5 ± 3.3**	**10.5 ± 3.4**	**18.4 ± 4.2**
C4 at conception	14.4 ± 3.5	**15.4 ± 4.1**	**13.9 ± 2.8**	**13.3 ± 3.2**	**15.7 ± 3.4**	**11 ± 1.3**	**17.8 ± 3**
C4 T1	15.8 ± 5.3	**15 ± 7.8**	**16.3 ± 2.8**	**12.5 ± 5.9**	**17.5 ± 4.2**	**9.3 ± 7.6**	**17.9 ± 6.2**
C4 T2	18.3 ± 4.4	**17.7 ± 4.7**	**18.7 ± 4.2**	**15.5 ± 4.3**	**19.8 ± 3.7**	**13.6 ± 4.1**	**19.6 ± 3.5**
C4 T3	17.6 ± 4.9	17.8 ± 4.4	17.5 ± 5.1	**15.7 ± 5.8**	**18.6 ± 4**	**15.8 ± 4.8**	**18.8 ± 3.9**
C4 3 months PP	18.3 ± 6.2	**16.2 ± 4.3**	**19.8 ± 6.9**	**14.9 ± 3.9**	**20 ± 6.4**	**13.3 ± 3.1**	**17.6 ± 4**
ΔC3(ΔC3 T1– at conception)	10.3 ± 43.2	**0.5 ± 53.2**	**16.6 ± 34.3**	**−6.7 ± 48.8**	**18.8 ± 37.6**	**−36.1 ± 42.6**	**17.3 ± 49.1**
ΔC4 (ΔC4 T1– at conception)	3.4 ± 7.6	**1.5 ± 9.1**	**4.5 ± 6.3**	**1.2 ± 8.1**	**4.4 ± 7.1**	**−1.1 ± 8.5**	**2.8 ± 9.1**

Results highlighted in bold are statistically significant.
Abbreviations: SLE, systemic lupus erythematosus; LN, lupus nephritis; T1, 1st trimester of gestation; T2, 2nd trimester of gestation, T3, 3rd trimester of gestation; PP, post-partum period (up to 3 months after delivery).

3.5. The fluctuations of C3 and C4 levels at T1 versus conception displayed the highest clinical significance in predicting disease flares

When analyzing the fluctuations of complement levels between different timepoints, the variations in both C3 and C4 between levels assessed at T1 *versus* those recorded at conception emerged as the most clinically significant. Indeed, the differential values in both C3 and C4 at T1 *versus* at conception (defined as $\Delta C3_{T1-conception}$ and $\Delta C4_{T1-conception}$, respectively) were significantly lower in patients with LN when compared to patients without renal involvement (ΔC3 0.5 ± 53 *versus* 16.6 ± 34.3, $p < 0.001$; ΔC4 1.5 ± 9.1 *versus* 4.5 ± 6.3, $p < 0.001$).

Women who experienced a flare during pregnancy had lower $\Delta C3_{T1-conception}$ and $\Delta C4_{T1-conception}$ ($\Delta C3_{T1-conception}$ − 6.7 ± 48.8 *versus* 18.8 ± 37.6, $p < 0.001$; ΔC4 1.2 ± 8.1 *versus* 4.4 ± 7.1, $p < 0.001$). The lowest levels of $\Delta C3_{T1-conception}$ and $\Delta C4_{T1-conception}$ were reported in patients that were diagnosed with LN and experienced flares during pregnancy ($\Delta C3_{T1-conception}$ − 36.1 ± 42.6; $\Delta C_{T1-conception}$ − 1.1 ± 8.5).

A decrease in $\Delta C3_{T1-conception}$ yielded an OR for flare during pregnancy of 3.1 (CI 95% 2.1–4.8) when below 5 mg/dL, an OR that increased up to 3.9 (CI 95% 2.5–6) when below 15 mg/dL.

