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Antenatal Glucocorticoids and Neonatal Inflammation-associated Proteins

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Abstract

Background—To date, studies of the relationship between antenatal glucocorticoids (AGC) and neonatal inflammation in preterm newborns have been largely limited to umbilical cord blood specimens.

Aim—To explore the association between exposure to antenatal glucocorticoids and concentrations of inflammation-related proteins in whole blood collected from very preterm newborns at multiple times during the first postnatal month.

Methods—We measured the protein concentrations on postnatal day 1 (N=1118), day 7 (N=1138), day 14 (N=1030), day 21 (N=936) and day 28 (N=877) from infants born before the 28th week of gestation and explored the relationship between antenatal steroid receipt and protein concentrations in the in the highest and lowest quartiles. The creation of multinomial logistic

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regression models (adjusted for potential confounders) allowed us calculate odds ratios and 95% confidence intervals.

Results—Twenty of 420 assessments [21 (proteins) $\times$ 2 (exposure levels: partial and full) $\times$ 2 (quartile levels: top and bottom) $\times$ 5 (days)] were statistically significant without any cohesive pattern.

Conclusion—Among infants born before 28 weeks of gestational age, neither full, nor partial courses of antenatal glucocorticoids have a sustained anti-inflammatory effect.

Keywords

Inflammation; infant; prematurity; preterm birth; antenatal glucocorticoids; blood proteins

1 Introduction

Antenatal glucocorticoids (AGC) was first introduced in 1972¹, and is now part of standard of care². AGC treatment has contributed to an appreciable reduction in the incidence of respiratory distress syndrome, and intra-ventricular hemorrhage, necrotizing enterocolitis, and systemic infections in the first 48 postnatal hours³, ultrasound-defined brain damage⁴ as well as cerebral palsy and better neurodevelopmental outcome^{5,6}.

Because glucocorticoids have anti-inflammatory properties⁷, and inflammation appears to be associated with a variety of adverse neurodevelopmental consequences⁸⁻¹¹, and disturbances to lung¹²⁻¹⁴, and intestine^{15,16}, we reasoned that perhaps AGC's benefits are due to diminished postnatal inflammation. Little is known about the effect of AGC on systemic inflammation in very preterm newborns, and the few existing studies are limited by small numbers of proteins evaluated in relatively small numbers of children, and by a focus on umbilical cord specimens only¹⁷⁻²¹. In one study of infants born before the 34th week of gestation, the 100 children exposed to AGC had umbilical cord concentrations of IL-1 β , interleukin-6 (IL-6) and interleukin-8 (IL-8) that did not differ appreciably from those of 100 children not exposed¹⁷. A study among infants before the 32nd week of gestation, non-exposure to AGC was associated with a higher IL-6 level in umbilical cord blood than those exposed¹⁸. Meanwhile, another study did not find such an association¹⁹.

The ELGAN Study of extremely low gestational age newborns (ELGANs)(i.e., born before the 28th week of gestation) collected information about the completeness of the gravida's course of antenatal corticosteroid and measured the concentration of inflammation-related proteins over the first four post-natal weeks. This provided us the opportunity to explore the relationship between AGC and inflammation-related proteins over the first four postnatal weeks.

2 Methods and materials

2.1 Participants

During the years 2002-2004, women who gave birth before 28 weeks gestation at one of 14 participating hospitals in 5 states in the U.S. were invited to enroll. The individual institutional review boards approved the enrollment and consent.

Mothers were approached for consent either upon antenatal admission or shortly after delivery. A total of 1506 infants born to 1249 mothers were enrolled. Information about the completeness of the course of antenatal corticosteroid receipt was available for 1501 newborns, of whom 1216 had measurements of the concentrations of proteins in at least one early postnatal blood spot. They comprise the sample for this report. The number of children on each day blood was routinely collected peaked on day 7 ($N = 1138$), and declined progressively to 877 day 28 (Supplement Table 1).

2.2 Demographic and pregnancy variables

After delivery, a trained research nurse interviewed each mother in her native language using a structured data collection form and following procedures defined in a manual. The mother's report of her own characteristics and exposures, as well as the sequence of events leading to preterm delivery, was taken as truth, even when her medical record provided discrepant information.

After the mother's discharge, the research nurse reviewed the maternal chart using a second structured data collection form. The medical record was relied on for events following admission.

