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MATERNAL-FETAL MEDICINE

Calprotectin in pregnancy and pregnancy-associated diseases: a systematic review and prospective cohort study

Günther A. Rezniczek[1] · Claudia Förster[1] · Ziad Hilal[1] · Timm Westhoff[2] · Clemens B. Tempfer[1]

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Abstract

Purpose Calprotectin, a marker of acute and chronic inflammation, may play a role in pregnancy-associated disorders. We aimed to summarize available clinical data on calprotectin in pregnancy and to establish normal values of calprotectin during the course of pregnancy.

Methods We performed a systematic review of the databases PubMed and Cochrane Central Register of Controlled Trials to identify experimental and clinical evidence assessing the role of calprotectin in pregnancy. In addition, we performed a prospective cohort study assessing serum and urine calprotectin throughout pregnancy.

Results We identified 17 studies investigating 1638 pregnant women, 151 newborns, and 99 non-pregnant controls, measuring calprotectin in different compartments. Calprotectin was present in meconium and elevated in fecal samples of pregnant women with active inflammatory bowel disease. In women with pregnancy-induced hypertension, mild and severe preeclampsia (PE), calprotectin was significantly elevated in maternal plasma and serum, but not in fetal serum, amniotic fluid, and umbilical cord blood. For the cohort study, we recruited 196 pregnant women. PE and concomitant renal disease were present in 6/196 (3%) and 11/196 (5.6%) of women, respectively. Throughout pregnancy, median serum and urine levels of calprotectin largely exceed reported concentrations of the healthy non-pregnant population, but showed no significant variations between trimesters 1–3 and post-partum. Calprotectin in serum was correlated with systolic blood pressure and in urine with leukocytes and total protein. No significant differences were found in subgroup analyses of smokers vs. non-smokers, PE vs. none, and renal disease (kidney stones, reflux) vs. none.

Conclusion Calprotectin concentrations in amnion fluid and stools serve as potential indicators of inflammatory states during pregnancy. Urinary calprotectin concentrations are continuously high during pregnancy and show no significant variations between trimesters 1–3 and post-partum.

Keywords Calprotectin · Pregnancy · Renal disease · Preeclampsia

Abbreviations

AF	Amniotic fluid
CAL	Calprotectin
CCS	Case–control study
CR	Case report
CS	Cesarean section
ELISA	Enzyme-linked immunosorbent assay
IBD	Inflammatory bowel disease
IFN	Interferon
IL	Interleukin
IUGR	Intrauterine growth restriction
PAHD	Pregnancy-associated hypertensive disorder
PCCS	Prospective case–control study
PCOS	Prospective cohort study
PCS	Prospective case series
PE	Preeclampsia
PIH	Pregnancy-induced hypertension
PROM	Premature rupture of membranes
RCT	Randomized controlled trial
SID	Systemic inflammatory disease

✉ Clemens B. Tempfer
clemens.tempfer@rub.de

Günther A. Rezniczek
guenther.rezniczek@rub.de

[1] Department of Obstetrics and Gynecology, Ruhr-Universität Bochum – Marien Hospital Herne, Hölkeskampring 40, 44625 Herne, Germany

[2] Department of Internal Medicine and Nephrology, Ruhr-Universität Bochum, Bochum, Germany

Introduction

Calprotectin, also known as leucocyte protein L1, calgranulin A/B, MRP 8/14, cystic fibrosis antigen, myeloid-histiocyte antigen, CP-10, and 27E10, is a heterodimer of the two calcium binding proteins S100A8 and S100A9. Calprotectin is present in the cytosol of various immune cell populations such as neutrophil granulocytes, monocytes, and macrophages and is expressed on the cell surface of monocytes and immature macrophages [1]. In neutrophils, for example, calprotectin is the main cytosolic component accounting for around 60% of the total soluble protein content. Upon activation of neutrophil granulocytes or after endothelial adhesion of monocytes, calprotectin is released into the interstitial tissue and the blood circulation as a pro-inflammatory response to infectious agents, tissue damage, and other cellular deviations such as cancer. It thereby serves as a danger-associated molecular pattern protein (DAMP) in the innate immune system [2]. Calprotectin has bacteriostatic and cytokine-like properties. Specifically, it enhances interleukin (IL)-1β secretion of interferon (IFN)-γ primed monocytes [3] and is associated with elevated levels of the pro-inflammatory serum cytokines IFN-γ, c-reactive protein, IL-6, tumor necrosis factor-β, and IL-17A [2]. In addition, calprotectin has been described to exert antimicrobial activity by depriving microorganisms of zinc [4].

