Abstract

Objective: S100B belongs to the family of danger signalling proteins. It is mainly expressed by glial-specific cells in the brain. However, S100B was also detected in other cell likewise immune cells. This molecule was suggested as biomarker for inflammation and fetal brain damage in spontaneous preterm birth (sPTB), preeclampsia (PE) and HELLP (hemolysis, elevated liver enzymes, and low platelet count).

Study design: The aim of our study was to determine the concentration of S100B in maternal and cord blood (CB) plasma and placenta supernatant as well as the expression of S100B in maternal and CB CD4+ T cells and CD19+ B cells in sPTB and patients delivering following PE/HELLP diagnosis compared to women delivering at term (TD). The S100B expression was further related to the birth weight in our study cohort.

Results: S100B concentration was enhanced in maternal and CB plasma of sPTB and PE/HELLP patients and positively correlated with IL-6 levels. Increased S100B was also confirmed in CB of SGA infants. S100B expression in maternal blood was elevated in CD4+ T cells of PE/HELLP patients and patients who gave birth to SGA newborns as well as in CD19+ B cells of sPTB and PE/HELLP patients and patients with SGA babies. In CB, the expression of S100B was increased in CD19+ B cells of sPTB, PE/HELLP and SGA babies.

Conclusion: Our results support the hypothesis that S100B expression is enhanced in inflammatory events associated with preterm birth and that S100B expression in immune cells is a relevant marker for inflammation during pregnancy complications.

Introduction

Preterm birth (PTB), the delivery of a living child before 37 completed weeks of gestation, is one of the most challenging problems in obstetrics. The syndrome PTB has multiple and complex causes. Most PTBs are spontaneous with preterm labor with or without preterm premature rupture of the membranes (PPROM) and about one third is medically indicated likewise in preeclampsia (PE) [1]. In order to develop new therapeutic options, it is crucial to understand the underlying pathophysiology of PTB. Term as well as preterm labor are associated with an inflammatory response. Increased levels of pro-inflammatory cytokines such as IL-6, TNF-α and IL-8 are produced by infiltrating immune cells in the amniotic fluid, the myometrium, cervix and fetal membranes [2-4]. Besides, the signals driving this inflammation differ: in PTB, often intraamniotic infections provide the stimulus for migration of pro-inflammatory cells into gestational tissues [5]. In term delivery (TD), the inflammatory response leading to labor at term is induced by an increased mechanical stretch forced by the growing fetus and by enhanced fetal signaling molecules produced at term [6-8]. The secreted cytokines activate the pro-inflammatory transcription factor nuclear factor κ-light-chain-enhancer of activated B-cells (NF-κB) in myometrial smooth muscle cells, which induces the expression of several genes that promote parturition by inducing uterine contractions [9-11].

PTB is often accompanied by inflammation and/or infection, although underlying infections are often subclinical and not clinically easy to proof [12, 13]. Immune cells detect invading pathogens through pathogen–associated molecular patterns (PAMPs) and tissue damage–associated molecular patterns (DAMPs) through Toll–like receptors (TLRs), leading to inflammation [14-17]. DAMPs such as S100 proteins are important danger signals that mediate inflammatory responses through interaction with the receptor for advanced glycation end–products (RAGE), a multi-ligand receptor of the immunoglobulin superfamily [18-20]. RAGE is a Pattern Recognition Receptor (PRR) and a central mediator of the innate immune response, but also expressed on T and B-lymphocytes [21-24], providing a link between the innate and adaptive immune system. RAGE activation leads to an immediate inflammatory response.

S100B is a 21kDa cytosolic calcium-binding protein with both intracellular and extracellular functions. Intracellular S100B stimulates cell proliferation and migration and inhibits apoptosis and differentiation, while RAGE mediates most of extracellular S100B effects. This interaction occurs in a variety of cell types and results in different

outcomes: It might exert beneficial or detrimental effects, induce proliferation or differentiation, depending on the S100B concentration, the cell type and the microenvironment [18]. Several cell types in the brain such as astrocytes, oligodendrocytes and neurons, but also immune cells like dendritic cells, NK cells, monocytes/macrophages and lymphocytes express S100B [25-28].

S100B was detected in amniotic fluid (AF), maternal and umbilical cord blood (CB) as well as in placenta samples [29-32]. Since S100B is a neurotrophic factor and could be used as a brain damage marker, it was investigated in healthy and high-risk pregnancies. S100B in AF correlated with gestational age, fetal brain development (head circumference and biparietal diameter) [33] and might be a promising tool to predict unexplained intrauterine death in the second trimester [34].

In CB of healthy newborns, the S100B level was higher than in their mothers, suggesting its high activity during fetal development [35]. However, enhanced S100B level in CB of patients with intrauterine growth restriction (IUGR), compared to uncomplicated pregnancies, correlated with abnormal umbilical artery Doppler findings [36]. In PTB, S100B concentrations in CB of obvious not neurologically injured newborns were higher and correlated with gestational age [37]. Increased S100B level were determined in maternal blood of preterm delivering patients suffering from severe PE [38].

