UNPUBLISHED DATA

TITLE

Antibiotic Prophylaxis for Caesarean Section: a pilot randomized controlled trial on the risk of neonatal and maternal infections

ABSTRACT

Introduction

To assess the effect of prophylactic antibiotics before or after cord clamping at caesarean section on neonatal and maternal infection.

Material and methods

A single-centre randomised controlled clinical trial allocated women delivering by caesarean section to prophylactic antibiotic administrated 15 to 30 minutes prior to skin incision or after umbilical cord clamping, and follow-up on mothers for 30 days post operative and infants for 9-months. The outcomes in this paper comprise results on neonatal expression of genes in blood related to myelopoiesis and maternal infection morbidity.

Results

Overall, 42 women and 44 infants were randomised. No significant differences were observed for neonatal gene expression at day 2-3 ($p >= 0.05$ for all) or on maternal infectious morbidity (relative risk 0.26, 95% CI 0.06–1.12; $P = 0.062$) within 30 days post-surgery.

Conclusion Timing of antibiotic exposure did not influence neonatal or maternal infections. Further, there were no clear effects of cefuroxime exposure on granulocyte-related gene expression in the neonate. Previous data from this study did not demonstrate effects on the microbiome of the child. We therefore find no reason to question the recommendation that cefuroxime should be administered before skin incision for CS.

Trial registration number CinicalTrials.gov (NCT02072798)

Keywords Caesarean Section, Cefuroxime, Granulocytosis, Gut, Half-life, Microbiota, Neonatal, Prophylactic Antibiotics.

Abbreviations

CS, cesarean section

SSI, surgical site infection

ITGAM, integrin subunit alpha M

ARG1 arginase 1

ELANE, elastase

INTRODUCTION

Women undergoing caesarean section (CS) have an increased risk of postpartum infections compared to women who give birth vaginally.[1] Endometritis, urinary tract infection, and wound infection are the most common surgical site infections (SSI) following CS.[2] Prophylactic antibiotics have proved effective in preventing SSIs.[3,4] The effect of prophylactic antibiotics is optimal if the dose is administrated in the hour before the surgical incision,[5] and the risk can be reduced further if the antibiotic is administrated in the 30 minutes prior to incision.[6] Administration more than two hours before or after the incision increases the risk of SSI due to insufficient concentration of antibiotics in the surgical field.[5] Previous recommendations were for a single dose of antibiotic administered immediately after umbilical cord clamping, rather than preoperatively, to avoid placental transfer because of putative neonatal adverse effects.[3] There were also concerns over the link between antibiotics administered before pregnancy and antenatally and the possibility of childhood obesity, asthma and atopic disease.[7] Subsequently, individual studies and systematic reviews have demonstrated that pre-incision antibiotic prophylaxis compared to that after cord clamping is advantageous for the mother with no apparent disadvantage to the neonate.[2,8,9] As a result, countries such as the USA, UK and Canada have changed their national guidelines to recommend that the timing should be 15 to 60 minutes prior to skin incision.[10-12] The neonatal outcomes most frequently studied are neonatal sepsis, neonatal septic work-up and admission to the special care baby unit.[2,9,13] We have previously published the effects of antibiotics dosing pre- or post cord clamping on cefuroxime clearance and the infant microbiota at age 10-days or 9-months, and did not find any significant differences.[14,15] In summary, the plasma cefuroxime concentrations in cord blood were low in infants exposed to cefuroxime before CS, corresponding to only 30–50% of the expected plasma concentration in the mother after administration of 1.5 g of IV cefuroxime. The half-life of cefuroxime was approximately three times longer (210 minutes) compared with adults (70 minutes),[16] but exposure to cefuroxime varied considerably between infants.[14] Further, we found no clear differences in gut microbiota composition between infants of mothers who received cefuroxime prior to skin incision or immediately after umbilical cord clamping at 10-days or 9-months postnatally, though at 9-months, although despite no overall gut microbiota compositional differences, the number of observed species was increased in the control group.[15] Differences in the microbiota at earlier time points cannot be excluded, but the microbial composition at earlier stages may be highly unstable and hence difficult to compare, or may require