Mononuclear phagocytes infiltrating a site of inflammation typically contain and express calprotectin, underlining its prominent role in the cellular immune response. Calprotectin has been associated with acute immunological activity, chronic inflammation, and other disorders, among them various human cancers [5]. Calprotectin can be measured in serum, plasma, and other body fluids such as meconium and amniotic fluid. In addition, high concentrations of calprotectin are found in stool. Fecal calprotectin has therefore been investigated as a marker of inflammatory activity in gastrointestinal disorders such as Crohn's disease, cystic fibrosis, ulcerative colitis, inflammatory bowel disease (IBD), and juvenile idiopathic arthritis [6, 7]. For example, in a meta-analysis of 49 studies in > 3000 IBD patients, fecal calprotectin demonstrated a pooled sensitivity of 85% and a specificity of 75% for diagnosing active IBD [8]. Beyond its diagnostic use in gastroenterology, it is also discussed as an inflammatory biomarker in rheumatology and nephrology. In rheumatoid arthritis, for example, both serum and synovial fluid calprotectin levels correlate with disease activity and radiographic disease progression [9]. In nephrology, calprotectin is a sensitive indicator of acute kidney injury in adult, pediatric, and transplant populations [10–12].

In contrast to IBD, rheumatology, and nephrology, the role of calprotectin in pregnancy and pregnancy-associated disorders is less well understood. Liosi et al., for example, assessed calprotectin in umbilical cord blood samples of term neonates and found that cord blood calprotectin concentrations at term were independent of intrauterine growth, gender, parity, and maternal age [13]. They concluded that calprotectin cord blood levels did not reflect the increased neutrophil activation and excessive apoptosis known to be associated with intrauterine growth restriction. On the other hand, plasma levels as well as placental expression of calprotectin have been observed to be elevated among pregnant women with hypertensive disorders [14, 15]. Since maternal, but not umbilical cord levels of calprotectin were found to be elevated, it has been hypothesized that calprotectin may be a potential marker of the maternal immune response to the pro-inflammatory endothelial activation in hypertensive disorders of pregnancy, but does not indicate changes in the fetal compartment [15]. Physiological levels of calprotectin during the course of normal pregnancy have not been established to date [13–15]. Therefore, we performed a systematic review of the literature assessing available clinical and experimental data examining the role of calprotectin in pregnant women to better define the role of calprotectin during pregnancy and pregnancy-related disease. In addition, we performed the first prospective study in a cohort of pregnant women assessing serum and urine calprotectin levels during all three trimesters of pregnancy and post-partum to establish normal values of serum and urine calprotectin during pregnancy.

Materials and methods

Systematic review of the literature

We performed a systematic literature search of the databases PubMed and Cochrane Central Register of Controlled Trials using the specific search terms "leukocyte L1 antigen complex"[MeSH Terms] OR ("leukocyte"[All Fields] AND "L1"[All Fields] AND "antigen"[All Fields] AND "complex"[All Fields]) OR "leukocyte L1 antigen complex"[All Fields] OR "calprotectin"[All Fields]) AND ("pregnancy"[MeSH Terms] OR "pregnancy"[All Fields]. Abstracts of all identified studies were retrieved and screened. Studies meeting the inclusion criteria for this review, i.e., reporting on calprotectin measurements in pregnant women in the maternal, placental, or fetal compartments, were obtained in full text. Calprotectin measurements in all body fluids or tissues were allowed, e.g., serum, plasma, amniotic fluid, placenta, and stool. A flowchart of the literature search is shown in Fig. 1. Full text papers were assessed according to PRISMA criteria [16]. Patient data

were extracted from studies and a pooled analysis was performed. Meta-analysis was not possible due to the heterogeneity of the identified studies.

Prospective cohort study

We performed a prospective cohort study of pregnant women treated at the Department of Obstetrics, Marien Hospital Herne, Ruhr-Universität Bochum, between 5/2015 and 1/2018. This study was approved by the Ethics Committee of the Ruhr-Universität Bochum Medical Faculty. All study participants provided written informed consent. We included healthy pregnant women and those with pregnancy-associated hypertensive disorders such as pregnancy-induced hypertension and preeclampsia and/or kidney disorders. Serum and urine specimens were obtained from all patients. Probands were recruited during the first, second, and third trimester in a 1:1:3 ratio, assuming that potential calprotectin changes would be best assessable during the third trimester of pregnancy [14, 15]. In addition, serum and urine samples were collected during the post-partum period until a maximum of 4 weeks after delivery. Calprotectin was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (PhiCal Calprotectin, catalog number K 6928, Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions as previously described [17].