Each mother was asked to provide her height and her pre-pregnancy weight. These were used to calculate her BMI. The United States government classifies BMIs as follows: < 18.5 is underweight, $18.5\text{--}24.9$ is normal, $25.0\text{--}29.9$ is overweight, $30.0\text{--}34.9$ is obese, $35.0\text{--}39.9$ is very obese, and ≥ 40 is extreme obesity²². We collapsed these groups into < 25 , $25\text{--}29.9$, and ≥ 30 .

The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record.²³ Each mother/infant pair was assigned to the category that described the primary reason for the preterm delivery. Preterm labor was defined as progressive cervical dilation with regular contractions and intact membranes. The diagnosis of preterm premature rupture of fetal membranes (pPROM) was defined as the presence of vaginal pooling with either documented nitrazine positive testing or ferning prior to regular uterine activity. Preeclampsia was defined as new onset hypertension and proteinuria of sufficient severity to warrant delivery for either a maternal or fetal indication. For a diagnosis of cervical insufficiency, a woman had to present with cervical dilation of greater than two centimeters, in the absence of membrane rupture and detected or perceived uterine activity. Placental abruption was defined as presentation with significant amount of vaginal bleeding (either documented in the medical record or a post-partum hematocrit $< 24\%$) and a clinical diagnosis of placental abruption in the absence of cervical change. Presentations under the category of fetal indication included severe intrauterine growth restriction based on antepartum ultrasound examination, non-reassuring fetal testing, oligohydramnios, and Doppler abnormalities of umbilical cord blood flow.

2.3 Newborn variables

The gestational age estimates were based on a hierarchy of the quality of available information. Most desirable were estimates based on the dates of embryo retrieval or

intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed on a 14 weeks fetal ultrasound (29%), LMP without fetal ultrasound (7%), and gestational age recorded in the log of the NICU (1%). The birth-weight Z-score is the number of standard deviations the infant's birth-weight is above or below the median weight of infants at the same gestational age in referent samples not delivered for preeclampsia or fetal indications.^{24,25}

Infections during the first postnatal week are considered “early,” while infections identified during weeks 2, 3 or 4 are considered “late.” An organism had to be recovered from the blood, tracheal aspirate, or cerebro-spinal fluid for an infection to be considered documented. Specific organisms were not identified.

2.4 Placentas

Placentas were placed in a sterile exam basin and transported to a sampling room, where they were biopsied under sterile conditions. Eighty-two percent of the samples were obtained within 1 hour of delivery.

The microbiologic procedures are described in detail elsewhere^{26,27}. Briefly, the frozen samples were allowed to thaw at room temperature, a portion approximately 1 cm squared was removed and weighed, then diluted 1:10 with sterile phosphate buffered saline (PBS), homogenized and aliquots plated on selective and non-selective media, including pre-reduced brucella-base agar with 5% sheep blood enriched with hemin and vitamin K₁, tryptic soy agar with 5% sheep blood, chocolate agar, and A-7 agar. After incubation, the various colony types were enumerated, isolated and identified by established criteria²⁸.

2.5 Placenta pathology

In keeping with the guidelines of the 1991 CAP Conference²⁹, representative sections were taken from all abnormal areas as well as routine sections of the umbilical cord and a membrane roll, and full thickness sections from the center and a paracentral zone of the placental disc. After training to minimize observer variability, study pathologists examined the slides for histologic characteristics listed on a standardized data form they helped create^{30,31}.

Chorionic plate inflammation was defined as > 20 neutrophils/20x. Chorioamnionitis required numerous large or confluent foci of neutrophils in the external membranes, as well as of the chorion/decidua. Neutrophilic infiltration into fetal stem vessels in the chorionic plate required that neutrophils appeared to have migrated towards the amnionic cavity.

2.6 Blood spot collection and protein measurement

Drops of blood were collected on filter paper on the first postnatal day (range: 1-3 days), the 7th postnatal day (range: 5-8 days), the 14th postnatal day (range: 12-15 days), the 21st postnatal day (range: 19-23 days), and the 28th postnatal day (range: 26-29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at -70°C in sealed bags with a desiccant until processed. Details about the elution of proteins from the blood spots are provided elsewhere³².

