Low serum lipocalin-2 in pregnant women with systemic lupus erythematosus

T.T. Pedersen[1,2], M.H. Fenstad[3], M. Wallenius[1,4], E. Hetlelid[5], T. Follestad[2], M. Langaas[5], M. Haug[2,6,7]

[1]Norwegian National Advisory Unit on Pregnancy and Rheumatic Diseases, Department of Rheumatology, St. Olavs University Hospital, Trondheim; [2]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Trondheim; [3]Department of Immunology and Transfusion Medicine, St. Olavs University Hospital, Trondheim; [4]Department of Neuromedicine and Movement Science, [5]Department of Mathematical Sciences, NTNU, Trondheim; [6]Centre of Molecular Inflammation Research (CEMIR), NTNU, Trondheim; [7]Department of Infectious Diseases, St. Olavs University Hospital, Trondheim, Norway.

Abstract

Objective

Systemic lupus erythematosus (SLE) pregnancies are considered high-risk due to risk of disease flare and pregnancy complications. A more in-depth understanding of the immunological alterations in SLE patients during pregnancy and identification of predictive biomarkers may help to achieve stable disease and to avoid pregnancy complications. Lipocalin-2 (LCN2) has been implicated as a potential biomarker for rheumatic diseases and preeclampsia, but remains unexplored in SLE pregnancies.

Methods

We measured LCN2 levels in serum samples from SLE pregnancies (n=25) at seven different time points. Samples were taken preconception, in each trimester, at 6 weeks, 6 months and 12 months postpartum. Serum LCN2 levels were compared to samples from rheumatoid arthritis (RA) (n=27) and healthy (n=18) pregnancies at each time point using t-test, and for all time points using a linear mixed effects model. In addition, we investigated the association between LCN2 levels and disease activity, CRP, kidney function, BMI, treatment regimen and adverse pregnancy outcome for SLE and RA patients.

Results

We found significantly lower serum LCN2 levels throughout pregnancy in SLE patients with quiescent disease compared to RA and healthy pregnancies. We did not find an association between serum LCN2 and disease activity or adverse pregnancy outcome in SLE pregnancies.

Conclusion

In a population of SLE women with low disease activity we have not found evidence that serum LCN2 levels predict disease activity or adverse pregnancy outcomes. Further studies are needed to elucidate a possible biological role of low LCN2 levels in SLE pregnancies.

Key words

systemic lupus erythematosus, rheumatoid arthritis, pregnancy, lipocalin-2, neutrophil gelatinase-associated lipocalin

Tina Therese Pedersen, MD
Mona H. Fenstad, MD, PhD
Marianne Wallenius, MD, PhD
Elisabeth Hetlelid, MSc
Turid Follestad, PhD
Mette Langaas, PhD
Markus Haug, PhD

Please address correspondence to:
Tina Therese Pedersen
Department of Rheumatology,
St. Olavs University Hospital,
Postboks 3250 Torgarden,
7006 Trondheim, Norway.
E-mail: tina.pedersen@stolav.no

Received on October 11, 2022; accepted in revised form on January 11, 2023.

Funding: this study was supported by grants from St. Olavs University Hospital and Research Fund of the Norwegian Organization for people with Rheumatic diseases (Norsk Revmatikerforbund).

Competing interests: none declared.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease mainly affecting women in their reproductive years. About 50% of women with SLE have disease flares during or after pregnancy (1, 2). In well-regulated SLE pregnancies, disease activity is low or absent during pregnancy, but found to increase six to twelve months postpartum (3). Pregnancies in women with SLE are considered high risk due to association with pregnancy complications and foetal morbidity (4, 5). Therefore, it is important to secure stable disease with low disease activity before pregnancy to prevent both disease flare and pregnancy complications. Biomarkers to predict disease flares and a more in-depth understanding of the immunological alterations during SLE pregnancies, are required to achieve the goal of stable disease throughout pregnancy and to avoid pregnancy complications.

Lipocalin-2 (LCN2) has become increasingly relevant as a potential biomarker for inflammatory diseases, including rheumatic diseases and preeclampsia (6, 7). LCN2, also known as neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein with multiple immune functions. Originally, LCN2 was described as acute-phase protein of the innate immune system that limits bacterial growth by limiting access to iron (8). Other LCN2 functions that were discovered in later years include a role as pro-inflammatory adipokine in metabolic diseases and in the adaptive immune system by inducing immune tolerance (6, 9). So far, most studies in SLE, have investigated urine LCN2 in lupus nephritis. A review article from 2020, identified urine LCN2 as a useful biomarker for diagnosis of nephritis, renal activity and prediction of renal flares (10). Only a few studies have explored a possible role of blood LCN2 as biomarker in SLE patients (11-17). A study in juvenile SLE showed that increase in plasma LCN2 anticipated worsening of both global disease activity and renal disease (11). Until now, LCN2 in SLE pregnancies have not been explored.

The objective of this study was to investigate the role of LCN2 in SLE pregnancies compared to RA and healthy pregnancies. First LCN2 levels at different time points in pregnancy and postpartum period were analysed and compared for the three groups. Then the association between LCN2 levels and disease activity, CRP, kidney function, medication, body mass index (BMI) and adverse pregnancy outcome (APO) was analysed. Our hypothesis was that LCN2 levels were associated with disease activity in pregnant SLE women and that LCN2 levels differ between SLE, RA and healthy controls.

Methods

The RevNatus biobank, RevNatus registry and patient population

The RevNatus registry is a Norwegian multicentre prospective quality registry following women with inflammatory rheumatic disease from preconception until one year postpartum (18). The women included at St. Olavs University Hospital, Trondheim give blood samples to the RevNatus biobank. In this study, patients were included from the RevNatus registry and biobank with the diagnosis of SLE and RA. Patient diagnosis was set by a rheumatologist, with the corresponding ICD-10 codes M32.1, M32.8 and M32.9 for SLE and M06.0, M05.8 and M05.9 for RA. Eighteen healthy pregnant women were recruited from local general practitioners` offices and via the St. Olavs University Hospital website.

Ethics

This study is approved by the Regional Committee for medical and health research ethics of Central Norway (REK-no.: 2015/2014-1). All women included in the RevNatus registry, and the research biobank have given their informed written consent.