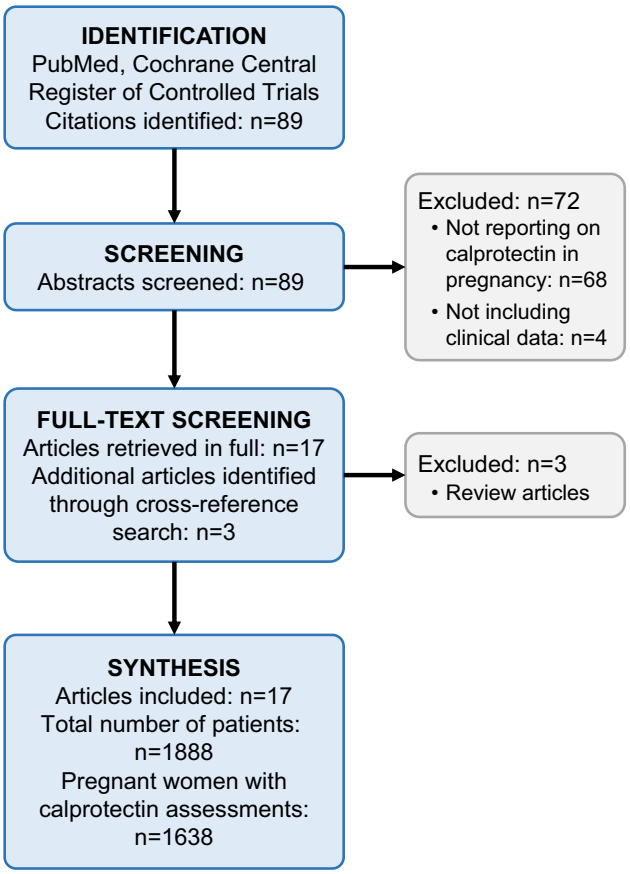

Fig. 1 Flowchart of the literature search

Statistics

All p values are two-tailed and a p value < 0.05 was considered statistically significant. The sample size was calculated based on previously published data on fecal calprotectin measurements in pregnant women with inflammatory bowel disease investigating calprotectin variations during pregnancy depending on inflammatory disease activity [18]. Analysis was performed using parametric or non-parametric tests when data were normally distributed or skewed, respectively. Values are given as means ± standard deviations or medians (interquartile ranges). Correlations were measured using the Pearson's product moment correlation and Spearman's rank order correlation. We used the statistical software SigmaPlot 14 (Systat Software Inc., San Jose, CA) for statistical analysis.

Results

Review of the literature

In a systematic literature search of the databases PubMed and Cochrane Central Register of Controlled Trials (search date 02-10-2018) using the search terms as defined above, we identified 89 citations. After screening all abstracts, 17 citations were found reporting on calprotectin in pregnant women, i.e., in the maternal, placental, or fetal compartments [13–15, 18–31]. Studies not reporting on calprotectin in pregnant women were excluded. The 17 identified studies were retrieved in full and cross reference searching was performed which identified a further 3 studies reporting on calprotectin in pregnant women [32–34]. Three review articles were excluded from analysis [21, 25, 28]. Therefore, in summary, 17 studies were analyzed for this review. Figure 1 shows a flow diagram of the literature search algorithm. Among the 17 studies were 9 retrospective case–control studies [13–15, 19, 27, 29, 32–34], 3 retrospective cohort studies [20, 30, 31], 1 case report [24], as well as 4 prospective studies (one cohort study [22], one case series [23], one case–control study [18], and one randomized controlled trial [26]). Experimental studies were not found. In summary, 1638 pregnant women, 151 newborns, and 99 non-pregnant controls were investigated in these 17 studies. Calprotectin was measured in different compartments, i.e., in fecal samples (5 studies), maternal plasma (5 studies), maternal serum (4 studies), amniotic fluid (2 studies), meconium (2 studies),

placenta (1 study), fetal plasma (1 study), and cord blood (1 study). Table 1 shows the study characteristics of the 17 studies with individual patient data. Calprotectin was present in meconium and correlated with birth weight, duration of pregnancy, and APGAR scores [23, 30]. Calprotectin in cord blood was higher after vaginal delivery than after cesarean section and increased with gestational age [13]. AF calprotectin was not elevated in the presence of preeclampsia, but was significantly elevated in the presence of intraamniotic infection, premature rupture of membranes (PROM), and preterm delivery [15, 31].

Studies assessing fecal samples [18, 20, 22, 24, 26] demonstrated that calprotectin levels were not elevated in healthy pregnant women, were unaffected by a dietary intervention, and did not significantly fluctuate throughout pregnancy. However, fecal calprotectin levels were elevated when IBD was present in pregnant women.

Table 2 shows the results of 8 studies comparing calprotectin levels of pregnant women with and without pregnancy-associated hypertensive disorders. In 8/8 studies, calprotectin levels in maternal plasma and/or serum were found to be significantly elevated in women with pregnancy-induced hypertension (PIH) [14], preeclampsia (PE) [15, 19, 27, 29], mild PE [14, 33, 34], as well as severe PE [14, 32–34] compared to healthy pregnant controls.