Each sample was analyzed in duplicate using the Meso Scale Discovery electrochemiluminescence multiplex platform and Sector Imager 2400. This platform has been validated by comparisons with traditional ELISA³³, and produces measurements that have high content validity³⁴⁻³⁸

The Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston Massachusetts measured the following 21 proteins: C-Reactive Protein (CRP), Serum Amyloid A (SAA), Myeloperoxidase (MPO), Interleukin-1beta (IL -1 β), Interleukin-6 (IL-6), Interleukin-6 Receptor (IL-6R), Tumor Necrosis Factor-alpha (TNF- α), Tumor Necrosis Factor Receptor-1 (TNF-R1), Tumor Necrosis Factor Receptor-2 (TNF-R2), Interleukin-8 (IL-8; CXCL8), Regulated upon Activation, Normal T-cell Expressed, and [presumably] Secreted (RANTES; CCL5), Intercellular Adhesion Molecule -1 (ICAM-1; CD54), Vascular Cell Adhesion Molecule-1 VCAM-1; CD106), Matrix Metalloproteinase-9 (MMP-9), Vascular Endothelial Growth Factor (VEGF), Vascular Endothelial Growth Factor Receptor-1 (VEGF-R1; Flt-1), Vascular Endothelial Growth Factor Receptor-2 (VEGF-R2; KDR), Insulin Growth Factor Binding Protein-1 (IGFBP-1), thyroid-stimulating hormone (TSH), and Erythropoietin (EPO), Insulin Growth Factor-1 (IGF-1). Because the volume of blood spots can vary, each protein measurement was normalized to milligrams of total protein. Total protein was measured by a photometric assay as described in previous ELGAN study³⁹. Measurements were made in duplicate, and the mean served as the basis for all tables and analyses.

The biomarker protein concentrations in the ELGAN study varied with gestational age, and with the postnatal day of specimen collection^{40,41}. In addition, one set of measurements was made in 2009-2010 and the second in 2015, and, while the distributions of each were similar, they were not identical. Consequently, we divided our sample into 30 groups defined by gestational age category (23-24, 25-26, 27 weeks), postnatal day of blood collection (1, 7, 14, 21 and 28), and measurement set (2009-2010, 2015). Because we were interested in the contribution of high concentrations, and the concentrations of most proteins did not follow normal distributions, the distribution of each protein's concentration was dichotomized into the highest quartile and the lower three quartiles in each of the 30 groups (3 gestational age groups, 5 collection days, 2 sets of measurements made years apart).

2.7 Data analyses

We evaluated two null hypotheses: that infants exposed to a full course of ACG were not more likely to have a concentration of each inflammation-related protein in the top quartile, and separately in the bottom quartile compared to those not exposed to any AGC or to an incomplete AGC course. Table 1 was prepared to identify potential confounders, and includes maternal, pregnancy and newborn characteristics that might be associated with the completeness of the AGC. We selected variables as confounders if identified in the literature, or if in our data they were associated with both the exposure and the outcome with probability $\geq .25$ ⁴². We did not calculate p values for each comparison. We are supported in this view by the American Statistical Association's recent statement on p-values⁴³. We created multivariable logistic regression models to calculate odds (risk) ratios and 95% confidence intervals that infants with each form of steroid exposure were no more likely than

their peers without any exposure to have a protein concentration in the top or bottom quartile. Odds ratios are from multinomial logistic regression models and are adjusted for mother not receiving magnesium sulfate for tocolysis or seizure prophylaxis, gestational age < 27 weeks, birth weight Z-score < -1, male sex, histologic evidence of chorioamnionitis, and neutrophils in the fetal stem vessels. These are displayed as forest plots (Figures 1-5)

3 Results

Of the 1216 infants for whom we had information about AGC exposure and the concentrations of proteins in at least one early postnatal blood spot, 773 (64%) were born to a mother who received a full course, 322 (26%) to a mother who received a partial course, and 121 (10%) to a mother who did not receive any (Table 1). Those most likely to be exposed to a full course were born to women who presented with premature rupture of membranes, or with evidence of cervical insufficiency, followed in descending order by those delivered for maternal indications or presumed abruption, fetal indications, and preterm labor (Table 1). Gestationally older infants and those with some degree of fetal growth restriction were also more likely than their peers to be exposed to a full course of AGC. Women who received a complete course were more likely than others to have a placenta parenchyma with inflammation of the membranes and the fetal stem vessels. In contrast, women who received a partial course had less risk. Infants exposed to a complete course were more likely than their peers to have an organism recovered from the trachea.