The aim of our study was to quantify S100B in maternal and CB plasma and in placenta supernatant by ELISA as well as to analyze its expression in CD4+ T cells and CD19+ B cells by flow cytometry. We aimed to compare the expression of the inflammation marker S100B in patients delivering at term with patients delivering preterm, either spontaneous or medically induced following diagnosis of PE/HELLP, which was also shown to have an underlying inflammation pathophysiology [39, 40] and in infants born appropriate-for-gestational-age (AGA) or small-for-gestational-age (SGA). We hypothesize that S100B levels and lymphocytic S100B expression is influenced by preterm delivery and/or the birth weight of the newborn.

Material and methods

Human subjects

The study was approved by the ethics committee of the Otto-von-Guericke University medical faculty (EK28/08). All patients were informed properly about the purpose of the study and gave written consent before participating. Seventeen patients undergoing planned cesarean section (CS) at term (term delivery, TD), 17 patients delivering preterm via CS and six patients delivering via CS following PE/HELLP diagnosis were included in this study and recruited between April 2016 and May 2019 at the University Hospital for Gynecology, Obstetrics, and Reproductive Medicine of the Otto-von-Guericke University, Magdeburg. No patient giving urgent birth was included. Patients with PE/HELLP and sPTB were diagnosed according to the ICD10 guidelines and the guidelines from the German, Austrian and Swiss society for Gynecology and Obstetrics. According to them, diagnosis of PE is any increased blood pressure (≥140/90 mm Hg) during pregnancy with at least one new organ manifestation that cannot be assigned to any other cause. Typically, the organ which is affected is the kidney, shown by proteinuria (≥300 mg/d) or a protein/ creatinine quotient ≥30 mg/mmol. Other organs that might be affected are the liver, the hematological system, the placenta (SGA/ IUGR) or the central nervous system. The presence of PE can also be assumed when beside hypertension, angiogenic factors (sFlt-1:PlGF ratio) [41] are present in pathological (ratio >85 in gestational weeks 20-34; ratio >110 from gestational week 35 on). HELLP is pregnancy-specific laboratory triad consisting of hemolysis, elevated liver enzymes (transaminases) and low platelet count. Diagnostic criteria for HELLP syndrome are hemolysis with increased lactate dehydrogenase (LDH; >600 U/l), aspartate transaminase (AST; ≥ 70 U/l), and platelets <100 000/µl. HELLP is often associated with PE. sPTB was defined as spontaneous onset of labor and delivery of a viable infant before 37 completed weeks of gestation. According to the guidelines from the German, Austrian and Swiss society for gynecology and obstetrics, intrauterine growth restriction (IUGR) was defined as estimated fetal weight <10th percentile and/ or non-percentile appropriate growth in the course of pregnancy and pathological Doppler sonography of the *Arteria umbilicalis* or *Arteriae uterinae* or oligohydramnios. Small-for-gestational-age (SGA) was applicable for newborns with either weight or length below 10th percentile for gestational age. The demographic data of the patients is summarized in Table 1.

Venous EDTA blood was taken from pregnant women within 30min. prior to delivery and cord blood immediately following birth. The blood was stored on ice and processed within one hour. Plasma was obtained and stored at -80°C.

Placenta explants

Placentas were collected immediately after delivery. Decidual tissue and large vessels were removed from villous placenta by dissection. The villous tissue was cut into 500 mg pieces and washed extensively with PBS. Human placental villous explants were transferred into 24-well plates and cultured for 24h in RPMI1640+ 3% charcoalized fetal bovine serum+ 1% penicillin/streptomycin as described before [42, 43]. Placenta supernatant was harvested and centrifuged at 2500g for 5min. Supernatant was transferred into a new tube and stored at -80°C before processing.

Cell staining and flow cytometry

$3x10^5$ peripheral blood mononuclear cells (PBMCs) and umbilical cord blood mononuclear cells (UCBMCs) were stained for cell surface markers for 30min. at 4°C in the dark. The following anti-human antibodies were used: eFluor506-labeled CD45 (clone H30), PE-labeled CD4 (clone RPA-T4) and APC-labeled CD19 (clone HIB19; all reagents ebioscience, Waltham, USA). Afterwards, cells were fixed for 30 min. with Fix and Perm (ebioscience, Waltham, USA) and stained with AlexaFluor 488-labeled S100B (clone EP1576Y, abcam, Cambridge, GB) for 30 min at 4°C. Measurements were performed on LSR Fortessa (BD Biosciences, Heidelberg, Germany) and Attune NxT flow cytometer (Thermo Fisher Scientific, Waltham, USA). Flow Cytometry data were analyzed with FlowJo software (Ashland, USA).

S100B ELISA

An ELISA (R&D systems, Minneapolis, USA) was used to determine the level of S100B in maternal and cord blood plasma as well as in placenta supernatants. The ELISA was performed according to the supplier's recommendation. The absorbance was measured at 450 nm using a microplate reader (BioTek Synergy HT, Winooski, USA).