Similar figures emerged when assessing the association between $\Delta C3_{T1-conception}$ and a prior diagnosis of LN: $\Delta C3_{T1-conception} \leq 5$ mg/dL conveyed an OR for a prior diagnosis of LN of 6.1 (CI 95% 3.9–9.6) while $\Delta C3_{T1-conception} \leq 10$ mg/dL conveyed an OR of 7.2 (CI 95% 4.5–11.7). Interestingly, even the lack of clinically relevant changes in the complement levels between T1 and conception was associated with both previous LN diagnosis (OR 2.2; CI 95% 1.3–3.6) and development of flare during pregnancy (OR 5.2; CI 95% 2.9–9.3). Table 2 resumes the results of the coefficient of risk conveyed by different $\Delta C3_{T1-conception}$ levels upon LN diagnosis or presence of flare.

3.6. The fluctuations of C3 and C4 levels at T1 versus conception displayed the highest clinical significance in predicting APO

Preterm delivery or miscarriage at <36 weeks were more frequent in women with a previous diagnosis of APS (39.7% *versus* 23%; $p = 0.003$), in patients who developed flares during pregnancy irrespectively of a concomitant diagnosis of LN (42.5% *versus* 28%; $p = 0.01$ in patients with LN and 34% *versus* 17.2%; $p = 0.01$ in those without a diagnosis of LN). Additionally, fetal death was more frequent in patients with a diagnosis of LN and positive aPL (4 out of 30 versus 6 out of 206; $p = 0.008$).

When computing all APO together, higher rates of complications were reported in patients with a previous diagnosis of APS (88.2% *versus* 56%; $p < 0.0001$) as well as LN (67.9% *versus* 53.9%; $p < 0.0001$) and occurrence of flare during pregnancy (91.2% *versus* 45.6%; $p < 0.0001$).

$\Delta C3_{T1-conception} \leq 5$ mg/dL and no changes of $\Delta C3_{T1-conception}$ were both associated with higher rates of overall APO (63.4% *versus* 45.6%; $p = 0.003$ and 58.5% *versus* 72.8%; $p = 0.02$, respectively).

4. Discussion

The present network meta-analysis, which includes more than 500 pregnant lupus patients from 4 international independent studies, allowed us to clearly assess the clinical relevance of complement monitoring during gestation to predict both disease flares and APO. Levels of C3 and C4 emerged as reliable biomarkers to identify those women who are at higher risk of developing disease flares and APO, even in case of a concomitant diagnosis of LN [19]. These findings are extremely relevant from a clinical perspective given that, despite the substantial improvements accomplished in the management of SLE patients, 50% of lupus women might develop a flare during gestation, with severe organ involvement occurring in up to 25% of cases [20,21]. Unfortunately, the current lack of reliable biomarkers and validated tools for the assessment of disease activity during pregnancy limits our ability to predict which subjects will experience disease worsening and/or APO. In the last few decades, several scoring systems have been developed to assess lupus activity and the risk of flare during pregnancy. Most of these tools, such as the LAI in Pregnancy (LAI-P), the SLE-Pregnancy Disease Activity Index (SLEPDAI), and the modified SLAM3 (m-SLAM) [22], include hypocomplementemia (C3 and C4). These clinimetric instruments have been created by modifying existing lupus activity indexes in order to differentiate between disease-specific features and physiologic changes occurring during gestation. Although promising, these pregnancy-adapted scores have not been extensively validated in large prospective cohorts and therefore their current employment in clinical practice is strongly limited. Similarly, C3 and C4 levels should be carefully evaluated in pregnant lupus women as complement serum levels rise throughout the course of normal gestation