Among newborns whose mother received a complete AGC course, the risk of a concentration in the top quartile was not reduced for any protein, but was increased for a concentration of IGFBP-1 in the lowest quartile. In contrast, the risk of a concentration in the lowest quartile was reduced for CRP. Among those exposed to a partial AGC course, IL-1 β and IGFBP-1 were the only proteins at reduced risk of a top quartile concentration on day 1 (Figure 1). Newborns exposed to a full AGC course were at reduced risk of having a CRP concentration in the lowest quartile on day-7 (Figure 2).

By day 14, newborns exposed to a full course were at increased risk of having top quartile concentrations of CRP and IGFBP-1, and at a decreased risk of a TSH concentration in the lowest quartile (Figure 3).

On day 21, both a complete and an incomplete course of AGC were associated with an increased risk of a top-quartile concentration of IGFBP-1 (Figure 4). Exposure to a partial, but not a complete course of AGC was associated with an increased risk of a top-quartile concentration of VEGF. Newborns exposed to any AGC were at decreased risk of having an EPO concentration in the lowest quartile. Infants exposed to either a complete or partial AGC course were at increased risk of a TSH concentration in the lowest quartile, while only those exposed to a complete AGC course were at increased risk of an IL-8 concentration in the bottom quartile.

On day 28, a complete course of AGC was associated with an reduced risk of a top-quartile concentration of SAA, while both complete and partial courses were associated with increased risk of a top quartile concentration of TNF- α (Figure 5). Infants exposed to a

complete course of AGC were also at reduced risk of a lowest quartile concentration of IGFBP-1.

4 Discussion

We found no evidence that complete, or partial AGC have anti-inflammatory effects on levels of inflammation-related protein concentrations that are sustained during the first 28 postnatal days. We view the twenty statistically significant findings among 420 comparisons [$21 \text{ (proteins)} \times 2 \text{ (exposure levels: partial and full)} \times 2 \text{ (quartile levels: top and bottom)} \times 5 \text{ (days)}$] as random phenomena.

The most likely interpretation of our findings is that AGC have no lasting anti-inflammatory effects, or have both anti- and pro-inflammatory properties. Support for both anti- and pro-inflammatory properties comes from both animal and human studies. In adult humans an infusion with 300 mg of hydrocortisone “a paradoxical increase in the mRNA expression of TLR 2, 5 and 9 and HMG-B1” was observed ⁴⁴. Similarly, in newborn lambs, AGC suppresses lung inflammation initially, followed 15 days later by increased lung inflammation ⁴⁵.

It is possible that a transient anti-inflammatory effect occurred at the systemic levels in utero and was still sporadically detectable during the first postnatal week when 3 of the 15 statistically significant reductions were seen in CRP and IL-1 β but did not affect downstream inflammatory cascade proteins e.g. chemokines and adhesion molecules. A suppression of immunity is seen during the first seven days in preterm lambs exposed to maternal betamethasone ⁴⁶, but not after day seven ⁴⁷.

In a recent meta-analysis of 12 studies, mothers exposed to a complete or partial course were not at increased risk of chorioamnionitis (RR 0.91, 95%CI 0.70 to 1.18). However, women who received both a full and a partial course of AGC at separate times were included in the analysis ³. In our study, women who received a full course were at increased risk of placenta inflammation, and women who received a partial course were at reduced risk. However, when we compared exposure to any AGC vs. none, we found that AGC was not associated with placenta inflammation. A possible explanation is that women who received a complete course presented with less “emergency preterm birth” and had higher exposure opportunity to AGC.

Women who received a full course were more likely to present with premature rupture of the membranes or cervical insufficiency, and consequently at increased risk of ascending infection leading to placenta inflammation. Thus, we are unable to attribute their increased risk of placenta inflammation to a full AGC course. They also probably provided their fetuses with the strongest inflammatory stimuli. It is possible that without the AGC their babies might have shown higher protein levels and thus, the lack of increased proteins levels after AGC may actually be a beneficial results of the AGC.

These observations lead us to the possibility that both epidemiologic bias and AGC biology account for what we observed. Our main observation remains. We did not identify a modulating effect of AGC on sustained inflammation.