Cytokine detection in plasma samples

Cytokines were quantified by the cytometric bead array (CBA) human Th1/Th2/Th17 Cytokine Kit (Biolegend, San Diego, USA) following supplier's recommendation. The

kit detected the cytokines IL-2, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α.

Data analysis and statistics

Statistical analysis was performed using GraphPad Prism 8.0 software. Normality of distribution was determined by Shapiro-Wilk test. In dependence of these results, data were analyzed by unpaired t-test/Mann-Whitney-test or One-way ANOVA/Kruskal-Wallis test followed by Holm-Sidak´s or Dunn´s multiple comparisons test. Significance was regarded when $p<0.055$ (*), $p<0.005$ (**), $p<0.0005$ (***) or $p<0.0001$ (****).

Results

Preterm delivery is accompanied by enhanced levels of S100B in maternal and cord plasma

Maternal blood was taken within 30min. prior to delivery, CB and placenta immediate after birth. Placenta explants were cultured for 24h. In maternal plasma, the level of S100B was enhanced in spontaneous PTB (sPTB) ($p<0.0001$) and medically induced PTB due to PE/HELLP ($p=0.0090$) when compared to term delivery (TD; Fig. 1A). Similarly, S100B was increased in cord plasma in the occurrence of sPTB ($p=0.0020$) and PTB associated with PE/HELLP ($p=0.0006$) as compared to TD (Fig. 1B). The concentration of S100B in placenta explant supernatant was higher than in plasma and enhanced in PE/HELLP ($p=0.0199$), but unaltered in sPTB compared to TD (Fig. 1C). Next, we divided the study cohort according to the birth weight into appropriate-for-gestational-age (AGA) and small-for-gestational-age (SGA) to understand whether its concentration also relates to the baby weight. The S100B maternal plasma level at a tendency to be higher when delivering a SGA baby ($p=0.0533$; Fig. 1D), S100B in CB plasma was higher in SGA than in AGA newborns ($p=0.0008$; Fig. 1E). Since S100B is involved in inflammatory immune responses, we determined IL-6 in plasma. IL-6 was highly expressed in maternal and CB plasma from sPTB patients ($p<0.0001$; Fig. 1F and Fig. 1G). The cytokine was also enhanced in PE/HELLP maternal plasma ($p=0.0296$; Fig. 1F), but not in CB (Fig. 1G). The spearman correlation between S100B and IL-6 received a positive correlation in maternal ($r=0.6268$; $p<0.0001$; Suppl. fig. 1A) and CB plasma ($r=0.5376$; $p=0.0002$; Suppl. fig. 1B).

Immune cell distribution and the expression of S100B in maternal peripheral immune cells differ between spontaneous or induced PTB and term delivery

We determined a decreased frequency of CD4+ T cells in sPTB ($p=0.0165$; Fig. 2A), but not in induced PTB due to PE/HELLP diagnosis, compared to TD. In sPTB, an enhanced frequency of CD19+ B cells was detected ($p=0.0393$), which was unaltered in PE/HELLP in comparison to TD (Fig. 2B). The frequency of S100B+CD4+ T cells was increased in maternal blood suffering from PE/HELLP ($p=0.0426$) and enhanced in sPTB compared to TD ($p=0.0598$; Fig. 2C). The percentage of S100B+CD19+ B cells was enhanced in patients with sPTB ($p=0.0209$) and PE/HELLP diagnosis ($p=0.0135$) in comparison to TD (Fig. 2D).

Neither the frequency of CD4+ T cells nor the percentage of CD19+ B cells correlated with the birth weight (Fig. 2E and Fig. 2F). Next, we concentrated in analyzing CD4+ T and CD19+ B cells expressing S100B according to birth weight. The frequency of S100B+CD4+ T cells (p= 0.0203; Fig. 2G) and of S100B-expressing CD19+ B cells were higher in maternal blood from patients delivering SGA babies compared to AGA-delivering patients (p=0.0147; Fig. 2H).

The cell distribution and the expression of S100B in immune cells of cord blood samples differ among spontaneous or induced PTB and term delivery

We found that the frequency of CD4+ T cells was decreased in CB from sPTB patients (p=0.00023) and unaltered in PE/HELLP (Fig. 3A). Additionally, the number of CD19+ B cells was enhanced in sPTB (p=0.0510) and unchanged in PE/HELLP compared to CB obtained following TD (Fig. 3B). The percentage of S100B+CD4+ T cells (p=0.0016; Fig. 3C) and S100B+CD19+ B cells (p=0.0320; Fig. 3D) was increased in CB of sPTB, but unchanged in PE/HELLP compared to CB from TD patients.

Similar to the results obtained in maternal blood, there were no differences in the percentage of CD4+ and CD19+ lymphocytes in CB (Fig. 3E and Fig. 3F). In addition, the frequency of S100B+CD4+ T cells was not changed (Fig. 3G), but in CB from SGA infants an enhanced number of S100B-expressing B cells were detected (p=0.0300; Fig. 3H).