Blood serum samples

We analysed serum samples from the RevNatus biobank in this study. Serum samples from women with SLE and RA were collected at seven different time points: before pregnancy (time point 0), in each trimester: week 10–12, week 23–25 and week 32–35 (time points 1–3) and 6 weeks, 6 months and 12 months postpartum (time points 4–6).

From healthy pregnant women we analysed serum samples from each trimester, 6 weeks and 6 months postpartum (time points 1–5). Serum samples were processed and stored frozen at -80 °C by Biobank1®, regional research biobank in Mid-Norway, until use.

Clinical variables

Clinical variables collected from the RevNatus registry were disease duration, previous pregnancies, BMI, CRP, disease activity, kidney function, use of medication and APO.

CRP values were measured at each visit. We defined all values <5 mg/l (the lower detection limit) as 2 mg/l. Kidney function was reported as creatinine in µmol/l at each visit.

Disease activity in SLE patients was evaluated by the Lupus Activity Index in Pregnancy (LAI-P), a modification of the Lupus Activity Index (LAI) validated for use in pregnancy (19). Disease activity was assessed on a scale from 0 (inactive disease) to 2.6 (very high disease activity), with a score above 0.5 considered moderate/high disease activity. We defined 3 groups of disease activity in SLE: None (LAI-P=0), low (LAI-P >0 to ≤0.5), moderate/high (LAI-P >0.5).

In RA disease activity was evaluated using the 3-variable Disease Activity Score in 28 joints (DAS28) using the CRP level (20). The 3-variable DAS28-CRP is considered to be the best index for evaluating disease activity in pregnant women with RA (21). Disease activity categories were defined according to the EULAR (European Alliance of Associations for Rheumatology) criteria using DAS28-score CRP as: Remission (DAS28-CRP ≤2.6), low disease activity (DAS28-CRP >2.6 to ≤3.2), moderate disease activity (DAS28-CRP >3.2 to ≤5.1), and high disease activity (DAS28-CRP >5.1) (22).

The medication variable was divided into two variables. The first was prednisolone use (yes/no) and the second was use of synthetic and/or biologic disease-modifying anti-rheumatic drugs (DMARDs). The medication variable synthetic and/or biologic DMARDs was further divided into three levels for SLE and RA pregnancies. In RA, the medication variable was divided into; no use of DMARDs = 0, use of a synthetic DMARDs = 1, use of biologic DMARDs = 2. In SLE, the medication variable was divided into; no use of DMARDs = 0, use of hydroxychloroquine = 1, use of azathioprine = 2.

We defined APO as preeclampsia/eclampsia, and/or preterm birth < week 37, and/or low birth weight <2500 gram. Preterm birth and/or low birth weight caused by infections, premature preterm rupture of membranes, chromosomal abnormality and anatomical malformations were excluded.

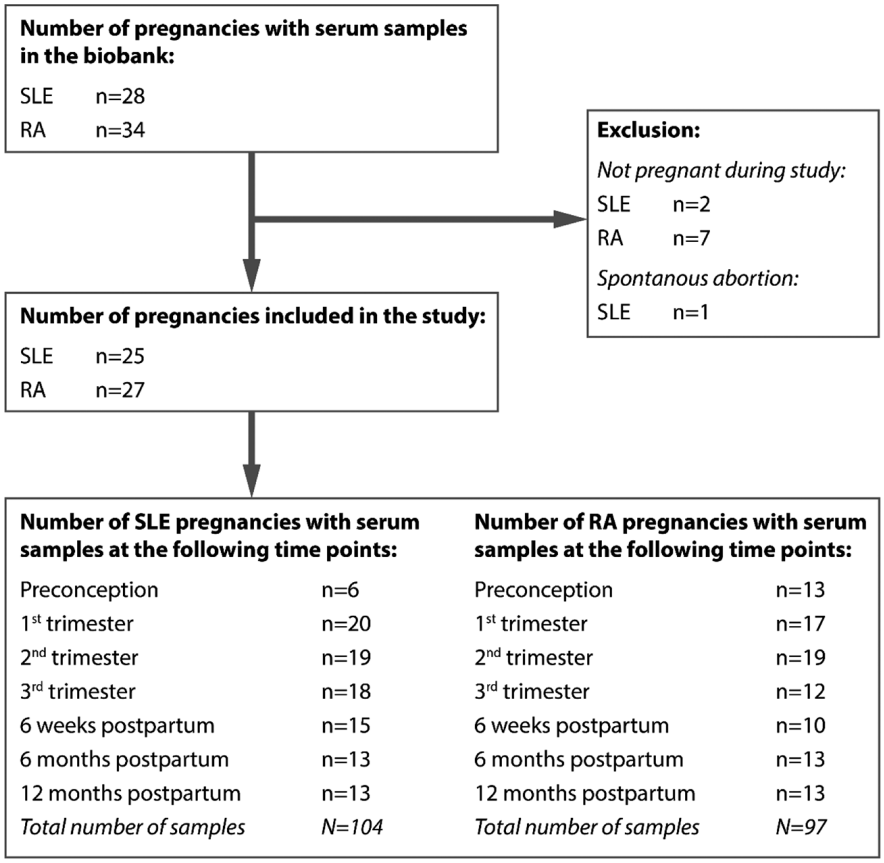

Fig. 1. Flow chart showing inclusion of SLE and RA pregnancies and number of serum samples at each time point.

LCN2 assay

Serum samples were diluted 1:120 in phosphate-buffered saline (PBS) with 1% bovine serum albumin. Samples were analysed for LCN2 in random order using a commercially available ELISA kit according to the manufacturer's protocol (DuoSet ELISA Human Lipocalin-2/NGAL, R&D Systems, Minneapolis, USA). A standard curve for LCN2 was performed on each assay plate. Sample analysis was performed in triplicates from which the mean LCN2 was calculated and used in further statistical analyses.

Statistical analyses

LCN2 levels were log-transformed (base 10) in all statistical analyses, so that observations or residuals from statistical models were approximately normally distributed assessed by histograms and quantile-quantile plots. Log-LCN2 levels were used to perform t-tests to compare two groups (SLE *vs*. healthy, SLE *vs*. RA, RA *vs*. healthy) at each time point. The same data was also analysed using linear mixed effects models (LMM) for all time points together. Here diagnosis (three levels: SLE, RA and healthy) and time point 0–6 for SLE and RA and time point 1–5 for healthy were included as fixed effects. In the LMM models measurements from the same pregnancy were modelled with a random intercept in pregnancy. In total 70 pregnancies in 64 women were included, six women had two pregnancies.