One of the study limitations is the lack of measurement of the time lag between the exposure to AGC and time of delivery. Apparently, AGC loses its effect on lung maturity after seven days⁴⁷. Among the many strengths of our study are a large sample size, prospective data collection, well-validated biomarker measurements^{28-34,48}, a large number of biomarkers measured, and the availability of measurements from five consecutive postnatal time points.

5 Conclusion

Among ELGANs, AGC exposure appears to have no consistent modulating effect on the concentrations of most inflammation-related proteins during the first postnatal month. However, AGC could have anti-inflammatory effect on the first 7 postnatal days. These results are based on a comprehensive list of systemic proteins that represent multiple stages of the inflammatory cascade and were therefore evaluated in the ELGAN study. However, other untested aspects of inflammation such as localized tissue/organ effects or microbiome changes could show a more significant result. AGC is now part of standard care for women who present with a pregnancy disorder likely to result in very preterm delivery. Also, AGC resulted in well establish improvement of premature infants outcome. With our finding of a possible anti-inflammatory effect in the first 7 days and a later pro-inflammatory effect marked with IL-8 on day 21 and TNF- β on day 28 after adjustment for confounders, more research on the pharmacokinetics and pharmacodynamics of AGC offers the hope of identifying how to improve the care of vulnerable very preterm newborns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics*. 1972; 50(4):515–525. [PubMed: 4561295]
2. ACOG committee opinion: antenatal corticosteroid therapy for fetal maturation. *Obstetrics and gynecology*. 2002; 99(5 Pt 1):871–873. [PubMed: 11978304]
3. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev*. 2006; (3):Cd004454. [PubMed: 16856047]
4. McElrath TF, Allred EN, Boggess KA, et al. Maternal antenatal complications and the risk of neonatal cerebral white matter damage and later cerebral palsy in children born at an extremely low gestational age. *Am J Epidemiol*. 2009; 170(7):819–828. [PubMed: 19713285]
5. Wong D, Abdel-Latif M, Kent A. Antenatal steroid exposure and outcomes of very premature infants: a regional cohort study. *Archives of disease in childhood. Fetal and neonatal edition*. 2014; 99(1):F12–20. [PubMed: 24142624]
6. Chawla S, Bapat R, Pappas A, Bara R, Zidan M, Natarajan G. Neurodevelopmental outcome of extremely premature infants exposed to incomplete, no or complete antenatal steroids. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet*. 2013; 26(15):1542–1547.
7. Vandevyver S, Dejager L, Tuckermann J, Libert C. New insights into the anti-inflammatory mechanisms of glucocorticoids: an emerging role for glucocorticoid-receptor-mediated transactivation. *Endocrinology*. 2013; 154(3):993–1007. [PubMed: 23384835]
8. Dammann O, Leviton A. Intermittent or sustained systemic inflammation and the preterm brain. *Pediatric research*. 2014; 75(3):376–380. [PubMed: 24429547]
9. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. *Pediatric research*. 1997; 42(1):1–8. [PubMed: 9212029]