Discussion

In the present study, we report enhanced S100B plasma concentrations in patients with sPTB and PE compared to normal pregnancy controls. Moreover, we confirmed higher S100B levels in CB than in maternal blood.

While some studies investigated plasma levels of S100B in PE, no studies exist about this protein in sPTB. Our results go in line with previous published data showing increased S100B level in maternal blood of preterm delivering patients due to severe PE [38]. This was associated with an enhanced risk for having CNS symptoms and an increased risk for having HELLP syndrome [38]. Others found that the increased S100B in PE patients was associated with visual disturbances, which might reflect CNS affection in these patients [44].

IUGR is common among babies from PE patients and also associated with sPTB [45-48], at least in part due to prenatal congenital infections that also account for IUGR cases [49]. Moreover, in CB of SGA infants, other pro-inflammatory markers such as IL-6 and CRP were elevated [50]. IUGR is associated with perinatal mortality, enhanced sepsis episodes and neurologic damage from intraventricular hemorrhage (IVH) [51]. In order to analyze the impact of IUGR in our study cohort, we divided the patients according to the birth weight [52] into AGA and SGA newborns. We found that the S100B concentration is higher in maternal and significantly higher in CB plasma from IUGR pregnancies compared to controls. However, contradictory results can be found in the literature. While Boutsikou et al. found that S100B levels did not differ between AGA and SGA groups in maternal and CB [53], Gazzolo et al. determined that S100B was enhanced in the maternal blood of IUGR pregnancies complicated by intraventricular hemorrhage than in those that were not and in controls [54]. In addition, an association between S100B expression and long-term consequences in the offspring were reported: enhanced IUGR-associated S100B level in CB was associated with negative results of cognitive tests and language composite scores in neurodevelopment tests performed in children at 2 years of age. Interestingly, S100B concentration in maternal serum correlated with results of adaptive behaviour tests [55].

Because of its neurotrophic activity, S100B measurements in biological fluids from fetuses and neonates might be useful for the laboratory evaluation of brain maturation. Observations from other studies in PTB showed increased S100B in CB of PTB newborns without underlying neurological lesions, indicating differences in the blood-

brain-barrier permeability and cerebral circulation between preterm and term newborns or a later stage of fetal brain maturation at term [37]. Moreover, enhanced CB S100B level correlated with gestational age [37]. CB S100B was shown to exhibit the highest sensitivity in predicting brain injury [56]. An association between S100B levels and chorioamnionitis in neonatal plasma without a later diagnosis of infant morbidities was also found [57].

It has to be considered that patients in risk of PTB are usually treated with glucocorticoids to induce lung maturation of the babies. This treatment has also the advantage to decrease the frequency of respiratory distress syndrome, neurological abnormalities, intraventricular hemorrhage, mechanical ventilation support and the duration of stay at the neonatal intensive care unit. A study investigating newborns from Glucocorticoid (GC)-treated mothers or untreated mothers but without brain damage found lower CB S100B levels in samples from both groups when compared to neonates who suffered brain damage born to untreated mothers [58].

Investigation of S100B expression in gestational tissues showed that S100B is located in trophoblast cells of placental tissue and is involved in trophoblast cell apoptosis [59]. We found that the concentration of S100B was higher in placenta supernatant than in plasma in all three groups and higher in PE/HELLP when compared to PTB patients and TD. Previous studies determined that oxidative stress induced S100B from placenta and amnion [29]. Interestingly, oxidative stress with elevated intracellular Ca^{2+} concentration as well as endothelial dysfunction are key players in the pathogenesis of PE, which might explain our results. Other groups found higher S100B mRNA in the amnion of PE patients with or without and higher AF S100B level in PE and normotensive IUGR compared to controls [31]. Friel et al. described that S100B was higher in AF of PTB patients than in term delivering women, even higher in PTB associated with intraamniotic infection and between patients following PPROM without than with intraamniotic infection [60].

PTB is associated with enhanced pro-inflammatory cytokines in maternal and CB plasma [61, 62]; in this study cohort we detected elevated IL-6 level in sPTB. Enhanced neonatal plasma concentrations of S100B and IL-6 were detected in brain injury in neonatal hypoxic-ischemic encephalopathy [63]. Increased CB level of S100B and IL-6 were determined in those subsequently experiencing neonatal hypoxic ischemic encephalopathy and in premature newborns suffering from brain injury [56]. S100B might contribute to Inflammatory events by activation of the endothelium, vascular

smooth muscle cells, monocytes and T cells via RAGE, resulting in the generation of cytokines and pro-inflammatory adhesion molecules [64, 65]. Moreover, S100B was shown to induce TNF-α and IL-6 in alveolar type I-like cells [66] which might contribute to the inflammatory events in the lung of preterm born babies. We detected a positive correlation between S100B and IL-6 in maternal and CB plasma, which supports the inflammatory role of S100B in PTB.