Prospective cohort study

Between 4/2015 and 1/2018, 196 pregnant women were included, and serum and urine levels of calprotectin were measured. Table 3 shows the patient characteristics of the study population. Of a total of 196 participants, 179 were healthy pregnant women, 6/196 (3%) had preeclampsia and 11/196 (5.6%) had a concomitant renal disease. There was no correlation between calprotectin levels in serum and urine, neither overall (Spearman rank order correlation coefficient = − 0.0358, $p = 0.63$, $n = 183$) nor per trimester or post-partum or controls (Fig. 2a). Table 4 and Fig. 2 show the serum (Fig. 2b) and urine (Fig. 2c) levels of calprotectin by trimester and post-partum. Specifically, during the 1st, 2nd, and 3rd trimesters of pregnancy and post-partum, serum levels of calprotectin were 1751 [813–4258] ng/mL, 2559 [902–5910] ng/mL, 1788 [1007–3347] ng/mL, and 2715 [1564–5169] ng/mL, respectively. Urine levels of calprotectin were 1348 [405–5864] ng/mL, 1360 [522–2394] ng/mL, 1624 [637–4614] ng/mL, and 1240 [347–3068] ng/mL, respectively. Both serum and urine levels showed no significant variations during pregnancy (all: p = n.s.). In a subgroup analysis, there were no significant differences in serum and urine calprotectin when comparing smokers vs. non-smokers, women with preeclampsia vs. no preeclampsia, and renal disease vs. no renal disease (Table 5). Table 6 gives blood pressure measurements and various serum and urine parameters by trimester and post-partum. This analysis showed a significant increase of serum c-reactive protein, leucocytes, and alkaline phosphatase during the course of pregnancy. In urine, a significant increase of microalbumin was observed.

Serum calprotectin was significantly correlated with systolic blood pressure (Pearson's product moment; cc = − 0.158; $p = 0.03$) and urine calprotectin showed a significant correlation with urine leukocytes (cc = 0.267, $p = 0.0003$) and urine total protein levels (cc = 0.192, $p = 0.009$).

Discussion

In this systematic review and prospective cohort study, we assessed the role of calprotectin in pregnancy. We identified data of > 1600 pregnant women from 17 studies demonstrating that calprotectin is present in various maternal and fetal compartments such as serum, urine, cord blood, amniotic fluid, maternal stool, and meconium. It is elevated in fecal samples of pregnant women with active IBD and in the serum and plasma of women with PIH and PE. In fetal serum, amniotic fluid, and umbilical cord blood, however, calprotectin is not elevated in the presence of PIH and PE. Of note, calprotectin in the AF of pregnant women is significantly elevated in the presence of intraamniotic infection, PROM, and preterm delivery underlining the role of calprotectin as a compartment-specific inflammatory marker.

Our prospective cohort study in pregnant women added new data to the literature in that we demonstrated that both serum and urine levels of calprotectin are stable throughout pregnancy with normal values between 1700 and 2500 ng/mL. We did not ascertain significant variations comparing all trimesters of pregnancy and the post-partum period. Urine concentrations are substantially higher than described in healthy non-pregnant subjects in the literature [10, 12, 35]. Urinary calprotectin concentration cut-off levels to detect intrinsic kidney injury range are consistently < 500 ng/mL and thereby lower than the observed concentrations in this pregnant population [10, 12, 36]. Hence, it is very unlikely that calprotectin will be able to serve as a biomarker for pregnancy-related kidney disease. Noteworthy, however, the present population did not comprise subjects with clinically manifest kidney disease like PE. Interestingly, in our collective of healthy pregnant women, serum calprotectin was significantly correlated with systolic

Table 1 Study characteristics and results of clinical studies describing calprotectin in serum/plasma, amniotic fluid, placenta, and meconium/stool of pregnant women and fetuses/newborns

References	Year	Study type	Number of pregnant women/pregnant controls/non-pregnant controls (*n*)	Type of calprotectin sampling (*n* studies)	Results
Abou Zaid et al. [33]	2014	CCS	40/20/0; cases had preeclampsia	Maternal plasma	CAL elevated in mild and severe preeclampsia vs. controls (305 ± 6 and 306 ± 8 vs. 106 ± 5 µg/L)
Bálint et al. [18]	2017	PCCS	48/0/87; controls were non-pregnant with/without IBD	Fecal samples	CAL not elevated in healthy pregnant women; CAL significantly elevated in active IBD patients vs. pregnant and non-pregnant healthy controls
Börekci et al. [34]	2009	CCS	61/20/0; cases had preeclampsia	Maternal serum	CAL elevated in mild and severe preeclampsia vs. controls (1677 ± 1222 and 2006 ± 1177 vs. 772 ± 451 µg/L)
Braekke et al. [15]	2005	CCS	31/38/0; cases had preeclampsia	Maternal and fetal plasma and AF	CAL elevated in maternal (1081 [865–1569] vs. 552 [471–651] µg/L) but not in fetal plasma (77 [66–173] vs. 110 [96–138]) and AF (3202 [1815–8948] vs. 3988 [96–138])
Espinoza et al. [31]	2003	COS	249/84/0; cases had premature labor/PROM/preterm delivery	AF	CAL elevated in intraamniotic infection, PROM, preterm delivery; association between CAL and intraamniotic inflammation as well as short interval to delivery in preterm labor with intact membranes
Feng et al. [14]	2011	CCS	60/32/0; cases had hypertensive disorder	Maternal plasma and placenta	CAL elevated in plasma of mild and severe preeclampsia vs. controls (751 ± 258 and 1012 ± 699 vs. 479 ± 103 µg/L) and overexpressed in placenta in gestational hypertension, mild and severe preeclampsia (immunohistochemical S-P staining)
Holthe et al. [29]	2005	CCS	20/20/12; cases had preeclampsia	Maternal plasma	CAL elevated in cases (768 [612–1016] vs. 445 [276–598] µg/L)
Julsgaard et al. [22]	2017	PCOS	46/21/0; cases had IBD	Fecal samples	CAL elevated in cases independent of gestational age; median CAL higher in pregnant IBD women vs. controls (131 µg/g [range 0–3600] vs. 0 µg/g [range 0–84]); CAL at a cutoff of 250 µg/g significantly correlated with active IBD ($p \leq 0.0002$). CRP significantly correlated with CAL ($p = 0.0007$)
Kammerlander et al. [20]	2018	COS	219/0/0; all subjects had IBD	Fecal samples	CAL elevated when IBD was active independent of gestational age (80–120, 259–349, and 778–1277 mg/kg in clinically inactive, mild, and moderate-severe disease activity); sensitivity of 69.7–80.0%, specificity of 66.7–73.3%, positive predictive value of 66.7–74.4% over the 4 gestational periods with a CAL cutoff of 200 mg/kg