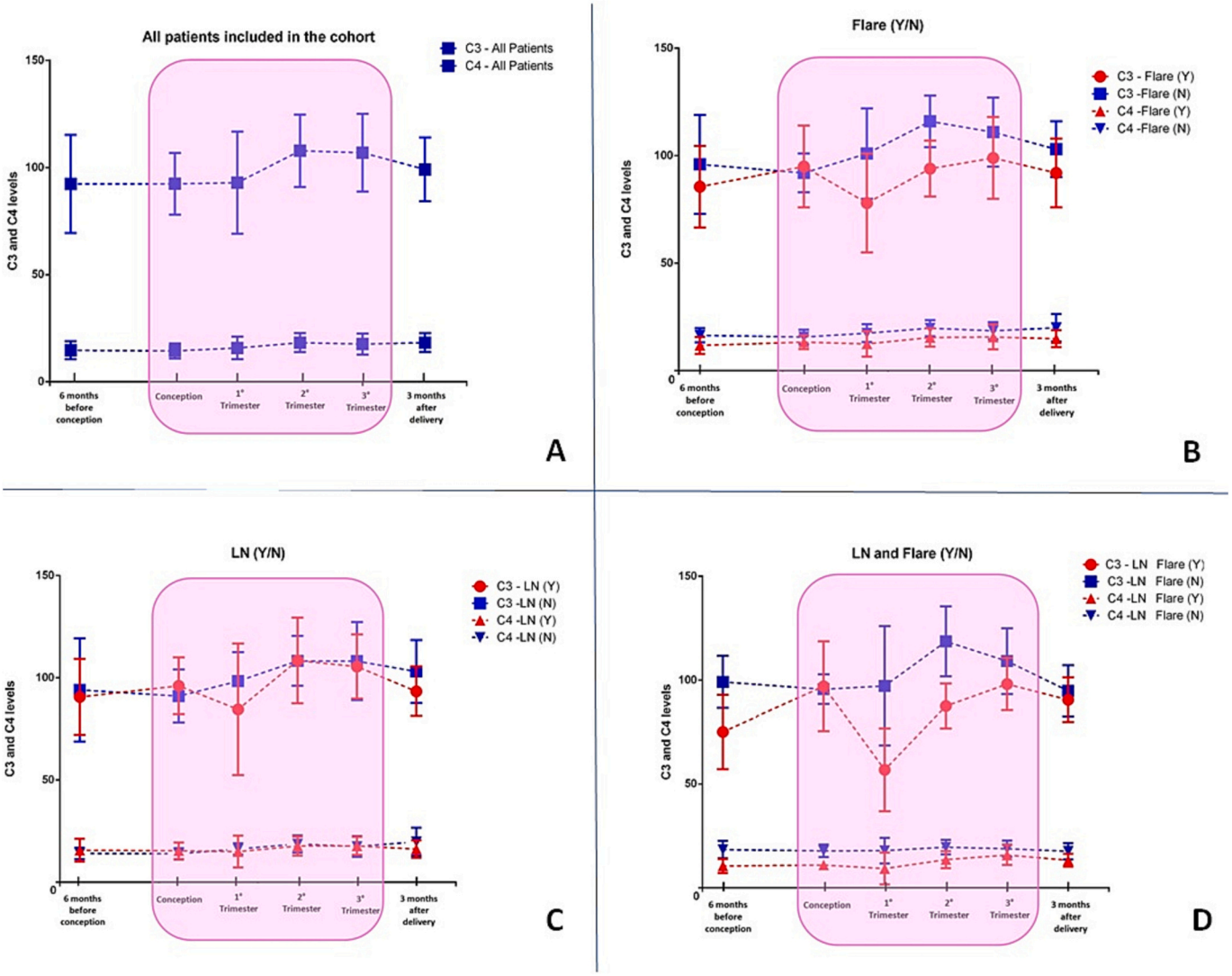

Fig. 2. Complement levels fluctuations over 6 time points (before conception, at conception, during each trimester of pregnancy, and after delivery).
Panel 2A. Linear representation of the complement levels overtime in the entire cohort of systemic lupus erythematosus patients (SLE). **Panel 2B.** Linear representation of the fluctuations of complement levels during pregnancy in patients with SLE with and without the occurrence of flares during pregnancy. **Panel 2C.** Linear representation of complement levels during time in patients with and without lupus nephritis (LN). **Panel 2D.** Linear representation of complement levels during time in patients with and without LN and presence, or absence, of flare during pregnancy.