An imbalance of immune cells contributes to the pathogenesis of PTB. We found that in sPTB, decreased CD4+ T cells and enhanced CD19+ B cells were present in maternal and CB. We have shown recently that in PTB, maternal B cells show a pro-inflammatory profile together with a decreased frequency of Breg cells [62]. Others found decreased numbers of CD4+ T cells in CB of PE patients [67] and an association between diminished CD4+ T cell frequencies in PTB CB in infants who later developed moderate bronchopulmonary dysplasia [68].

We found that while in maternal and CB the frequency of S100B-expressing CD4+ T cells was enhanced in PE/HELLP, S100B+ B cells were higher in maternal and CB both in sPTB and PE/HELLP. We and others have shown that sPTB and PE/HELLP were both associated with B and T cells that secrete high level of pro-inflammatory cytokines [62, 69-71]. For S100B+CD8+ T cells, it was shown that following stimulation these cells secrete S100B [27]. Thereby, T cells and probably B cells might contribute to the enhanced S100B plasma level.

B and T cells expressing S100B were augmented in in maternal blood; the same was true for B cells positive for S100B in CB of SGA infants. S100B is a marker for inflammation and IUGR was shown to be associated with increased pro-inflammatory cytokines in maternal blood lymphocytes [72]. Nevertheless, since most SGA born infants were also preterm, differences between S100B expression in AGA and SGA infants might rather be due to the prematurity than the birth weight. However, IUGR might have profound impact in infants' life since it is associated with several disorders and thereby a leading cause of perinatal mortality and morbidity.

Taken together, our data strengthen the importance of S100B in spontaneous and indicated preterm delivery due to PE/HELLP and add the finding that S100B is expressed by T cells and B cells. Further studies should analyze the frequency of S100B-expressing B and T cells in maternal blood in different gestational weeks to determine their potential to predict pregnancy-associated complications. Additionally,

these studies should investigate the function of the S100B-expressing B and T cells in more detail.

Conflict of interest

The authors declare no competing interests.

All authors declare responsibility for the entire content of the manuscript.

References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
2. Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod. 2003;9(1):41-5.
3. Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod. 1999;14(1):229-36.
4. Cox SM, Casey ML, MacDonald PC. Accumulation of interleukin-1beta and interleukin-6 in amniotic fluid: a sequela of labour at term and preterm. Hum Reprod Update. 1997;3(5):517-27.
5. Rauk PN, Chiao JP. Interleukin-1 stimulates human uterine prostaglandin production through induction of cyclooxygenase-2 expression. Am J Reprod Immunol. 2000;43(3):152-9.
6. Toyoshima K, Narahara H, Furukawa M, Frenkel RA, Johnston JM. Platelet-activating factor. Role in fetal lung development and relationship to normal and premature labor. Clin Perinatol. 1995;22(2):263-80.
7. Gao L, Rabbitt EH, Condon JC, Renthal NE, Johnston JM, Mitsche MA, et al. Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. J Clin Invest. 2015;125(7):2808-24.
8. Sooranna SR, Lee Y, Kim LU, Mohan AR, Bennett PR, Johnson MR. Mechanical stretch activates type 2 cyclooxygenase via activator protein-1 transcription factor in human myometrial cells. Mol Hum Reprod. 2004;10(2):109-13.
9. Soloff MS, Cook DL, Jr., Jeng YJ, Anderson GD. In situ analysis of interleukin-1-induced transcription of cox-2 and il-8 in cultured human myometrial cells. Endocrinology. 2004;145(3):1248-54.
10. Olson DM. The role of prostaglandins in the initiation of parturition. Best Pract Res Clin Obstet Gynaecol. 2003;17(5):717-30.
11. Fuchs AR, Fuchs F, Husslein P, Soloff MS. Oxytocin receptors in the human uterus during pregnancy and parturition. Am J Obstet Gynecol. 1984;150(6):734-41.
12. Nadeau HC, Subramaniam A, Andrews WW. Infection and preterm birth. Semin Fetal Neonatal Med. 2016;21(2):100-5.
13. Cappelletti M, Della Bella S, Ferrazzi E, Mavilio D, Divanovic S. Inflammation and preterm birth. J Leukoc Biol. 2016;99(1):67-78.
14. O'Neill LA. How Toll-like receptors signal: what we know and what we don't know. Curr Opin Immunol. 2006;18(1):3-9.
15. Kirschning CJ, Bauer S. Toll-like receptors: cellular signal transducers for exogenous molecular patterns causing immune responses. Int J Med Microbiol. 2001;291(4):251-60.
16. Janeway CA, Jr., Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197-216.
17. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Immunol. 2001;13(1):114-9.
18. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, et al. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta. 2009;1793(6):1008-22.

19. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001;108(7):949-55.
20. Donato R. RAGE: a single receptor for several ligands and different cellular responses: the case of certain S100 proteins. Curr Mol Med. 2007;7(8):711-24.
21. Moser B, Desai DD, Downie MP, Chen Y, Yan SF, Herold K, et al. Receptor for advanced glycation end products expression on T cells contributes to antigen-specific cellular expansion in vivo. J Immunol. 2007;179(12):8051-8.
22. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. Nat Immunol. 2007;8(5):487-96.
23. Chen Y, Akirav EM, Chen W, Henegariu O, Moser B, Desai D, et al. RAGE ligation affects T cell activation and controls T cell differentiation. J Immunol. 2008;181(6):4272-8.
24. Akirav EM, Preston-Hurlburt P, Garyu J, Henegariu O, Clynes R, Schmidt AM, et al. RAGE expression in human T cells: a link between environmental factors and adaptive immune responses. PLoS One. 2012;7(4):e34698.
25. Miki Y, Gion Y, Mukae Y, Hayashi A, Sato H, Yoshino T, et al. Morphologic, flow cytometric, functional, and molecular analyses of S100B positive lymphocytes, unique cytotoxic lymphocytes containing S100B protein. Eur J Haematol. 2013;90(2):99-110.
26. Steinman RM, Pack M, Inaba K. Dendritic cells in the T-cell areas of lymphoid organs. Immunol Rev. 1997;156:25-37.
27. Steiner J, Marquardt N, Pauls I, Schiltz K, Rahmoune H, Bahn S, et al. Human CD8(+) T cells and NK cells express and secrete S100B upon stimulation. Brain Behav Immun. 2011;25(6):1233-41.
28. Houtman M, Ekholm L, Hesselberg E, Chemin K, Malmstrom V, Reed AM, et al. T-cell transcriptomics from peripheral blood highlights differences between polymyositis and dermatomyositis patients. Arthritis Res Ther. 2018;20(1):188.
29. Tskitishvili E, Sharentuya N, Temma-Asano K, Mimura K, Kinugasa-Taniguchi Y, Kanagawa T, et al. Oxidative stress-induced S100B protein from placenta and amnion affects soluble Endoglin release from endothelial cells. Mol Hum Reprod. 2010;16(3):188-99.
30. Serpero LD, Bianchi V, Pluchinotta F, Conforti E, Baryshnikova E, Guaschino R, et al. S100B maternal blood levels are gestational age- and gender-dependent in healthy pregnancies. Clin Chem Lab Med. 2017;55(11):1770-6.
31. Tskitishvili E, Komoto Y, Temma-Asano K, Hayashi S, Kinugasa Y, Tsubouchi H, et al. S100B protein expression in the amnion and amniotic fluid in pregnancies complicated by pre-eclampsia. Mol Hum Reprod. 2006;12(12):755-61.
32. Michetti F, Gazzolo D. S100B testing in pregnancy. Clin Chim Acta. 2003;335(1-2):1-7.
33. Gazzolo D, Bruschettini M, Corvino V, Oliva R, Sarli R, Lituania M, et al. S100b protein concentrations in amniotic fluid correlate with gestational age and with cerebral ultrasound scanning results in healthy fetuses. Clin Chem. 2001;47(5):954-6.
34. Florio P, Michetti F, Bruschettini M, Lituania M, Bruschettini P, Severi FM, et al. Amniotic fluid S100B protein in mid-gestation and intrauterine fetal death. Lancet. 2004;364(9430):270-2.
35. Amer-Wahlin I, Herbst A, Lindoff C, Thorngren-Jerneck K, Marsal K, Alling C. Brain-specific NSE and S-100 proteins in umbilical blood after normal delivery. Clin Chim Acta. 2001;304(1-2):57-63.