Table 1 (continued)

References	Year	Study type	Number of pregnant women/pregnant controls/non-pregnant controls (*n*)	Type of calprotectin sampling (*n* studies)	Results
Laforgia et al. [30]	2003	COS	0/0/0; cases ($n=131$) were healthy neonates	Meconium	CAL correlated with birth weight ($r=-0.333$; $p<0.001$), gestational age ($r=-0.206$; $p=0.018$) and 5′-Apgar score ($r=-0.243$, $p=0.035$). No differences in relation to gender, mode of delivery and maternal conditions
Li et al. [19]	2018	CCS	25/30/0; cases had preeclampsia	Maternal serum	CAL elevated in cases; CAL correlated with duration of hypertension and pregnancy duration
Liosi et al. [13]	2010	CCS	50/110/0; cases had IUGR	Cord blood	No CAL difference IUGR vs. controls; CAL increased in IUGR samples with gestational age; higher CAL levels in vaginal deliveries vs. CS ($b=-74.5$, 95% confidence interval −115.2 to −33.9; $p<0.001$); CAL increased with every gestational week ($b=45.3$, 95% confidence interval 13.5–77.1; $p=0.006$)
Lisowska-Myjak et al. [23]	2018	PCS	0/0/0; cases ($n=20$) were healthy neonates	Meconium	CAL in meconium was (mean ± SD) 286.5 ± 214.6 µg/g (range 34–1067). CAL in the last portions passed were 3-fold higher than in first portions ($p=0.0004$); total CAL content of 3668.7 ± 1819.0 µg (range 1158.9–8155.5) was related to birth weight ($r=0.46$; $p=0.042$)
Protic et al. [24]	2017	CR	1/0/0; case had ulcerative colitis	Fecal sample	CAL showed high level in 2nd trimester
Ramma et al. [32]	2012	CCS	61/27/0; cases had preeclampsia or inflammation	Maternal serum	CAL elevated in cases vs. controls (median 18.8 (interquartile range 13.0–39.4) vs. 40.7 (16.2–86.4)
Sugulle et al. [27]	2011	CCS	138/64/0; cases had diabetes mellitus; controls with/without preeclampsia	Maternal plasma and serum	CAL elevated in diabetic pregnant women, but lower compared to preeclampsia (729 vs 552 and 1081 µg/L; $p=0.006$); in diabetic women with PE, median plasma CAL was elevated vs. controls, but not different from PE alone (969 vs 552 and 1081 µg/L; $p=0.01$)
Urwin et al. [26]	2014	RCT	123/0/0; randomized in salmon diet vs. no diet	Fecal samples	CAL not affected by salmon diet
Pooled analysis	–	–	1172/466/99; others: 151	Fecal samples (5), maternal plasma (5), maternal serum (4), AF (2), meconium (2), placenta (1), fetal plasma (1), cord blood (1)	–

CCS case–control study, *IUGR* intrauterine growth restriction, *AF* amniotic fluid, *CAL* calprotectin, *CS* cesarean section, *COS* cohort study, *IBD* inflammatory bowel disease, *PCOS* prospective cohort study, *PCS* prospective case series, *PCCS* prospective case–control study, *CR* case report, *RCT* randomized controlled trial, *PROM* premature rupture of membranes

Table 2 Studies describing calprotectin in serum/plasma, amniotic fluid, placenta, and meconium/stool of pregnant women with and without pregnancy-associated hypertensive disorders