[23]. This study confirms that complement levels fluctuate over the gestational course also in SLE women: values of C3 and C4 remained stable at early stages of pregnancy, then progressively increased during the second trimester of gestation; once reached the highest levels, both C3 and C4 showed a decline with discrepant behaviors after delivery, resulting in a constant rise of C4 values and a progressive decrease of C3. Interestingly, we observed that lupus patients who experienced a clinical flare during pregnancy had significantly lower mean values of C3 and C4 throughout the entire gestation compared with patients with stable disease activity. If our data confirm the relevance of complement as a monitoring tool of lupus disease activity even during gestation, it should be mentioned that the consensus about the reliability of complement in predicting SLE flare is not unanimous. Indeed, its relevance has been questioned by few studies [24–27], most likely due to the methodological challenges of accurately measuring circulating complement levels as well as to the inappropriate designs of clinical studies [28]. Nevertheless, despite these inconsistencies, it is universally accepted that complement activation in SLE is mirrored by a secondary decline of circulating complement levels and a parallel increase in complement split products and circulating levels of complement proteins (C3 and C4) are extensively used in clinical practice for classification and diagnostic purposes, monitoring of disease activity and follow-up. Similarly, the clinical significance of low C3 and C4 circulating levels as biomarkers for LN is still matter of research [29]. Whereas a significant drop in C4 levels can be observed even two months prior to renal flare occurrence, a decline in C3 was shown to be influenced by genetic variants of factor H, which regulates C3-convertase in the alternative pathway [30]. In addition, elevated titers of autoantibodies directed against C1q have been described as better predictors of renal involvement in SLE patients compared to C3 and C4, although with inconclusive results [30]. Further analysis of our data revealed significantly lower levels of C3 and C4 in pregnant patients with flare at all time-points considered, from conception throughout pregnancy and until 3 months following delivery, consistently with what reported by other authors [30]. Most importantly, the present study also highlights that those patients with both previous LN and disease flare during gestation had the lowest complement levels, suggesting that levels of C3 and C4 below the normal threshold before conception can serve as predictor of flare during pregnancy in this high-risk group of patients [31].

To better evaluate the fluctuations of C3 and C4 by minimizing the

Table 2
Odds Ratios (OR) according to lupus nephritis (LN) diagnosis or presence of flare and different ΔC3 levels (first trimester –at conception).

	FLARE OR	FLARE CI 95%	LN OR	LN CI 95%	LN & FLARE OR	LN & FLARE CI 95%
ΔC3 ≥ 15 mg/dL	0.3	0.2–0.5	1.2	0.7–2.5	0.06	0.02–0.3
ΔC3 ≥ 10 mg/dL	0.5	0.3–0.7	0.4	0.3–0.5	0.03	0.01–0.1
ΔC3 ≥ 5 mg/dL	0.4	0.3–0.6	0.2	0.1–0.3	0.02	0.01–0.07
ΔC3 no change defined as [−2;+4] mg/dL	1.1	0.6–1.9	**2.2**	**1.3–3.6**	**5.2**	**2.9–9.3**
ΔC3 ≤ 5 mg/dL	**3.1**	**2.1–4.8**	**6.1**	**3.9–9.6**	**6.5**	**3.9–11.2**
ΔC3 ≤ 10 mg/dL	**3.3**	**2.2–5.1**	**7.2**	**4.5–11.7**	**5.6**	**3.3–9.7**
ΔC3 ≤ 15 mg/dL	**3.9**	**2.5–6**	**6.4**	**4–10.3**	**6.2**	**3.6–10.7**

Results highlighted in bold are statistically significant.

confounding effect of cut-off variability and inter-assay heterogeneity among the four different cohorts, as well as the potential influence of genetic variants, the analysis in this network meta-analysis also focused on the differential levels of circulating C3 and C4 values (ΔC3 and ΔC4) between different trimesters of gestation, rather than the mere absolute levels or the dichotomous categorization into hypocomplementemia *versus* normocomplementemia. This approach allowed us to determine that the most informative data in clinical practice consists in the lack of physiological increase in C3 and C4 values in the first trimester of gestation as compared to conception: women who experienced a lupus flare during gestation displayed the lowest ΔC3 and ΔC4 during the first trimester *versus* at conception. In addition, the less pronounced the increase in C3 levels is from conception throughout the first trimester of gestation, the higher the risk of developing disease flare with an OR up to 3.9 when ΔC3 is below 15 mg/dl. The same conclusion can be extrapolated to pregnant women with renal involvement and the occurrence of flare during gestation, a subset of patients in which a small ΔC3 carried an even higher risk of disease flare (OR 5.2). Despite the significance of C4 variations during pregnancy in predicting both APO and disease flare, we decided to emphasize the results obtained when focusing on C3 variations. In fact, from a practical point of view, and based on the more extended range of C3 values, ΔC3 might be easier to assess and more informative for clinicians.