36. Gazzolo D, Marinoni E, di Iorio R, Lituania M, Bruschettini PL, Michetti F. Circulating S100beta protein is increased in intrauterine growth-retarded fetuses. Pediatr Res. 2002;51(2):215-9.
37. Gazzolo D, Vinesi P, Marinoni E, Di Iorio R, Marras M, Lituania M, et al. S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries. Clin Chem. 2000;46(7):998-1000.
38. Artunc-Ulkumen B, Guvenc Y, Goker A, Gozukara C. Maternal Serum S100-B, PAPP-A and IL-6 levels in severe preeclampsia. Arch Gynecol Obstet. 2015;292(1):97-102.
39. Amaral LM, Wallace K, Owens M, LaMarca B. Pathophysiology and Current Clinical Management of Preeclampsia. Curr Hypertens Rep. 2017;19(8):61.
40. Tenorio MB, Ferreira RC, Moura FA, Bueno NB, de Oliveira ACM, Goulart MOF. Cross-Talk between Oxidative Stress and Inflammation in Preeclampsia. Oxid Med Cell Longev. 2019;2019:8238727.
41. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom M, et al. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. N Engl J Med. 2016;374(1):13-22.
42. Jensen F, Wallukat G, Herse F, Budner O, El-Mousleh T, Costa SD, et al. CD19+CD5+ cells as indicators of preeclampsia. Hypertension. 2012;59(4):861-8.
43. Ehrentraut S, Sauss K, Neumeister R, Luley L, Oettel A, Fettke F, et al. Human Miscarriage Is Associated With Dysregulations in Peripheral Blood-Derived Myeloid Dendritic Cell Subsets. Front Immunol. 2019;10:2440.
44. Bergman L, Akhter T, Wikstrom AK, Wikstrom J, Naessen T, Akerud H. Plasma levels of S100B in preeclampsia and association with possible central nervous system effects. Am J Hypertens. 2014;27(8):1105-11.
45. Tiensuu H, Haapalainen AM, Karjalainen MK, Pasanen A, Huusko JM, Marttila R, et al. Risk of spontaneous preterm birth and fetal growth associates with fetal SLIT2. PLoS Genet. 2019;15(6):e1008107.
46. Zeitlin J, Ancel PY, Saurel-Cubizolles MJ, Papiernik E. The relationship between intrauterine growth restriction and preterm delivery: an empirical approach using data from a European case-control study. BJOG. 2000;107(6):750-8.
47. Lackman F, Capewell V, Richardson B, daSilva O, Gagnon R. The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. Am J Obstet Gynecol. 2001;184(5):946-53.
48. Srinivas SK, Edlow AG, Neff PM, Sammel MD, Andrela CM, Elovitz MA. Rethinking IUGR in preeclampsia: dependent or independent of maternal hypertension? J Perinatol. 2009;29(10):680-4.
49. Longo S, Borghesi A, Tzialla C, Stronati M. IUGR and infections. Early Hum Dev. 2014;90 Suppl 1:S42-4.
50. Amarilyo G, Oren A, Mimouni FB, Ochshorn Y, Deutsch V, Mandel D. Increased cord serum inflammatory markers in small-for-gestational-age neonates. J Perinatol. 2011;31(1):30-2.
51. Simchen MJ, Beiner ME, Strauss-Liviathan N, Dulitzky M, Kuint J, Mashiach S, et al. Neonatal outcome in growth-restricted versus appropriately grown preterm infants. Am J Perinatol. 2000;17(4):187-92.
52. Salomon LJ, Bernard JP, Ville Y. Estimation of fetal weight: reference range at 20-36 weeks' gestation and comparison with actual birth-weight reference range. Ultrasound Obstet Gynecol. 2007;29(5):550-5.

53. Boutsikou T, Mastorakos G, Kyriakakou M, Margeli A, Hassiakos D, Papassotiriou I, et al. Circulating levels of inflammatory markers in intrauterine growth restriction. Mediators Inflamm. 2010;2010:790605.
54. Gazzolo D, Marinoni E, Di Iorio R, Lituania M, Marras M, Bruschettini M, et al. High maternal blood S100B concentrations in pregnancies complicated by intrauterine growth restriction and intraventricular hemorrhage. Clin Chem. 2006;52(5):819-26.
55. Mazarico E, Llurba E, Cabero L, Sanchez O, Valls A, Martin-Ancel A, et al. Associations between neural injury markers of intrauterine growth-restricted infants and neurodevelopment at 2 years of age. J Matern Fetal Neonatal Med. 2019;32(19):3197-203.
56. Lu H, Huang W, Chen X, Wang Q, Zhang Q, Chang M. Relationship between premature brain injury and multiple biomarkers in cord blood and amniotic fluid. J Matern Fetal Neonatal Med. 2018;31(21):2898-904.
57. Rogers LK, Graf AE, Bhatia A, Leonhart KL, Oza-Frank R. Associations between maternal and infant morbidities and sRAGE within the first week of life in extremely preterm infants. PLoS One. 2013;8(12):e82537.
58. Gazzolo D, Kornacka M, Bruschettini M, Lituania M, Giovannini L, Serra G, et al. Maternal glucocorticoid supplementation and S100B protein concentrations in cord blood and urine of preterm infants. Clin Chem. 2003;49(7):1215-8.
59. Weng Z, Liu Z, Zhang S, Tao H, Ji X. Zinc protection in fetal rats for maternal mercury exposure-induced growth retardation is probably associated with S100B expression. J Obstet Gynaecol Res. 2017;43(1):73-7.
60. Friel LA, Romero R, Edwin S, Nien JK, Gomez R, Chaiworapongsa T, et al. The calcium binding protein, S100B, is increased in the amniotic fluid of women with intra-amniotic infection/inflammation and preterm labor with intact or ruptured membranes. J Perinat Med. 2007;35(5):385-93.
61. Boyle AK, Rinaldi SF, Norman JE, Stock SJ. Preterm birth: Inflammation, fetal injury and treatment strategies. J Reprod Immunol. 2017;119:62-6.
62. Busse M, Campe KJ, Redlich A, Oettel A, Hartig R, Costa SD, et al. Regulatory B Cells Are Decreased and Impaired in Their Function in Peripheral Maternal Blood in Pre-term Birth. Front Immunol. 2020;11:386.
63. Massaro AN, Wu YW, Bammler TK, Comstock B, Mathur A, McKinstry RC, et al. Plasma Biomarkers of Brain Injury in Neonatal Hypoxic-Ischemic Encephalopathy. J Pediatr. 2018;194:67-75 e1.
64. Yan SF, Ramasamy R, Naka Y, Schmidt AM. Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. Circ Res. 2003;93(12):1159-69.
65. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell. 1999;97(7):889-901.
66. Piazza O, Leggiero E, De Benedictis G, Pastore L, Salvatore F, Tufano R, et al. S100B induces the release of pro-inflammatory cytokines in alveolar type I-like cells. Int J Immunopathol Pharmacol. 2013;26(2):383-91.
67. Sava F, Toldi G, Treszl A, Hajdu J, Harmath A, Tulassay T, et al. Expression of lymphocyte activation markers of preterm neonates is associated with perinatal complications. BMC Immunol. 2016;17(1):19.
68. Misra R, Shah S, Fowell D, Wang H, Scheible K, Misra S, et al. Preterm cord blood CD4(+) T cells exhibit increased IL-6 production in chorioamnionitis and decreased CD4(+) T cells in bronchopulmonary dysplasia. Hum Immunol. 2015;76(5):329-38.

69. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, et al. Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol. 2009;183(11):7023-30.
70. Azizieh FY, Raghupathy RG. Tumor necrosis factor-alpha and pregnancy complications: a prospective study. Med Princ Pract. 2015;24(2):165-70.
71. Arenas-Hernandez M, Romero R, Xu Y, Panaitescu B, Garcia-Flores V, Miller D, et al. Effector and Activated T Cells Induce Preterm Labor and Birth That Is Prevented by Treatment with Progesterone. J Immunol. 2019;202(9):2585-608.
72. Al-Azemi M, Raghupathy R, Azizieh F. Pro-inflammatory and anti-inflammatory cytokine profiles in fetal growth restriction. Clin Exp Obstet Gynecol. 2017;44(1):98-103.

Figure Legends

Table 1:

Study cohort

Seventeen women delivering at term (term delivery, TD), 17 women delivering preterm spontaneous (sPTB) and 6 patients delivering preterm following PE/HELLP diagnosis were included in the study. Maternal characteristics included age of the pregnant women, gestational age (GA; weeks), number of pregnancies and parities. Neonatal features included birth weight (grams, g), body length (cm), APGAR scores at 1min., 5 min. and 10min. after birth, the cord blood pH value and base excess. Data were analyzed with Kruskal-Wallis-test, followed by Dunn´s multiple comparisons test.

Figure 1:

Detection of S100B and IL-6 in term and preterm deliveries

S100B was detected using ELISA in maternal (A) and cord blood plasma (B) and placenta supernatant (C) in term deliveries (TD), spontaneous preterm births (sPTB) and preterm births following PE/HELLP diagnosis (PE/HELLP). S100B was determined in maternal (D) and cord blood plasma (E) in the study cohort separated due to birth weight into appropriate-for-gestational-age (AGA) and small-for-gestational-age (SGA). IL-6 was determined using cytometric bead array in maternal (F) and cord blood plasma (G) in TD, sPTB and PE/HELLP. Data were analyzed with Kruskal-Wallis-test, followed by Dunn´s multiple comparisons test (A, B, C, F, G) or Mann-Whitney U-test (D, E). $p<0.055$ (*), $p<0.005$ (**), $p<0.0005$ (***) or $p<0.0001$ (****).

Figure 2:

Detection of S100B expression by lymphocytes in maternal blood from term and preterm deliveries

The frequency of CD4+ T cells (A) and CD19+ B cells (B) and the percentage of S100B-expressing CD4+ T cells (C) and CD19+ B cells (D) were determined by flow cytometry in TD, sPTB and PE/HELLP. Flow cytometry results of the frequency of CD4+ T cells (E) and CD19+ B cells (F) and the percentage of S100B-expressing CD4+ T cells (G) and CD19+ B cells (H) in appropriate-for-gestational-age (AGA) and small-for-gestational-age (SGA) born infants. Data were analyzed with Kruskal-Wallis-

test, followed by Dunn´s multiple comparisons test (A-D) or Mann-Whitney U-test (E-H). $p<0.055$ (*)

Figure 3:

Detection of S100B expression by lymphocytes in cord blood from term and preterm deliveries

The percentage of CD4+ T cells (A) and CD19+ B cells (B) and the frequency of S100B-expressing CD4+ T cells (C) and CD19+ B cells (D) were determined by flow cytometry in TD, sPTB and PE/HELLP. The frequency of CD4+ T cells (A) and CD19+ B cells (B) and the percentage of S100B-expressing CD4+ T cells (C) and CD19+ B cells (D) was analyzed according to the birth weight as appropriate-for-gestational-age (AGA) and small-for-gestational-age (SGA) born infants. Data were analyzed with Kruskal-Wallis-test, followed by Dunn´s multiple comparisons test (A-D) or Mann-Whitney U-test (E-H). $p<0.055$ (*), $p<0.005$ (**)