Table I. Characteristics of study population (n=number of pregnancies).

Characteristics	SLE (n=25)	RA (n=27)	Healthy (n=18)
Age, mean ± SD (years)	30.0 ± 5.7	32.0 ± 4.0	30.1 ± 2.5
Disease duration, mean ± SD (years)	6.52 ± 4.12	6.74 ± 4.96	n.a.
BMI in 1st trimester, mean ± SD	23.8 ± 3.0	26.8 ± 4.7	23.5 ± 4.7
Creatinine µmol/l in 1st trimester, median (Q1-Q3)	51 (47-54)	47 (41-51)	–
Nulliparous* n (%)	9 (36)	12 (44.4)	7 (38.9)
Adverse pregnancy outcome (APO) n (%)	2 (8)	4 (15)	0 (0)

*Never given birth to a live child.

Associations between log-LCN2 and BMI in SLE, RA and healthy in 1st, 2nd and 3rd trimester in pregnancy (time points 1–3) were analysed using LMM in three separate analyses. The analysis was restricted to time points 1–3 since we only had BMI measurements from healthy at these time points. In addition, the overall association between log-LCN2 and BMI for all three groups were evaluated using LMM, again with pregnancy as a random intercept.

Data from the SLE and RA pregnancies alone were in addition analysed with a LMM model where the following explanatory clinical variables were included as fixed effects: time point (factor with levels 0–6), diagnosis (SLE or RA), BMI (continuous measurement), CRP (continuous measurement), disease activity (three levels), medication variables (prednisolone use, synthetic and biologic DMARDs) and adverse pregnancy outcome (0=normal, 1=APO). Kidney function was normal for all SLE and RA women and therefore not included in the LMM analysis. In total 52 pregnancies in 46 women were included; six women had two pregnancies.

A significance level of 0.05 was used. Data wrangling was done in R (23). Statistical analyses were performed in SPSS v. 27.0.1.0 (IBM). Graphs and plots were produced using GraphPad Prism (v. 9.1.2, GraphPad Software, Inc.)

Results

Patient inclusion data and characteristics

We included 25 SLE pregnancies in 23 women; three of the 28 pregnancies in the biobank were excluded because the women had not conceived (n=2) at the time of analysis or had a spontaneous abortion (n=1) (Fig. 1). The mean age of SLE women was 30.0 years with a mean disease duration of 6.5 years. As control populations, we included 27 RA pregnancies in 23 women and 18 healthy pregnancies in 18 women. Seven RA pregnancies were excluded because they had not conceived. The mean age of women in the RA group was 32.0 years, while in the healthy women group 30.1 years (Table I). The mean BMI in the SLE and healthy pregnant group in the 1st trimester was 23.8 and 23.5, respectively (Table I). In the RA group, mean BMI was 26.8 in the 1st trimester. All SLE women had normal

Table II. Disease activity, medication and CRP from preconception until 12 months postpartum in SLE and RA.

	Preconception	1st trimester	2nd trimester	3rd trimester	6 weeks postpartum	6 months postpartum	12 months postpartum
SLE							
Disease activity, number/total number (%)							
None	5/6 (83)	12/20 (60)	17/19 (89)	16/18 (89)	14/15 (93)	7/13 (54)	10/13 (77)
Low	1/6 (17)	8/20 (40)	2/19 (11)	2/18 (11)	1/15 (7)	6/13 (46)	3/13 (23)
Moderate	0/6 (0)	0/20 (0)	0/19 (0)	0/18 (0)	0/15 (0)	0/13 (0)	0/13 (0)
Immunosuppressive medication, number/total number (%)							
Synthetic DMARDs	5/6 (83)	19/20 (95)	18/19 (95)	17/18 (94)	15/15 (100)	12/13 (92)	12/13 (92)
Biologic DMARDs	0/6 (0)	0/20 (0)	0/19 (0)	0/18 (0)	0/15 (0)	0/13 (0)	0/13 (0)
Prednisolone	1/6 (17)	8/20 (40)	9/19 (47)	8/18 (44)	9/15 (60)	8/13 (62)	7/13 (54)
No medication	1/6 (17)	0/20 (0)	1/19 (5)	1/18 (6)	0/15 (0)	1/13 (8)	1/13 (8)
CRP mg/l, median (Q1-Q3)	2 (2-2)	2 (2-2)	2 (2-2)	2 (2-2)	2 (2-2)	2 (2-2)	2 (2-2)
RA							
Disease activity, number/total number (%)							
Remission	5/13 (38)	10/17 (59)	12/19 (63)	8/12 (67)	4/10 (40)	7/13 (54)	5/13 (38)
Low	3/13 (23)	1/17 (6)	2/19 (11)	1/12 (8)	5/10 (50)	5/13 (38)	4/13 (31)
Moderate	5/13 (38)	6/17 (35)	5/19 (26)	3/12 (25)	1/10 (10)	1/13 (8)	4/13 (31)
Immunosuppressive medication, number/total number (%)							
Synthetic DMARDs	13/13 (100)	5/17 (29)	13/19 (68)	10/12 (83)	7/10 (70)	11/13 (85)	10/13 (77)
Biologic DMARDs	8/13 (62)	5/17 (29)	3/19 (16)	4/12 (33)	3/10 (30)	6/13 (46)	6/13 (46)
Prednisolone	4/13 (31)	3/17 (18)	6/19 (32)	6/12 (50)	5/10 (50)	5/13 (38)	3/13 (23)
No medication	0/13 (0)	8/17 (47)	5/19 (26)	2/12 (17)	2/10 (20)	1/13 (8)	3/13 (23)
CRP mg/l, median (Q1-Q3)	2 (2-9)	2 (2-14)	2 (2-10)	2 (2-6)	2 (2-2)	2 (2-2)	2 (2-2)

Values for disease activity and medication are number/total number (%).
In SLE we evaluated disease activity using LAI-P: None (LAI-=0), low (LAI-P > 0 to ≤0.5) and moderate (LAI-P >0.5). In RA we used DAS28-CRP: Remission (DAS28-CRP ≤2.6), low disease activity (DAS28-CRP >2.6 to ≤3.2), moderate disease activity DAS28-CRP >3.2 to ≤5.1).