10. Leviton A, Allred EN, Dammann O, et al. Systemic Inflammation, Intraventricular Hemorrhage, and White Matter Injury. *Journal of Child Neurology*. 2013; 28(12):1637–1645. [PubMed: 23112243]
11. Leviton A, Kuban K, O'Shea TM, et al. The relationship between early concentrations of 25 blood proteins and cerebral white matter injury in preterm newborns: the ELGAN study. *The Journal of pediatrics*. 2011; 158(6):897–903. e891–895. [PubMed: 21238986]
12. Yoon BH, Romero R, Kim KS, et al. A systemic fetal inflammatory response and the development of bronchopulmonary dysplasia. *Am J Obstet Gynecol*. 1999; 181(4):773–779. [PubMed: 10521727]
13. Bose C, Laughon M, Allred EN, et al. Blood protein concentrations in the first two postnatal weeks that predict bronchopulmonary dysplasia among infants born before the 28th week of gestation. *Pediatric research*. 2011; 69(4):347–353. [PubMed: 21150694]
14. Ambalavanan N, Carlo WA, D'Angio CT, et al. Cytokines associated with bronchopulmonary dysplasia or death in extremely low birth weight infants. *Pediatrics*. 2009; 123(4):1132–1141. [PubMed: 19336372]
15. Maheshwari A, Schelonka RL, Dimmitt RA, et al. Cytokines associated with necrotizing enterocolitis in extremely-low-birth-weight infants. *Pediatr. Res*. 2014; 76(1):100–108. [PubMed: 24732104]
16. Lodha A, Asztalos E, Moore AM. Cytokine levels in neonatal necrotizing enterocolitis and long-term growth and neurodevelopment. *Acta Paediatr*. 2010; 99(3):338–343. [PubMed: 19922507]
17. Kumar P, Venners SA, Fu L, Pearson C, Ortiz K, Wang X. Association of antenatal steroid use with cord blood immune biomarkers in preterm births. *Early human development*. 2011; 87(8):559–564. [PubMed: 21576006]
18. Goepfert AR, Andrews WW, Carlo W, et al. Umbilical cord plasma interleukin-6 concentrations in preterm infants and risk of neonatal morbidity. *Am J Obstet Gynecol*. 2004; 191(4):1375–1381. [PubMed: 15507968]
19. Caldas JP, Vilela MM, Braghini CA, Mazzola TN, Marba ST. Antenatal maternal corticosteroid administration and markers of oxidative stress and inflammation in umbilical cord blood from very low birth weight preterm newborn infants. *Jornal de pediatria*. 2012; 88(1):61–66. [PubMed: 22344368]
20. Arad I, Bar-Oz B, Ergaz Z, Nir A, Barak V. Interleukin-6 and N-terminal pro-brain natriuretic peptide cord blood levels in premature infants: correlations with perinatal variables. *The Israel Medical Association journal : IMAJ*. 2010; 12(7):419–423. [PubMed: 20862823]
21. Kavelaars A, van der Pompe G, Bakker JM, et al. Altered immune function in human newborns after prenatal administration of betamethasone: enhanced natural killer cell activity and decreased T cell proliferation in cord blood. *Pediatric research*. 1999; 45(3):306–312. [PubMed: 10088646]
22. Lim R, Morwood CJ, Barker G, Lappas M. Effect of silibinin in reducing inflammatory pathways in in vitro and in vivo models of infection-induced preterm birth. *PLoS One*. 2014; 9(3):e92505. [PubMed: 24647589]
23. McElrath TF, Hecht JL, Dammann O, et al. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. *Am J Epidemiol*. 2008; 168:980–989. [PubMed: 18756014]
24. Yudkin PL, Aboualfa M, Eyre JA, Redman CW, Wilkinson AR. New birthweight and head circumference centiles for gestational ages 24 to 42 weeks. *Early Hum. Dev*. 1987; 15(1):45–52. [PubMed: 3816638]
25. Leviton A, Paneth N, Reuss ML, et al. Maternal infection, fetal inflammatory response, and brain damage in very low birthweight infants. *Pediatr. Res*. 1999; 46(5):566–575. [PubMed: 10541320]
26. Onderdonk AB, Delaney ML, DuBois AM, Allred EN, Leviton A. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. *Am J Obstet Gynecol*. 2008; 198(1):110, e111–117. [PubMed: 18166321]
27. Onderdonk AB, Hecht JL, T.F. M, et al. Colonization of second-trimester placenta parenchyma. *Am. J. Obstet. Gynecol*. 2008; 199(1):52.e51–52.e10. [PubMed: 18313635]
28. Murray, P.; Baron, E.; Jorgensen, J.; Pfaller, M. *Manual of Clinical Microbiology*. 8th ed.. ASM Press; Washington DC: 2003.