greater numbers to demonstrate significant differences. However, the putative effects of antibiotic intervention may take place, or become evident earlier, in the first few days of life. Neonatal granulocytosis is a well-established postpartum event, seen as an increase in the concentration of white blood cells, and in particular granulocytes, in neonatal blood that peaks one day postnatally, then decreases during the following 5-6 days, reaching a concentration below the level at birth.[17] This postnatal peak is partly caused by the microbial challenge taking place during and after birth as seen in a study comparing germ-free and wild bred mice [18] and in antibiotics treated mice.[19,20] Accordingly, antibiotics may affect the level of white blood cells, in particular the level of granulocytes in the first days postpartum. The overall aim of this study was to investigate the timing of prophylactic antibiotics in women undergoing CS, with particular focus on neonatal outcomes. In this article, we report the outcomes neonatal granulocytosis in blood taken on day 2-3 postnatally determined by qPCR and maternal infectious morbidiy within 30 days post-surgery, the study design and feasibility.

MATERIAL AND METHODS

Study design

A single centre unblinded, parallel, randomised clinical trial with equal randomisation 1:1 was conducted between February 2014 and July 2014 at Odense University Hospital, Denmark. The trial was approved by the Danish Health and Medicines Authority (EudraCT 2012-002068-29), the Regional Scientific Ethical Committees for Southern Denmark (S-20130117), monitored by the local Good Clinical Practice Research Unit, and registered at cinicaltrials.gov (NCT02072798).

Participants

Eligible participants were women, age 18 or older, with a pre-gestational BMI less than 30kg/m^2, who could read and understand Danish, and were due to have a planned birth by CS at Odense University Hospital. Exclusion criteria were: i) hypersensitivity to cefuroxime or other cephalosporins; ii) previous immediate and/or severe hypersensitivity reaction to penicillin or other betalactam antibiotics; iii) systemic exposure to any antimicrobial agent within the week before delivery; and iv) women who were immunosuppressed such as those who were HIV seropositive or using systemic steroid therapy. Written information was sent to women two weeks prior to their scheduled CS at Odense University Hospital, Denmark. A trained study nurse

approached eligible subjects at a pre-planned information session the day before their scheduled CS and informed consent was obtained if participation was accepted.

Interventions

Participants were randomly assigned to one of two groups, both of whom received a single dose of intravenous (IV) cefuroxime 1.5 g dissolved in 100 ml NaCl. In the intervention group cefuroxime was administrated 15 to 30 minutes prior to the skin incision and in the control group cefuroxime were administrated immediately after umbilical cord clamping, as per standard practice at Odense University Hospital at the time the study was conducted. The exact timing was recorded in each case. Cefuroxime, a second-generation cephalosporin, is the standard prophylaxis recommended in the Danish National Guidelines [21] and was selected because it is active against streptococci, staphylococci, and most enterobacteria.[22] All IV infusions were prepared and administered by the anaesthesia staff. The surgical technique was the same for both groups.

Outcomes

The primary outcome was the neonatal gut microbiome on day-10 of life, as reported elsewhere.[15] The secondary neonatal outcomes were cefuroxime half-life in neonates in the intervention group,[14] neonatal granulocytosis day 2-3, and the neonatal gut microbiome at 9-months follow-up.[15] Secondary maternal outcomes were the incidence of post-CS SSIs (endometritis, urinary tract infection and wound infection), length of hospitalization, and readmissions to hospital, or contact with the general practitioner due to suspicion of infection after CS, and antibiotic treatment.

Data collection

In the intervention group one blood sample was taken from the umbilical cord, and two blood samples (0.5 ml of capillary blood) were taken from the infant at 3-4 hours and 8-10 hours postnatally. Details are described elsewhere.[14] All infants in both groups had a blood sample (0.05 ml of capillary blood) taken on days 2-3 postpartum for immunological analysis. Stool samples were collected from the infants during the nurse visit 10-days and 9-months postnatally. Details are described elsewhere.[15] A data collection form was used to collect intraoperative data. Data on patient characteristics and hospitalisation were obtained from the medical records. The study nurse followed enrolled participants (mother and children) throughout their hospital stay. Data about

symptoms of maternal infection, contacts with the healthcare professionals, and the need for antibiotics after discharge were obtained by a self-administered questionnaire sent electronically to all participating women within 30 days of CS. The questionnaire data were subsequently compared with hospital diagnostic codes and prescriptions for antibiotics. Data on diagnostic codes and prescriptions were obtained from the Danish National Patient Registry [23] and the Odense University Pharmacoepidemiological Database,[24] respectively. The parents were also asked to fill out a nutritional questionnaire from birth to 10-days postpartum.