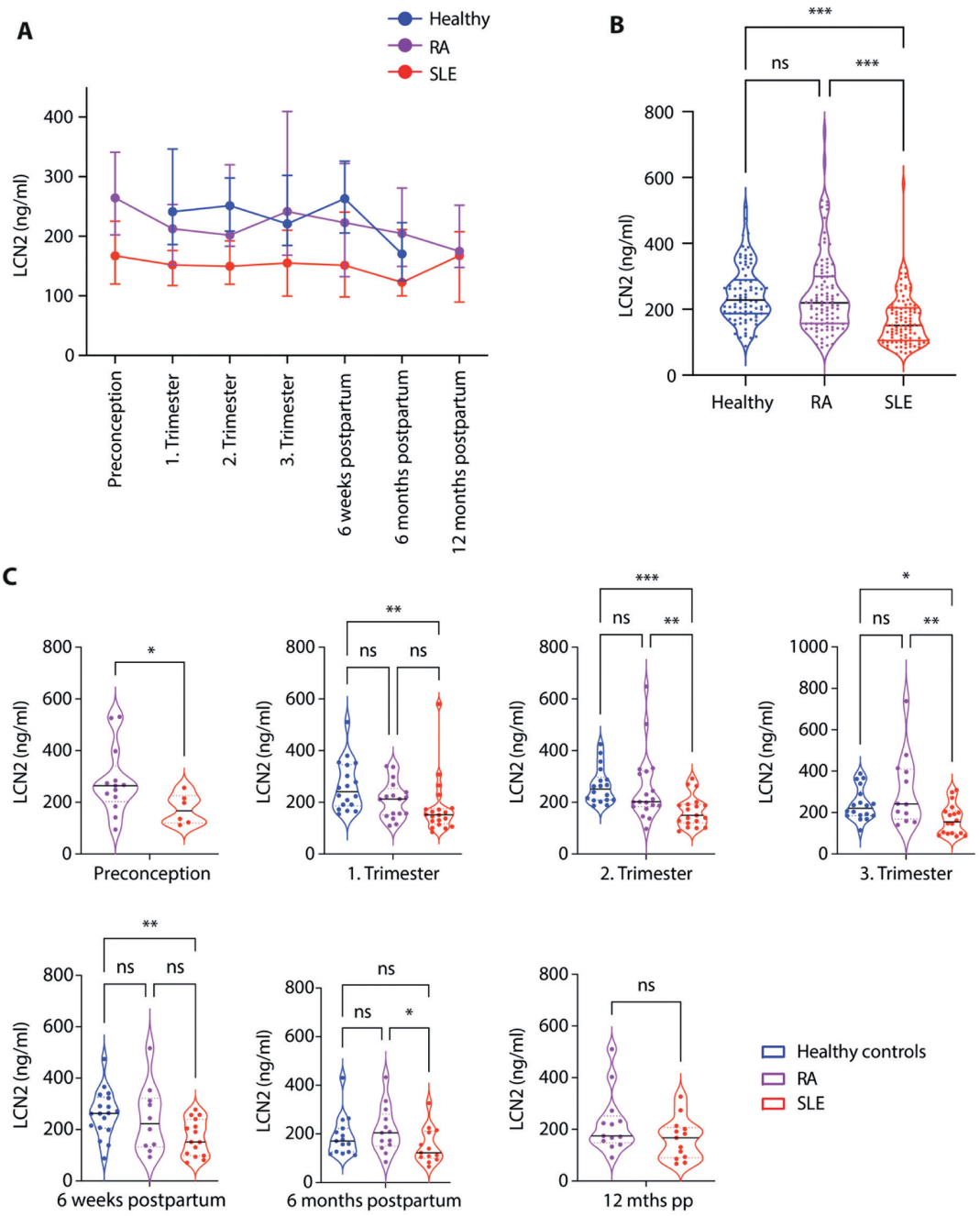

Fig. 2. Serum LCN2 levels in SLE, RA and healthy pregnancies.
LCN2 was measured in serum from SLE, RA and healthy study participants at seven time points before, during and after pregnancy.
A: Serum LCN2 concentration in SLE, RA and healthy pregnancies over time. Data points and bars indicate median LCN2 measurements and interquartile range at each time point.
B: Combined analysis of LCN2 measurements for SLE, RA and healthy irrespective of the time point of sample acquisition. Statistical analyses were performed using LMM.
C: Serum LCN2 concentrations at the individual time points. Log-transformed datasets were analysed by t-test.
p-values: **p*<0.05; ***p*<0.01; ****p*<0.001, and ns= non-significant, *p*-values >0.05. Serum LCN2 was measured in triplicates for all samples by ELISA. Violin plots in **B** and **C** show all data points (mean of triplicates), median and quartiles (horizontal bars). No samples were available for healthy before pregnancy and 12 months postpartum.

kidney function, and only one woman had a prior history of lupus nephritis.

ALL SLE pregnancies were well-regulated, with none or low disease activity both before pregnancy, in each trimester and postpartum. In 1st trimester 60% had low LAI-P disease activity and 40% had no LAI-P disease activity. In both 2nd and 3rd trimester as many as 89% had no disease activity and 11% had low disease activity. RA women had higher disease activity. In 1st trimester, 35% had moderate disease activity, while 65% had low disease activity or were in remission according to DAS28-CRP (Table II). In SLE pregnancies, the use of synthetic DMARDs, mainly hydroxychloroquine, were high

at all time points (83–100%). The use of prednisolone increased from 17% preconception, 47% in the 2nd trimester, to as high as 62% at six months postpartum. No biologic DMARDs were used in SLE patients (Table II). In RA pregnancies, 47% of patients did not have any immunosuppressive medication and 18% used prednisolone in the 1st trimester. The proportion of RA patients using synthetic DMARDs increased to 68% in 2nd trimester, and 46% used biologic DMARDs six and twelve months postpartum (Table II).
The incidence of adverse pregnancy outcome was small in both groups, with 8% APO in SLE and 14.8% in RA patients (Table I). Only one of the two APOs in the SLE group where in the first pregnancy, all the APOs in the RA group where in the subsequent pregnancy.