The data gathered in this meta-analysis allowed us to investigate also the role of complement levels in predicting obstetric morbidity among lupus women. Women with lower levels of both C3 and C4 prior to conception and during the entire gestation are more likely to experience APO: a ΔC3 below 5 mg/dl between the first trimester and at conception as well as no changes in ΔC3 at these time-points were associated with an overall higher rate of APO. These findings are consistent with the available literature, which traditionally enlists hypocomplementemia, together with active LN at conception, previous history of LN, aPL positivity and high disease activity before conception, as major determinants of poor maternal and fetal outcomes in lupus women [6].The relationship between complement levels and APO should not be surprising, given the multifaceted role of the complement cascade in pregnant lupus women. On one hand, the complement system, with more than30 plasma proteins and receptors, represents a key element of the innate immunity response that contributes to the progression of SLE through the stimulation of inflammation and the removal of immune complexes, cells, and apoptotic debris [32]. Importantly, SLE onset, disease activity and organ damage have been all linked to complement activation and consumption, as well as to complement deficiencies [33]. On the other hand, a consistent stream of data has progressively shown that the complement cascade exerts a pivotal role throughout all the stages of physiologic gestation (conception, embryo implantation, placentation, fetal growth, and labor) and the fine tuning of the expression of complement factors, receptors and inhibitors during gestation, with their increased hepatic synthesis, is mandatory to ensure a successful pregnancy [34].

This study presents some limitations that should be acknowledged. First, the limited number of included studies does not encompass the whole prospective experience in lupus pregnancy available in the literature. Second, the geopolitical representation of the included cohorts does not comprehend North America, Asia-Pacific and Africa, thus reducing the generalizability of our conclusions. Third, since SLE is an extremely heterogeneous condition, the inclusion of patients with distinct clinical profile might limit the reproducibility of the observed results. Fourth, given the nature of the study, the lack of a control group (*e.g.* healthy subjects) represents another limitation. Despite the acknowledged limitations, our study has indeed some strengths: the high number of included patients, the prospective design of the considered studies, and lupus diagnosis assessed with homogeneous criteria across different cohorts [12–14]. Moreover, despite the absence of complement levels adjustment for gestational state or trimester [35], cut-off values for circulating levels C3 and C4 were comparable among different cohorts.

This network meta-analysis suggests the role of C3 and C4 levels/fluctuations before conception and in early pregnancy as predictors of SLE flares and APO later in the pregnancy course. Particularly, the lack of increase in C3 and C4 levels during the first 13 weeks of gestation appeared as a strong predictor of flare, especially in women with previous LN. These findings deserve further validation in order to define the role of complement as a biomarker that can inform risk stratification and guide individualized treatment decisions in women with SLE who are pregnant or planning to get pregnant.

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Patients and public involvement statement

Patients were not involved in this study.

CRediT authorship contribution statement

Massimo Radin: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Irene Cecchi:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Francesca Crisafulli:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Evandro Mendes Klumb:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Guilherme Ramires de Jesús:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Marcela Ignacchiti Lacerda:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Miguel Ángel Saavedra:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Geraldine Vanessa Reyes-Navarro:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Luca Iaccarino:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Maddalena Larosa:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Gabriella Moroni:** Data curation, Investigation, Methodology,

Visualization, Writing – review & editing. **Francesco Tamborini:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **Dario Roccatello:** Conceptualization, Supervision, Methodology, Writing – review & editing. **Laura Andreoli:** Conceptualization, Supervision, Methodology, Writing – original draft, Writing – review & editing. **Savino Sciascia:** Conceptualization, Supervision, Methodology, Writing – original draft, Writing – review & editing. **Cecilia Chighizola:** Conceptualization, Supervision, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None declared.

Data availability

Data will be available upon reasonable request.

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