References	Type of PAHD (*n*)	Type of controls (*n*)	Type of body fluid	Calprotectin in pregnant women with PAHD (*n*)	Calprotectin in controls (*n*)	*p* value
Abou Zaid et al. [33]	Mild PE (20), severe PE (20)	Healthy pregnant (20)	Maternal plasma	Mild PE: 305.2 ± 6.8 µg/l Severe PE: 306.1 ± 8.1 µg/l	106.8 ± 5.3 µg/l	< 0.05 vs. controls < 0.05 vs. controls
Börekci et al. [34]	Mild PE (22), severe PE (39)	Healthy pregnant (20)	Maternal plasma	Mild PE: 1677.4 ± 1222.0 µg/l Severe PE: 2006.0 ± 1177.6 µg/l	772.1 ± 451.4 µg/l	< 0.05 vs. controls < 0.05 vs. controls
Braekke et al. [15]	PE (31)	Healthy pregnant (38)	Maternal plasma	1081 (865–1569) µg/l	552 (471–6519) µg/l	< 0.001
Feng et al. [14]	PIH (12), mild PE (22), severe PE (26)	Healthy pregnant (12)	Maternal plasma	PIH: 519 ± 327 µg/l Mild PE: 751 ± 258 µg/l Severe PE: 1012 ± 699 µg/l	479 ± 103 µg/l	< 0.05 vs. controls < 0.05 vs. PIH < 0.05 vs. mild PE
Holthe et al. [29]	PE (20)	Healthy pregnant (20)	Maternal plasma	768 (612–1016) µg/l	445 (276–598) µg/l	0.002
Li et al. [19]	PE (25)	Healthy pregnant (30)	Maternal serum	2656 ± 1724 µg/l	1877 ± 905 µg/l	0.03
Ramma et al. [32]	Severe PE (45), SID (16)	Healthy pregnant (27)	Maternal serum	Severe PE: 40.7 (16–86) µg/ml SID: 56.8 (16–65) µg/ml	18.8 (13–39) µg/ml	0.01 vs. controls 0.01 vs. controls
Sugulle et al. [27]	Preexisting DM (64), GDM (63), PE and GDM (11)	Healthy pregnant (37), PE (27)	Maternal plasma	GDM: 729 µg/l PE and GDM: 969 µg/l	Healthy pregnant: 552 µg/l PE: 1081 µg/l	0.006 vs. GDM 0.01 vs. PE and GDM 0.001 vs. GDM 0.1 vs. PE and GDM

PAHD pregnancy-associated hypertensive disorder, *PIH* pregnancy-induced hypertension, *PE* preeclampsia, *SID* systemic inflammatory disease
Values are means ± standard deviations or medians (interquartile range)

blood pressure and urine calprotectin with urine leukocytes and urine total protein levels. This is in line with data in the literature showing that calprotectin increases in the presence of preeclampsia, which is a combination of high blood pressure and proteinuria [14]. In addition to its association with preeclampsia [14, 15, 19, 32–34], which is consistent with a pro-inflammatory endothelial activation, calprotectin also reflects various other inflammatory states during pregnancy, i.e., bacterial infections as in intraamniotic infection [15, 31] as well as auto-immune-mediated endogenous inflammation as in Crohn's disease and ulcerative colitis [20, 22, 24]. However, calprotectin is not specific for any of these disorders and is thus unsuitable as a disease-specific marker during pregnancy. In addition, urinary leukocytosis is frequent in pregnancy and calprotectin is correlated with leukocytosis, thus making calprotectin unsuitable as a urinary marker of renal damage.

The best documented clinical aspect of calprotectin in pregnancy is its correlation with PIH and PE. It is of note that 8/8 studies identified in the literature found that calprotectin levels in maternal plasma and/or serum were significantly elevated in women with PIH, mild PE as well as severe PE compared to healthy pregnant controls. This is promising, and further studies of calprotectin are warranted especially regarding the potential role of calprotectin as a prognostic and/or predictive marker of PIH/PE. At this time, however, there are no data demonstrating that calprotectin may be able to predict the later development of PIH/PE in early pregnancy. On the contrary, our prospective cohort study showed no elevated serum or urine levels of calprotectin among women who later developed PIH/PE. In addition, there are no data in the literature to date demonstrating that calprotectin has a prognostic value, e.g., by predicting fetal outcome, time of delivery, or the development of severe PE.

Table 3 Patient characteristics

Patient characteristic	Value
Number of patients	196
Age (years)	28.7 ± 5.9 (range: 15.5–46.2) [3]
Para	1 (0–2) [6]
Gravida	2 (1–3) [6]
Body mass index (kg/m^2)	26.0 (22.0–30.8) [13]
Smoking (yes/no)	54 (33.5)/107 (66.5) [35]
Concurrent disease (yes/no)	81 (47.6)/89 (52.4) [26]
Taking medication(s) (yes/no)	28 (17.3)/134 (82.7) [34]
Preeclampsia (yes/no)	6 (3.1)/196 (96.9)
Gestational diabetes (yes/no)	19 (9.7)/177 (90.3)
Renal disease (yes/no)	11 (5.6)/185 (94.4)
Timepoint of sample collection[a]	[5]
First trimester	31 (15.3)
Second trimester	30 (14.9)
Third trimester	106 (52.5)
Post-partum	35 (17.3)

Data are numbers (percentage), mean ± standard deviation, or median (interquartile range). Numbers in square brackets indicate the number of missing values

[a]For 11 patients, samples at 2 time points were taken

Therefore, based on the available evidence, calprotectin is an experimental marker in pregnant women with PIH/PE without a proven role in clinical practice.