29. Driscoll SG, Langston C. College of American Pathologists Conference XIX on the Examination of the Placenta: report of the Working Group on Methods for Placental Examination. *Arch. Pathol. Lab. Med.* 1991; 115(7):704–708. [PubMed: 2064531]
30. Hecht JL, Onderdonk A, Delaney M, et al. Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues. *Pediatr. Dev. Pathol.* 2008; 11(1):15–22. [PubMed: 18237241]
31. Hecht JL, Allred EN, Kliman HJ, et al. Histological characteristics of singleton placentas delivered before the 28th week of gestation. *Pathology.* 2008; 40(4):372–376. [PubMed: 18446627]
32. Fichorova RN, Beatty N, Sassi RRS, et al. Systemic inflammation in the extremely low gestational age newborn following maternal genitourinary infections. *Am. J. Reprod. Immunol.* 2015; 73(2): 162–174. [PubMed: 25164433]
33. Fichorova RN, Richardson-Harman N, Alfano M, et al. Biological and technical variables affecting immunoassay recovery of cytokines from human serum and simulated vaginal fluid: a multicenter study. *Anal Chem.* 2008; 80(12):4741–4751. [PubMed: 18484740]
34. Hecht JL, Fichorova RN, Tang VF, Allred EN, McElrath TF, Leviton A. Relationship between neonatal blood protein profiles and placenta histologic characteristics in ELGANs. *Pediatr Research.* 2011; 69:68–73.
35. McElrath TF, Fichorova RN, Allred EN, et al. Blood protein profiles of infants born before 28 weeks differ by pregnancy complication. *Am. J. Obstet. Gynecol.* 2011; 204(5):418.e411–418.e412. [PubMed: 21349490]
36. Leviton A, Hecht JL, Allred EN, et al. Persistence after birth of systemic inflammation associated with umbilical cord inflammation. *J. Reprod. Immunol.* 2011; 90(2):235–243. [PubMed: 21722967]
37. Leviton A, O'Shea TM, Bednarek FJ, et al. Systemic responses of preterm newborns with presumed or documented bacteraemia. *Acta Paediatr.* 2012; 101(4):355–359. [PubMed: 22085230]
38. Bose CL, Laughon MM, Allred EN, et al. Systemic inflammation associated with mechanical ventilation among extremely preterm infants. *Cytokine.* 2013; 61(1):315–322. [PubMed: 23148992]
39. Fichorova RN, Onderdonk AB, Yamamoto H, et al. Maternal microbe-specific modulation of inflammatory response in extremely low-gestational-age newborns. *MBio.* 2011; 2(1):e00280–00210.
40. Leviton A, Fichorova R, Yamamoto Y, et al. Inflammation-related proteins in the blood of extremely low gestational age newborns. The contribution of inflammation to the appearance of developmental regulation. *Cytokine.* 2011; 53(1):66–73. [PubMed: 20934883]
41. Leviton A, Allred EN, Yamamoto H, Fichorova RN. Investigators. ftES. Relationships among the concentrations of 25 inflammation-associated proteins during the first postnatal weeks in the blood of infants born before the 28th week of gestation. . *Cytokine.* 2012
42. Dales LG, Ury HK. An improper use of statistical significance testing in studying covariables. *Int. J. Epidemiol.* 1978; 7(4):373–375. [PubMed: 744677]
43. Wasserstein RL, Lazar NA. The ASA's Statement on p-Values: Context, Process, and Purpose. *The American Statistician.* 2016; 70(2):129–133.
44. Dandona P, Ghanim H, Sia CL, et al. A mixed anti-inflammatory and pro-inflammatory response associated with a high dose of corticosteroids. *Curr Mol Med.* 2014; 14(6):793–801. [PubMed: 25056537]
45. Kallapur SG, Kramer BW, Moss TJM, et al. Maternal glucocorticoids increase endotoxin-induced lung inflammation in preterm lambs. *American Journal of Physiology-Lung Cellular and Molecular Physiology.* 2003; 284(4):L633–L642. [PubMed: 12471018]
46. Kramer BW, Ikegami M, Moss TJ, Nitsos I, Newnham JP, Jobe AH. Antenatal betamethasone changes cord blood monocyte responses to endotoxin in preterm lambs. *Pediatric research.* 2004; 55(5):764–768. [PubMed: 14973182]
47. Peaceman A, Bajaj K, Kumar P, Grobman W. The efficacy of a single course of antenatal steroids beyond seven days. *American Journal of Obstetrics and Gynecology.* 2004; 191(6):S112–S112.

48. McElrath TF, Fichorova RN, Allred EN, et al. Blood protein profiles of infants born before 28 weeks differ by pregnancy complication. *Am J Obstet Gynecol.* 2011; 204(5):418.e411–418.e412. [PubMed: 21349490]

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Highlights

- Antenatal glucocorticoids have no sustained anti-inflammatory effects on extremely low gestational age infants.
- Preterm Infants born to mothers with strong inflammatory stimuli are more likely to be exposed to a complete course of antenatal glucocorticoids.
- Antenatal glucocorticoids could depress the preterm infant immunity during the 1st week of life.

Figure 1.