Serum LCN2 levels in SLE, RA and healthy pregnancies

LCN2 concentrations were quantified in serum samples from SLE, RA and healthy pregnancies. Seven time points were measured for SLE and RA patients, while serum of healthy pregnancies were measured at five time points. Following LCN2 levels in the three groups over time, we found that LCN2 levels were lowest in SLE patients throughout the observed time period (Fig. 2A). When we combined LCN2 measurements from all time points for each of the groups, the overall median LCN2 concentration was 150.9 ng/ml in SLE pregnancies, while overall median LCN2 in RA and healthy pregnancies were 219.5 ng/ml and 228.3 ng/ml respectively (Fig. 2B). From the LMM analysis we found that the overall serum LCN2 levels were significantly lower in SLE pregnancies compared to RA ($p<0.001$) and healthy pregnancies ($p<0.001$) (Table III). No overall significant difference was found between the log-LCN2 level of healthy and RA pregnancies. Log-LCN2 levels were stable throughout pregnancy and postpartum, except from 6 months postpartum (time point 5) where log-LCN2 was significantly lower (Table III).
Full LMM analysis for SLE and RA patients at all time points, which included the clinical explanatory variables BMI, CRP, disease activity, use of medication and adverse pregnancy outcome (APO), also confirmed a significantly lower overall level of LCN2 in SLE compared to RA pregnancies ($p=0.009$) (Table IV). A total of six women, two SLE and four RA women had two pregnancies. When adjusting the LMM models to include nesting of pregnancies within women (double nesting) we had the same results as in the simple nesting within pregnancies.
Next, we addressed differences in serum LCN2 levels between the SLE, RA and healthy women at individual time points (Fig. 2C). SLE patients showed significantly lower serum LCN2 levels than RA and healthy pregnancies also at individual time points (Fig. 2C). Compared to healthy pregnancies, SLE

Table III. Results from the LMM analyses with dependent variable log-LCN2 for SLE, RA and healthy pregnancies.

		Estimate	95% CI	*p*-value
Diagnosis	SLE *vs*. RA	-0.159	-0.243 to -0.074	<0.001
	SLE *vs*. healthy	-0.178	-0.269 to -0.087	<0.001
	RA *vs*. healthy	-0.019	-0.111 to 0.072	0.674
Time	1 *vs*. 0	-0.026	-0.101 to 0.049	0.497
	2 *vs*. 0	-0.005	-0.081 to 0.070	0.888
	3 *vs*. 0	-0.004	-0.082 to 0.074	0.915
	4 *vs*. 0	-0.024	-0.103 to 0.055	0.557
	5 *vs*. 0	-0.100	-0.179 to -0.021	0.013
	6 *vs*. 0	-0.022	-0.106 to 0.062	0.603

Fixed effects are diagnosis (SLE, RA and healthy) and time (time points 0-6; preconception, 1st-3rd trimester, 6 weeks, 6 months and 12 months postpartum).

Table IV. Results from the full LMM analysis of log10-LCN2 for SLE and RA pregnancies.

		Estimate	95% CI	*p*-value
Diagnosis	SLE *vs*. RA	-0.130	-0.226 to -0.033	0.009
Time	1 *vs*. 0	-0.041	-0.125 to 0.042	0.332
	2 *vs*. 0	-0.032	-0.118 to 0.053	0.458
	3 *vs*. 0	-0.010	-0.105 to 0.085	0.831
	4 *vs*. 0	-0.040	-0.131 to 0.052	0.393
	5 *vs*. 0	-0.052	-0.141 to 0.036	0.247
	6 *vs*. 0	-0.010	-0.100 to 0.079	0.818
CRP		0.003	-0.001 to 0.007	0.184
BMI		0.008	-0.002 to 0.020	0.138
Disease activity	low/moderate *vs*. none/remission	-0.005	-0.053 to 0.042	0.820
Prednisolone use	no *vs*. yes	0.020	-0.046 to 0.085	0.554
Synthetic or biologic DMARDs				
	level 0 *vs*. 2	-0.005	-0.091 to 0.080	0.899
	level 1 *vs*. 2	0.041	-0.029 to 0.111	0.248
Adverse pregnancy outcome	no *vs*. yes	-0.059	-0.196 to 0.079	0.395

Included in this model are the fixed effects CRP, BMI, disease activity, prednisolone use, synthetic and biologic disease-modifying anti-rheumatic drugs (DMARDs) and adverse pregnancy outcome (APO).

Table V. Association between log-LCN2 and BMI in SLE, RA and healthy pregnancies.

		Estimate	95% CI	*p*-value
All	(Time 0-6)	0.011	0.004 to 0.018	0.003
SLE	(Time 1-3)	-0.001	-0.016 to 0.013	0.850
RA	(Time 1-3)	0.014	0.002 to 0.026	0.018
Healthy	(Time 1-3)	0.007	-0.003 to 0.017	0.152

All, include RA and SLE at time points 0–6 and healthy time points 1–3. Then, each group (SLE, RA and healthy) is analysed separately at time points 1–3.

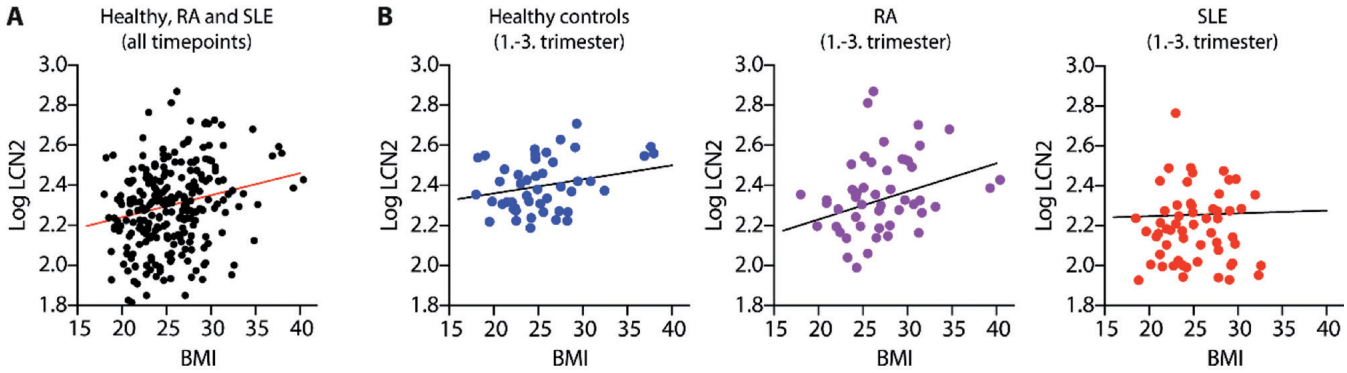

Fig. 3. Association between BMI and serum log-LCN2 levels.
BMI were measured in healthy in each trimester (time points 1–3). In SLE and RA patients BMI were in addition measured at preconception, 6 weeks, 6 months and 12 months postpartum (time points 0, 4–6).
A: Association between BMI and log-LCN2 for all time points for SLE, RA and healthy.
B: Association between BMI and log-LCN2 in healthy, RA and SLE at time points 1–3.