Sample size

The power calculation for this pilot/feasibility study was based on the cefuroxime half-life analysis where a sample size of 20 is normal for pharmacokinetic studies. Thus 20 patients should have cefuroxime before clamping the cord. Furthermore, the sample size of 40, randomised 1:1 in two groups, was chosen to be able to detect a 10-25% difference in the microbiota in the infants' stool with p-value of 0,05 and a power of 80%.

Randomisation and blinding

A restricted block randomization sequence was created with a 1:1 allocation using a fixed block size of four. The block size was unknown to the investigators, study nurse and participants during the trial. No stratification was used. A data manager with no clinical involvement in the trial prepared the randomization sequence using a computerised random number generator. When the study nurse had obtained the woman´s consent, she used a computer link to the random number generator to randomise the woman. As it would have been unethical to take blood samples for the measurement of cefuroxime in newborn infants from the control group, blinding was abandoned.

Quantitative real time PCR on whole blood samples in infants.

The relative expression of the following genes; integrin subunit alpha M (ITGAM) encoding the surface marker CD11b, ARG1 encoding arginase 1, ELANE encoding elastase, and HP encoding haptoglobin in blood was measured by qPCR.[25] Briefly, heel-prick blood taken on day-2 or 3 postnatally was transferred to a tube with MacMAX lysis/binding solution and stored at -20ºC. RNA was extracted using Mag-MAX-96 Blood RNA Isolation kit (Ambion, AM1837) and MagMAX Express Magnetic Particle Processor. RNA concentration and purity was assessed using

Thermo Scientific NanoDrop 2000. Aliqouts of RNA (50 ng) were subjected to cDNA synthesis with random hexamer primers in a total volume of 20 µL according to manufacturer's instructions, using Applied Biosystems High Capacity cDNA Reverse Transcriptase Kit (Thermofisher Technologies). Gene expression analysis was performed on 2µL cDNA, corresponding to 2.5 ng of RNA, in a total volume of 10 µL using TaqMan assays from Applied Biosystems.for ITGAM, ARG1, ELANE and HP and with ACTB encoding Gene expression of ACTB b-actin and PKG1 encoding β-actin and PKG1 encoding protein kinase G1 were used as reference genes. The expression of target genes were normalized to each reference gene [ΔCq = Cq(target)– Cq(reference)].

Statistical methods

Baseline characteristics and maternal outcomes were presented descriptively. Continuous variables were summarised as mean and standard deviation (SD) or as mean and the interquartile range (25th to 75th percentile) if the distribution was asymmetrical. Categorical variables were summarised as numbers and percentages. Gene expression data were analysed by unpaired t-test and correlation analysis assuming Gaussian populations in Graphpad Prism (version 5.03 for Windows, Graphpad software, La Jolla, CA, www.graphpad.com). Maternal infectious morbidiy were estimated by relative risks (RR) with 95% confidence intervals (95% CI). A significance level of 0.05 (two-sided) was chosen. The statistical software Stata, version 15.1 (StataCorp, College Station, TX, USA) was used for statistical analyses. No core outcome set was used, and no participants were involved when designing the study. However, a website was made available, describing the study design, and relevant information about the post-operative course.

Ethics approval

This trial was approved by the Danish Health and Medicines Authority (EudraCT 2012-002068-29), the Regional Scientific Ethical Committees for Southern Denmark (S-20130117), monitored by the local Good Clinical Research Unit, and registered at cinicaltrials.gov (NCT02072798).