In non-pregnant individuals, calprotectin is best known for its role as a marker of inflammatory activity with gastrointestinal disorders such as Crohn's disease, cystic fibrosis, ulcerative colitis, and inflammatory bowel disease (IBD) in juvenile idiopathic arthritis [6, 7]. In our systematic review we found that this is also true for pregnant women. For example, Kammerlander et al. showed that fecal calprotectin is reliably elevated in the presence of IBD activity independent of gestational age [20]. This was independently confirmed by Julsgaard et al. who found that calprotectin was elevated in IBD cases independent of gestational age [22]. In accordance, Bálint et al. suggested that fecal calprotectin may be a useful noninvasive diagnostic tool in pregnancy for monitoring mucosal inflammation based on their finding that fecal calprotectin was significantly elevated in active IBD patients but remained stable during pregnancy among healthy women [18]. Together, these data suggest that fecal calprotectin can be used as a reliable marker of active IBD in pregnancy with the same accuracy as demonstrated in > 3000 non-pregnant IBD patients with a pooled sensitivity of 85% and a specificity of 75% [8].

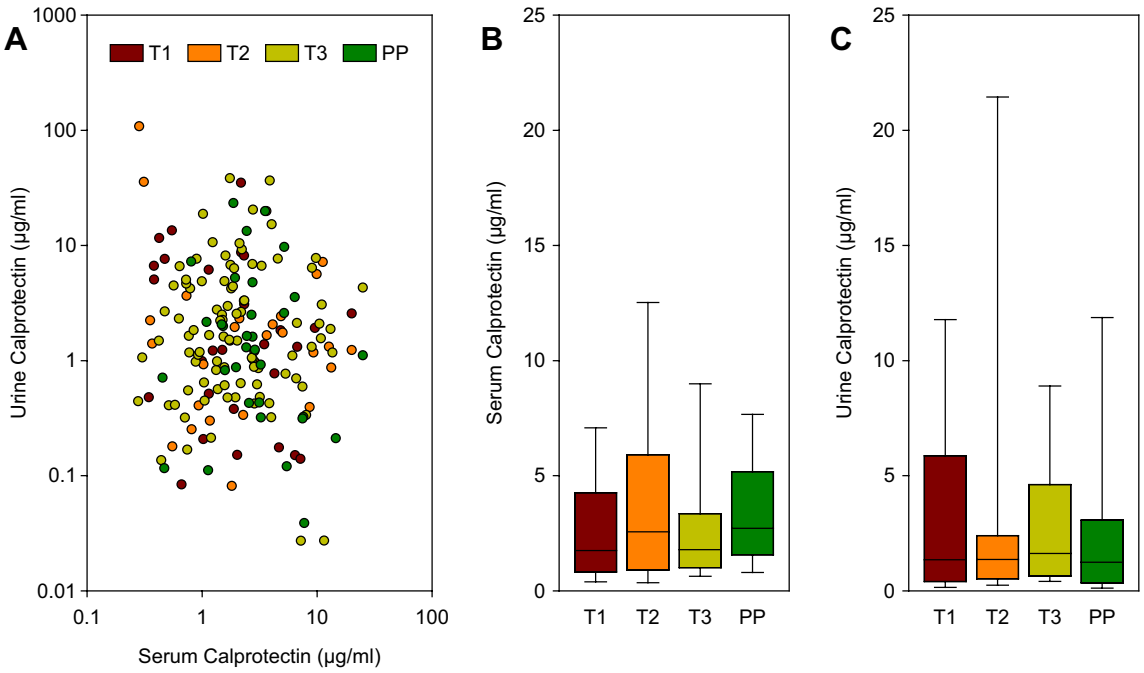

Fig. 2 Serum and urine calprotectin levels during pregnancy (trimesters 1–3) and post-partum. **a** Scatter plot of serum vs. urine calprotectin levels. For better visualization, a logarithmic scale was used. Box plots of serum (**b**) and urine (**c**) calprotectin levels by trimester and post-partum. Boundaries of the boxes indicate the 25th/75th percentiles, black lines within the boxes mark the medians. Whiskers indicate the 10th and 90th percentiles; *T1–3* first, second, and third trimester, respectively, *PP* post-partum

Table 4 Serum and urine calprotectin levels in pregnant women (by trimester) and post-partum

Subgroup	Serum calprotectin (ng/mL)	Urine calprotectin (ng/mL)
Trimester 1 ($n=31$)	1751 (813–4258)	1348 (405–5864) [3]
Trimester 2 ($n=30$)	2559 (902–5910)	1360 (522–2394) [2]
Trimester 3 ($n=106$)	1788 (1007–3347) [1]	1624 (637–4614) [14]
Post-partum ($n=35$)	2715 (1564–5169) [1]	1240 (347–3068) [2]