patients had significantly lower serum LCN2 concentrations in each trimester as well as six weeks postpartum. We did not find significant differences in LCN2 levels between SLE and healthy pregnancies at six months postpartum. Compared to RA pregnancies, we found in SLE patients significantly lower serum LCN2 concentrations before pregnancy, in 2nd and 3rd trimester and six months postpartum. No significant differences in serum LCN2 were observed between RA and healthy pregnancies at any time point during or after pregnancy (Fig. 2C). Interestingly, we observed large variations of LCN2 levels in 2nd and 3rd trimester in RA patients (Fig. 2C).

Serum LCN2 levels and BMI

We found an overall significant association between log-LCN2 and BMI when including available BMI data from SLE, RA and healthy pregnancies at all time points, not adjusting for other variables (p=0.003) (Table V, Fig. 3A). BMI increased with increasing levels of log-LCN2. BMI were measured in healthy pregnancies in each trimester (time points 1–3), while for SLE and RA pregnancies we also had BMI before (time point 0) and after pregnancy (time points 4–6). Analysis of log-LCN2 in relation to BMI in each trimester (time points 1–3) in SLE, RA and healthy pregnancies showed a significant association between log-LCN2 and BMI in RA women (p=0.018), but not in SLE (p=0.850) or healthy pregnancies (p=0.152) (Table V, Fig. 3B).

When BMI was included in the full LMM model for SLE and RA pregnancies, that includes clinical variables, we found no significant overall association with LCN2 (Table IV).

Serum LCN2 levels and disease activity, CRP, medication and adverse pregnancy outcome

LCN2 levels were not significantly associated with disease activity, CRP or medication status (Table IV). SLE women had a quiescent disease throughout pregnancy, all with low disease activity or remission at all time points. CRP levels were also low in both SLE and RA pregnancies (Table II). Median CRP in 1st trimester was 2 in both groups, with quartiles 2-14 (Q1-Q3) in RA pregnancies, and even lower in SLE pregnancies with quartiles 2-2.

No association were found neither between LCN2 levels and use of prednisolone (yes/no) or LCN2 levels and use of synthetic/biologic DMARDs.

We found no association between adverse pregnancy outcome and LCN2, but the numbers for adverse pregnancy outcome were small (2 in SLE, 4 in RA, 0 in healthy) in all groups (Table IV).

Discussion

In this study we investigated serum LCN2 levels longitudinally throughout pregnancy and postpartum in SLE compared to RA and healthy pregnancies. We found significantly lower LCN2 levels in SLE at all time points compared to RA and healthy. In our cohort of SLE women the disease activity was low or absent throughout pregnancy and postpartum and the numbers of adverse pregnancy outcomes were low. We could not find an association between LCN2 and disease activity or adverse pregnancy outcome in either SLE or RA pregnancies. However, we observed an overall significant association between LCN2 levels and BMI when including BMI from all three groups at all time points. To our knowledge, this is the first study of LCN2`s role in SLE pregnancies.

Few studies have evaluated the role of LCN2 blood levels in association with disease activity and flare in SLE. Most studies on LCN2 in SLE, have investigated urine LCN2 as a biomarker of lupus nephritis, reviewed by Gao *et al.* (10). In studies evaluating LCN2 in blood as a biomarker, a large proportion of the SLE patients had active disease and a majority of patients had lupus nephritis. In a study by Torres-Salido *et al.*, a significant difference in serum LCN2 was found between active lupus nephritis and non-active SLE (13). Two studies show that SLE patients with nephritis have higher serum LCN2 levels than those without nephritis (14, 16). In our cohort of pregnant SLE patients with low or absent disease activity and only one patient with prior lupus nephritis, LCN2 levels are low, in accordance with these findings.

In pregnancy, studies have shown an association between LCN2 and the presence and severity of preeclampsia (7, 24). Most studies are done regarding preeclampsia, there is only a few

studies on other adverse pregnancy outcomes as small for gestational age (SGA). In a study by Karampas *et al*. no association between LCN2 and SGA were found (25). In our study, only 2 of 25 SLE pregnancies had adverse pregnancy outcomes, both had preterm birth and babies with low birth weight, one caused by preeclampsia. Skorpen *et al*. with a larger SLE cohort from the same RevNatus registry has shown that APO is associated with disease activity. In inactive disease 4 out of 85 (4.7%) pregnancies had preeclampsia and 11 out of 85 (12.9%) pregnancies had preterm birth (26). Even if the majority of SLE patients in our cohort had inactive disease, the numbers of APOs found by Skorpen *et al*. is higher than in our study. We could not find an association between adverse pregnancy outcome and LCN2 levels, but the numbers were small.

LCN2 is also an adipokine, one of many pro-inflammatory adipokines that contribute to the low-grade inflammatory state in obese subjects. An association between LCN2 and obesity and insulin resistance are established (27). Our finding of a significant overall association between LCN2 and BMI is in line with previous observations. When we looked at the groups separately, we only saw this association between LCN2 levels and BMI in RA patients. RA women had a higher mean BMI indicating overweight, while SLE and healthy women had a BMI in the range of normal weight. When adjusting for other clinical variables, the association between BMI and LCN2 was no longer significant, but a positive estimated association was found. The association we found to BMI was stronger than the association to the more general marker of inflammation investigated, CRP. LCN2 was estimated to increase with higher CRP levels, but the association was not significant. A strength in our study is the longitudinally design; we followed SLE pregnancies with both clinical data and serum LCN2 from preconception until 12 months postpartum using both disease and healthy controls. When studying pregnancy in rheumatic diseases, biologic samples in general and longitudinal cohorts in particular are scarce. Though our cohort is large compared to other similar published studies, the sample size is still small. The comparison of SLE with RA and healthy pregnancies is another strength of our study. SLE do not have the phenomenon of pregnancy-related improvement which is observed in RA (28). Thus, a deeper understanding of how the immunologic processes differs between pregnant women with SLE and RA is important. Comparative analyses of immune-modulation during pregnancy in RA and SLE have been called for (28).