RESULTS

A total of 140 women were assessed for eligibility, of which 67 (48%) declined to participate and 30 women (21%) were excluded, mainly due to failure to fulfil the inclusion criteria or because

they had an emergency CS. One woman randomised to the control group was excluded after randomisation because she had an emergency CS prior to her planed CS. Participants were recruited until 20 evaluable infants in each group were reached. Two women in the intervention group gave birth to twins. Overall, we enrolled 42 women and 44 infants. There was no loss to follow-up but in four infants, the capillary blood samples taken 8-10 hours postnatally were lost and one set of twins withdrew from the study prior to 9-months follow-up. Figure 1 illustrates the participant flow. There was only one protocol violation in a woman in the control group who received a double dose of IV cefuroxime of 1.5 g and a single dose of IV metronidazole of 1.5 g due to a prolonged and complicated delivery. There were no reports of adverse events related to the use of cefuroxime during the trial. Table 1 summarizes the baseline maternal and infant characteristics. No significant differences were observed between the mothers in the two groups. The main reason for CS was maternal request (43%), followed by breech presentation (21.5%) and maternal indication (21.5%). In the intervention group, prophylactic antibiotics were administrated with a mean time of 23.8 minutes prior to skin incision with an interquartile range of 28 to 20 minutes. In the control group antibiotic was administrated with a mean time of 0.8 minute (48 seconds) after cord clamping with a range from 0-3 minutes.

Outcomes

In the infants, blood samples taken on days 2-3 postnatally, showed an expression of ITGAM encoding the myeloid marker CD11b that was marginally higher in the pre-, compared to the post-treated group (1.38+/- 0.94 vs 1.03 +/- 0.83, p= 0.057). The expression of the genes encoding elastase and arginase 1 expressed in different states of not fully differentiated granulocytes did not differ between the two groups (p= 0.202 and p=0.591, respectively). The expression of HP was un detectable or close to the detection limit in all samples (data not shown). Expression of ARG1 but not of ELANE correlated with the expression of ITGAM (Figure 2). None of the infants were readmitted to hospital or treated with antibiotics during the first 10-days of life. In total, 15 infants were exclusively breastfed (34%), four received only infant formula (9%) and 25 infants (57%) were fed with both breast milk and formula during the first 10 days of life.

In the mothers, a total of six women (27%) in the intervention group and three (15%) in the control group were in contact with their general practitioner due to suspicion of infection. One woman in the control group, who received prophylactic antibiotic three minutes following cord clamping,

developed a urinary tract infection, endometritis, and mastitis. She was readmitted to the hospital on day six postpartum and treated with antibiotics. Overall, seven women were treated with antibiotics for urinary tract infection or mastitis within the first 30 days after surgery (intervention, n=2; control, n=5). Although there were no statistically significant differences in the number of maternal SSI (endometritis, urinary tract infection and wound infection) between the two groups, overall, more cases of infection were seen in the control group than in the intervention group (RR 0.26, 95% CI 0.06–1.12; P = 0.062) (Table 2). Finally, there was no difference in length of hospital stay between the two groups.

DISCUSSION

To our knowledge this is the first randomised clinical trial investigating the timing of prophylactic antibiotic for CS with a focus on the possible impact on the composition of the infant gut microbiome. The gut microbiota results, and cefuroxime clearance, has been published previously.[14,15] As an alternative early measure of the effect of antibiotic treatment, we measured the whole blood expression of genes related to granulopoiesis on day-2-3 postnatally. This method offers a well-established and convenient method to assess differences in cell populations in blood.[26] The expression of ITGAM encoding the myelocyte specific surface marker CD11b tended to be higher in the group receiving antibiotics treatment prior to CS. This may indicate that the neonatal granulocytosis may be slightly delayed in the group receiving antibiotic treatment prior to intervention, due to reduced or delayed colonization. The lack of statistical correction for multiple comparisons, however, should be borne in mind. The correlation with the ARG1 but not the ELANE gene expression, supports the difference in ITGAM expression. ARG1 is expressed myelocytes and meta-myelocytes, while ELANE is expressed in the earlier differentiation state pro-myelocytes These not fully differentiated granulocytes are primarily present in the bone marrow but in granulocytosis, these not fully differentiated granulocytes may be present in blood signified by an increase in the ARG1 and ELANE expression. However, our findings should be confirmed in larger studies and preferentially also by other methods.