Forest plots of odds ratios and 95% confidence intervals of a protein measure in the highest or lowest quartile on **day 1** associated with maternal receipt of a complete or partial course of antenatal glucocorticoids (AGC). Odds ratios are adjusted for mother not receiving magnesium sulfate for tocolysis or seizure prophylaxis, gestational age < 27 weeks, birth weight Z-score < -1, male sex and histologic evidence of chorioamnionitis and neutrophils in the fetal stem vessels.

Figure 2.

Forest plots of odds ratios and 95% confidence intervals of a protein measurement in the highest or lowest quartile on **day 7** associated with maternal receipt of a complete or partial course of antenatal glucocorticoids (AGC). Odds ratios are adjusted for mother not receiving magnesium sulfate for tocolysis or seizure prophylaxis, gestational age < 27 weeks, birth weight Z-score < -1, male sex and histologic evidence of chorioamnionitis and neutrophils in the fetal stem vessels.

Figure 3.

Forest plots of odds ratios and 95% confidence intervals of a protein measure in the highest or lowest quartile on day 14 associated with maternal receipt of a complete or partial course of antenatal glucocorticoids (AGC). Odds ratios are adjusted for mother not receiving magnesium sulfate for tocolysis or seizure prophylaxis, gestational age < 27 weeks, birth weight Z-score < -1, male sex and histologic evidence of chorioamnionitis and neutrophils in the fetal stem vessels.

Figure 4.

Forest plots of odds ratios and 95% confidence intervals of a protein measure in the highest quartile on **day 21** associated with maternal receipt of a complete or partial course of antenatal glucocorticoids (AGC). Odds ratios in the top panel are unadjusted while those in the bottom panel are adjusted for mother not receiving magnesium sulfate for tocolysis or seizure prophylaxis, gestational age < 27 weeks, birth weight Z-score < -1, male sex and histologic evidence of chorioamnionitis and neutrophils in the fetal stem vessels.

Figure 5.

Forest plots of odds ratios (ORs) and 95% confidence intervals of a protein measure in the highest or lowest quartile on **day 28** associated with maternal receipt of a complete or partial course of antenatal glucocorticoids (AGC). Odds ratios are from multinomial logistic regression models and are adjusted for mother not receiving magnesium sulfate for tocolysis or seizure prophylaxis, gestational age < 27 weeks, birth weight Z-score < -1, male sex and histologic evidence of chorioamnionitis and neutrophils in the fetal stem vessels.

Table 1

Percent of infants whose mother received a complete or partial course, or no antenatal glucocorticoids who had the characteristics listed on the left. These are row percents.

		Antenatal corticosteroid course			Row N
		Complete	Partial	None	
Maternal					
Body Mass Index (BMI) ≥ 30	Yes	65	28	7	254
	No	63	26	1	917
Delivery					
Initiator of delivery	Preterm labor	55	32	13	543
	pPROM	75	19	6	261
	Preeclampsia	66	30	4	162
	Abruption	66	17	17	129
	Cervical insufficiency	78	20	1	69
	Fetal indication	62	25	13	52
Magnesium sulfate	No	56	20	24	385
	Tocolysis	68	29	3	665
	Seizure prophylaxis	64	31	4	156
Multifetal gestation	Yes	65	28	7	392
	No	63	26	11	824
Gestational age, weeks	23-24	60	26	14	259
	25-26	62	29	9	562
	27	68	24	9	395
Birth weight Z-score	< -2	67	27	7	75
	$-2, < -1$	67	26	6	163
	-1	63	26	11	978
Male sex	Yes	65	25	10	645
	No	61	28	10	571
Infection/inflammation					
# bacterial species isolated from placenta	0	62	31	8	565
	1	70	24	6	266
	2	65	20	15	274
Plate inflammation	Yes	68	23	8	214
	No	63	27	10	897
Chorioamnionitis	Yes	72	19	9	409
	No	59	31	11	703
Neutrophils in fetal stem vessels	Yes	74	18	8	275
	No	60	29	11	829
Early sepsis	Yes	61	32	7	75
	No	64	26	10	1140

		Antenatal corticosteroid course			Row N
		Complete	Partial	None	
Late sepsis	Yes	61	28	10	311
	No	64	26	10	900
Early tracheal infection	Yes	69	22	8	49
	No	63	27	10	1158
Late tracheal infection	Yes	63	26	11	240
	No	64	27	10	960
Cerebrospinal fluid (CSF) infection	Yes	62	35	4	26
	No	63	26	10	1169
Maximum N		773	322	121	1216