Well-planned pregnancies and mainly White European patients in our cohort contribute to low disease activity and few adverse pregnancy outcomes compared to what is seen in other cohorts (29, 30). Health services in Norway are universal, and a close follow-up in pregnancy is offered to all women with rheumatic diseases. In addition, a main goal of NKSR and the RevNatus registry is to encourage to well-planned pregnancy and secure remission or low disease activity six months prior to gestation. SLE is a disease with different manifestations and severity depending on ethnicity. In our cohort of SLE patients, 19 out of 23 were White European. White have more often a non-severe SLE and a smaller proportion have lupus nephritis (31, 32). This cohort with pregnant SLE patients with low disease activity provides us with supplementary data to what is reported in less well-regulated cohorts. However, the low disease activity and few adverse pregnancy outcomes are the main limitations of our study. This limits the possibility to investigate if LCN2 may have a value as a potential biomarker in SLE pregnancies with moderate to high disease activity and cohorts that show higher frequencies of adverse pregnancy outcomes.

We found significantly lower LCN2 levels in SLE pregnancies at all timepoints compared to both RA and healthy pregnancies. This finding was true also when BMI, as well as medication, pregnancy outcome and disease activity were included in the model. It has been suggested that LCN2 may have a role in the adaptive immunity by inducing immune tolerance. A study supporting this theory, found LCN2-deficient mice more susceptible to induction of autoimmunity (33). Another study, done in an *in vitro* model, reported that LCN2 increased human leukocyte antigen G (HLA-G) and $FOXP3^+$ T-regulatory cells, two well-known parameters for immune tolerance (9). Findings of low levels of LCN2 in SLE pregnancies might thus indicate low tolerance through low HLA-G and T-regulatory cell levels. Low LCN2 in our cohort of quiescent disease might be a constitutional hallmark of SLE. We wonder if low LCN2 can be a marker of altered immune tolerance seen in SLE in general and in pregnancy. Our findings warrant further exploration to the role of LCN2 as a part of the pathogenesis and immune modulation in SLE.

From our study we conclude that serum LCN2 is not useful as a marker of disease activity in SLE pregnancies with relatively quiescent disease. However, our finding of lower levels of serum LCN2 in pregnant SLE women in remission compared to both RA and healthy controls, may implicate LCN2 in the pathogenesis of altered immune tolerance seen in SLE.

Acknowledgments

The authors appreciate the valuable advice and practical assistance provided by expert personnel at Biobank1®, the research biobank of Central Norway, in collection, processing and storage of the biological material. We thank Hege Svean Koksvik, Bente Jakobsen, Ingrid Rekaa Nilssen and Tone Moksnes at the Norwegian National Advisory Unit on Pregnancy and Rheumatic Diseases, Trondheim for practical help collecting data from the RevNatus registry and Biobank1.

References

1. JAKOBSEN IM, HELMIG RB, STENGAARD-PEDERSEN K: Maternal and foetal outcomes in pregnant systemic lupus erythematosus patients: an incident cohort from a stable referral population followed during 1990-2010. *Scand J Rheumatol* 2015; 44(5): 377-84. https://doi.org/10.3109/03009742.2015.1013982
2. OSTENSEN M, BRUCATO A, CARP H *et al*.: Pregnancy and reproduction in autoimmune rheumatic diseases. *Rheumatology* (Oxford)