Evaluation of the feasibility of the study showed, that the randomisation, data collection and monitoring procedures worked well and we did not encounter any safety problems. However, a high proportion of eligible women declined to participate (consent rate of 30%). The main reasons

for declining participation were: i) participation in multiple studies during pregnancy; ii) the project did not appeal to the parents; iii) participation required additional blood samples from the infants. The last reason was consistent with the findings of a previous study showing that women are less willing to allow their newborns to participate in research, particularly if it involves physical or biological data collection.[27]

We were not able to show an effect on maternal infectious morbidity though the overall RR showed a tendency towards an reduced risk of maternal infectious morbidity in the intervention group. This was probably because of the small sample size. Nevertheless, the evidence of prophylactic antibiotic administrated prior to skin incision in women undergoing CS to reduce the risk of maternal infectious morbidities has been well established in the literature.[8,9,13,28] The same studies showed that neonatal outcomes, such as sepsis, sepsis workup, and admission to the neonatal intensive care unit, did not differ between infants of mothers who received prophylactic antibiotics prior to skin incision or after cord clamping.[8,9,13,28] As a result, many national guidelines have changed their recommendation for timing of prophylactic antibiotics, suggesting that antibiotics should be administrated before skin incision.[10-12] However the lack of robust evidence for neonatal outcomes means that hospital practices still differ because of concerns about the long-term consequences for children. We questioned whether neonatal exposure to antibiotics during a CS would affect the composition of the infant gut microbiota. This is relevant as the use of antibiotics during pregnancy has been shown to increase the risk of asthma in early childhood and is likely that the link is through alteration of gut microbiota.[7,29] The strengths of our study are in the randomised design, the 100% response rate and adherence to protocol, and very few missing data. The limitations were the small sample size (albeit that this was a pilot/feasibility study), the unblinded design which can introduce observer and patient bias,[30] and the narrow inclusion criteria (planned CS and pre-pregnancy BMI <30 kg/m^2) that might have affected the extrapolation of maternal results.

CONCLUSION

Timing of antibiotic exposure did not influence neonatal or maternal infections. Further, there were no clear effects of cefuroxime exposure on granulocyte-related gene expression in the neonate. Previous data from this study did not demonstrate effects on the microbiome of the child. We

therefore find no reason to question the recommendation that cefuroxime should be administered before skin incision for CS.

Legends of figures and tables

Figure 1: Flow diagram for timing of prophylactic antibiotics prior to skin incision vs after umbilical cord clamping in women giving birth by caesarean section.

Figure 2: A: Relative expression of genes related to myelopoiesis in whole blood from neonates on the 4th postnatal day. Gene expression is shown as relative fold expression relative to the average of all samples in the Pre- group. B: Correlation between the expression of integrin subunit alpha M (ITGAM) and arginase 1 (ARG1).

Table 1: Maternal, Infant and Perioperative Characteristics by Antibiotic Timing Group