Values are medians (interquartile ranges). Numbers in square brackets indicate the number of missing values. There are no statistically significant differences between groups (Kruskal–Wallis one-way analysis of variance on ranks; serum, $p=0.164$; urine, $p=0.113$)

Table 5 Serum and urine calprotectin concentrations in patient subgroups

	Serum calprotectin (ng/mL)	Urine calprotectin (ng/mL)
Smoking		
Yes ($N=57$)	1929 (1346–3291) [2]	2108 (716–6046) [9]
No ($N=111$)	1971 (933–3991)	1334 (469–4559) [9]
p	0.741	0.181
Preeclampsia		
Yes ($N=8$)	1189 (392–3087)	2117 (880–7643) [1]
No ($N=199$)	2108 (1085–4068) [2]	1445 (509–4245) [21]
p	0.150	0.251
Renal disease		
Yes ($N=14$)	1618 (687–3651)	2041 (840–5464) [4]
No ($N=193$)	2108 (1077–4023) [2]	1488 (515–4293) [18]
p	0.387	0.599

N refers to samples, not patients and may thus be higher than the number of patients. Values are medians (interquartile range). Numbers in square brackets indicate the number of missing values. Statistics: Mann–Whitney *U* test

We noted a marked heterogeneity in the range of calprotectin levels in different studies published in the literature. For example, Braekke et al. [15] and Feng et al. [14] measured calprotectin plasma levels in healthy pregnant women of around 400–500 µg/L, whereas Ramma et al. measured 18.8 µg/mL, i.e., 18 800 µg/L [32]. We found serum levels of 2000 ng/mL, i.e., 2000 µg/L. This wide variation suggests that differences between calprotectin assays used in the literature are significant limiting the external validity and comparability of the published data. However, the internal validity of the data in the literature is high and internal results are consistent throughout the published studies.

In summary, the results of this literature review and prospective cohort study indicate that calprotectin may be used in pregnant women with IBD as a monitoring marker of disease activity and that calprotectin is a worthwhile subject of further studies regarding its prognostic and/or predictive value in pregnant women with PIH/PE. The observed high urinary concentrations, however, prevent a diagnostic use of urinary calprotectin as a marker of pregnancy-related kidney disease.

Table 6 Blood pressure and serum and urine parameters by trimester and post-partum

Parameter	Trimester 1 ($N=31$)	Trimester 2 ($N=30$)	Trimester 3 ($N=106$)	Post-partum ($N=35$)
Blood pressure				
Systolic (mmHg)	120 (110–120) [2]	120 (110–130) [2]	120 (110–130) [7]	120 (110–120) [5]
Diastolic (mmHg)	70 (60–80) [2]	70 (60–80) [2]	70 (70–80) [7]	70 (68.75–80) [5]
Serum				
C-reactive protein (mg/dL)***	0.3 (0.2–0.6) [2]	0.7 (0.4–2.9) [3]	0.4 (0.2–1.2) [42]	0.6 (0.4–1.3) [16]
Leukocytes (10^3/µL)***	9.3 (8.2–10.6)	10.2 (7.4–11.9)	11.1 (9.3–12.8) [3]	11.3 (9.1–13.9)
Creatinine (mg/dL)	0.6 (0.4–0.6)	0.5 (0.4–0.6) [1]	0.6 (0.5–0.6) [3]	0.6 (0.5–0.6)
AST (IU/L)	19 (17–23) [1]	19 (16–24) [3]	19 (16–25) [3]	19 (16–24)
ALT (IU/L)**	17 (13–24) [1]	14 (10–22) [2]	12 (9–19) [3]	12 (10–15)
GGT (IU/L)*	13 (11–17) [1]	8 (7–11) [9]	10 (7–15) [25]	8 (7–14) [6]
Alkaline phosphatase (IU/L)***	55 (50–62) [4]	54 (45–71) [12]	135 (106–168) [34]	167 (147–195) [9]
Urine				
Leukocytes (μL^{-1})	75 (25–300) [2]	50 (0–500) [4]	25 (0–150) [8]	25 (0–75) [2]
Erythrocytes (μL^{-1})***	0 (0–38) [2]	0 (0–10) [4]	0 (0–18) [9]	250 (18–250) [2]
Creatinine (µmol/L)	73 (31–120) [3]	70 (47–110) [3]	74 (46–133) [15]	90 (56–162) [2]
Microalbumin (mg/L)***	3.0 (3.0–6.6) [3]	3.0 (2.1–6.0) [3]	7.9 (3.0–25.5) [15]	30.3 (12.3–102.8) [2]
Total protein (mg/dL)	40 (6–61) [3]	13 (6–61) [3]	18 (11–60) [14]	18 (11–60) [2]

Values are medians (interquartile range). Numbers in square brackets indicate the number of missing values

*$p<0.05$, **$p<0.01$, ***$p<0.001$ (Kruskal–Wallis one-way analysis of variance on ranks with Dunn's pairwise multiple comparison method)

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Ruhr-Universität Bochum Ethics Committee. Written informed consent was obtained from all patients described in the prospective cohort study.

Data availability The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

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