2011; 50(4): 657-64. https://doi.org/10.1093/rheumatology/keq350
3. GÖTESTAM SKORPEN C, LYDERSEN S, GILBOE IM *et al*.: Disease activity during pregnancy and the first year postpartum in women with systemic lupus erythematosus. *Arthritis Care Res* (Hoboken) 2017; 69(8): 1201-8. https://doi.org/10.1002/acr.23102
4. BUNDHUN PK, SOOGUND MZ, HUANG F: Impact of systemic lupus erythematosus on maternal and fetal outcomes following pregnancy: A meta-analysis of studies published between years 2001-2016. *J Autoimmun* 2017; 79: 17-27. https://doi.org/10.1016/j.jaut.2017.02.009
5. CLOWSE ME, JAMISON M, MYERS E, JAMES AH: A national study of the complications of lupus in pregnancy. *Am J Obstet Gynecol* 2008; 199(2): 127.e1-6. https://doi.org/10.1016/j.ajog.2008.03.012
6. ABELLA V, SCOTECE M, CONDE J *et al*.: The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases. *Biomarkers* 2015; 20(8): 565-71. https://doi.org/10.3109/1354750x.2015.1123354
7. ARTUNC-ULKUMEN B, GUVENC Y, GOKER A, GOZUKARA C: Relationship of neutrophil gelatinase-associated lipocalin (NGAL) and procalcitonin levels with the presence and severity of the preeclampsia. *J Matern Fetal Neonatal Med* 2015; 28(16): 1895-900. https://doi.org/10.3109/14767058.2014.972926
8. FLO TH, SMITH KD, SATO S *et al*.: Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature* 2004; 432(7019): 917-21. https://doi.org/10.1038/nature03104
9. LA MANNA G, GHINATTI G, TAZZARI PL *et al*.: Neutrophil gelatinase-associated lipocalin increases HLA-G(+)/FoxP3(+) T-regulatory cell population in an *in vitro* model of PBMC. *PLoS One* 2014; 9(2): e89497. https://doi.org/10.1371/journal.pone.0089497
10. GAO Y, WANG B, CAO J, FENG S, LIU B: Elevated urinary neutrophil gelatinase-associated lipocalin is a biomarker for lupus nephritis: a systematic review and meta-analysis. *Biomed Res Int* 2020; 2020: 2768326. https://doi.org/10.1155/2020/2768326
11. HINZE CH, SUZUKI M, KLEIN-GITELMAN M *et al*.: Neutrophil gelatinase-associated lipocalin is a predictor of the course of global and renal childhood-onset systemic lupus erythematosus disease activity. *Arthritis Rheum* 2009; 60(9): 2772-81. https://doi.org/10.1002/art.24751
12. PITASHNY M, SCHWARTZ N, QING X *et al*.: Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. *Arthritis Rheum* 2007; 56(6): 1894-903. https://doi.org/10.1002/art.22594
13. TORRES-SALIDO MT, CORTÉS-HERNÁNDEZ J, VIDAL X, PEDROSA A, VILARDELL-TARRÉS M, ORDI-ROS J: Neutrophil gelatinase-associated lipocalin as a biomarker for lupus nephritis. *Nephrol Dial Transplant* 2014; 29(9): 1740-9. https://doi.org/10.1093/ndt/gfu062
14. LA'ULU SL, SUH-LAILAM BB, DAVIS KW, STRASESKI JA, TEBO AE: Comparative analysis of neutrophil gelatinase-associated lipocalin and other laboratory markers for lupus nephritis: a cross-sectional investigation. *Ann Clin Biochem* 2017; 54(2): 240-5. https://doi.org/10.1177/0004563216651888
15. EL SHAHAWY MS, HEMIDA MH, ABDEL-HAFEZ HA, EL-BAZ TZ, LOTFY AM, EMRAN TM: Urinary neutrophil gelatinase-associated lipocalin as a marker for disease activity in lupus nephritis. *Scand J Clin Lab Invest* 2018; 78(4): 264-8. https://doi.org/10.1080/00365513.2018.1449242
16. JAFARI NAKHJAVANI MR, ABEDIAZAR S, GHORBANIHAGHJO A, HANAFIZADEH B, ZUNUNI VAHED S, POURLAK T: The importance of serum neutrophil gelatinase-associated lipocalin level in patients with lupus nephritis. *J Renal Inj Prev* 2019; 8(2): 133-9. https://doi.org/10.15171/jrip.2019.25
17. MIRIOGLU S, CINAR S, YAZICI H *et al*.: Serum and urine TNF-like weak inducer of apoptosis, monocyte chemoattractant protein-1 and neutrophil gelatinase-associated lipocalin as biomarkers of disease activity in patients with systemic lupus erythematosus. *Lupus* 2020; 29(4): 379-88. https://doi.org/10.1177/0961203320904997
18. MEISSNER Y, STRANGFELD A, COSTEDOAT-CHALUMEAU N *et al*.: European Network of Pregnancy Registers in Rheumatology (EuNeP) - an overview of procedures and data collection. *Arthritis Res Ther* 2019; 21(1): 241. https://doi.org/10.1186/s13075-019-2019-3
19. RUIZ-IRASTORZA G, KHAMASHTA MA, GORDON C *et al*.: Measuring systemic lupus erythematosus activity during pregnancy: validation of the lupus activity index in pregnancy scale. *Arthritis Rheum* 2004; 51(1): 78-82. https://doi.org/10.1002/art.20081
20. PREVOO ML, VAN 'T HOF MA, KUPER HH, VAN LEEUWEN MA, VAN DE PUTTE LB, VAN RIEL PL: Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38(1): 44-8. https://doi.org/10.1002/art.1780380107
21. DE MAN YA, HAZES JM, VAN DE GEIJN FE, KROMMENHOEK C, DOLHAIN RJ: Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. *Arthritis Rheum* 2007; 57(5): 716-22. https://doi.org/10.1002/art.22773
22. VAN GESTEL AM, PREVOO ML, VAN 'T HOF MA,VAN RIJSWIJK MH, VAN DE PUTTE LB, VAN RIEL PL: Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis Rheum* 1996; 39(1): 34-40. https://doi.org/10.1002/art.1780390105
23. R CORE TEAM. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2021.
24. KIM SM, PARK JS, NORWITZ ER *et al*.: Circulating levels of neutrophil gelatinase-associated lipocalin (NGAL) correlate with the presence and severity of preeclampsia. *Reprod Sci* 2013; 20(9): 1083-9. https://doi.org/10.1177/1933719113477480
25. KARAMPAS G, ELEFTHERIADES M, PANOULIS K *et al*.: Maternal serum levels of neutrophil gelatinase-associated lipocalin (NGAL), matrix metalloproteinase-9 (MMP-9) and their complex MMP-9/NGAL in pregnancies with preeclampsia and those with a small for gestational age neonate: a longitudinal study. *Prenat Diagn* 2014; 34(8): 726-33. https://doi.org/10.1002/pd.4337
26. SKORPEN CG, LYDERSEN S, GILBOE IM *et al*.: Influence of disease activity and medications on offspring birth weight, pre-eclampsia and preterm birth in systemic lupus erythematosus: a population-based study. *Ann Rheum Dis* 2018; 77(2): 264-9. https://doi.org/10.1136/annrheumdis-2017-211641
27. RECINELLA L, ORLANDO G, FERRANTE C, CHIAVAROLI A, BRUNETTI L, LEONE S: Adipokines: new potential therapeutic target for obesity and metabolic, rheumatic, and cardiovascular diseases. *Front Physiol* 2020; 11: 578966. https://doi.org/10.3389/fphys.2020.578966
28. FORGER F, VILLIGER PM: Immunological adaptations in pregnancy that modulate rheumatoid arthritis disease activity. *Nat Rev Rheumatol* 2020; 16(2): 113-22. https://doi.org/10.1038/s41584-019-0351-2
29. STOJAN G, BAER AN: Flares of systemic lupus erythematosus during pregnancy and the puerperium: prevention, diagnosis and management. *Expert Rev Clin Immunol* 2012; 8(5): 439-53. https://doi.org/10.1586/eci.12.36
30. SMYTH A, OLIVEIRA GH, LAHR BD, BAILEY KR, NORBY SM, GAROVIC VD: A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. *Clin J Am Soc Nephrol* 2010; 5(11): 2060-8. https://doi.org/10.2215/cjn.00240110
31. GONZALEZ LA, TOLOZA SM, MCGWIN G JR, ALARCON GS: Ethnicity in systemic lupus erythematosus (SLE): its influence on susceptibility and outcomes. *Lupus* 2013; 22(12): 1214-24. https://doi.org/10.1177/0961203313502571
32. PARIKH SV, ALMAANI S, BRODSKY S, ROVIN BH: Update on Lupus Nephritis: Core Curriculum 2020. *Am J Kidney Dis* 2020; 76(2): 265-81. https://doi.org/10.1053/j.ajkd.2019.10.017
33. PAWAR RD, GOILAV B, XIA Y *et al*.: Serum autoantibodies in pristane induced lupus are regulated by neutrophil gelatinase associated lipocalin. *Clin Immunol* 2014; 154(1): 49-65. https://doi.org/10.1016/j.clim.2014.06.007