Table 2: Maternal Infectious Morbidity

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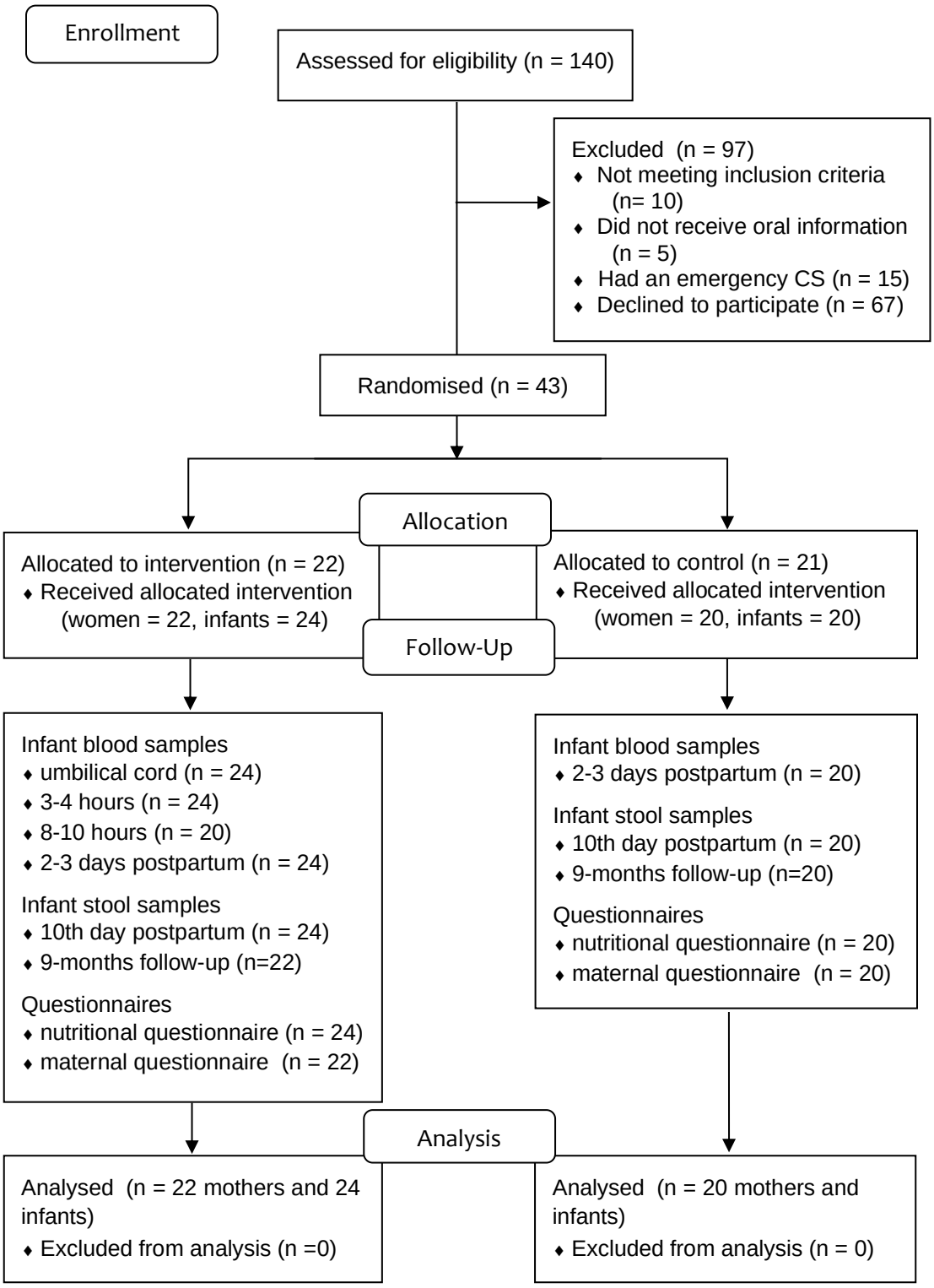

Figure 1: Flow diagram for timing of prophylactic antibiotics prior to skin incision vs after umbilical cord clamping in women giving birth by caesarean section.

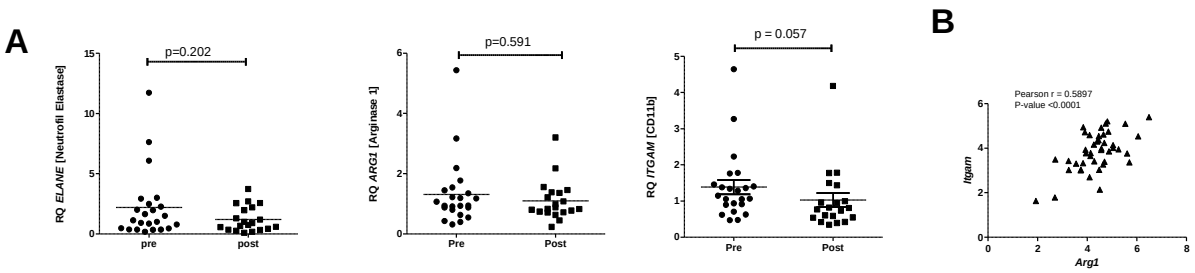

Figure 2: A: Relative expression of genes related to myelopoiesis in whole blood from neonates on the 4th postnatal day. Gene expression is shown as relative fold expression relative to the average of all samples in the Pre- group. B: Correlation between the expression of integrin subunit alpha M (ITGAM) and arginase 1 (ARG1).

Table 1. Maternal, Infant and Perioperative Characteristics by Antibiotic Timing Group

	Prior to skin incision	After cord clamp
Maternal characteristics	(n= 22)	(n= 20)
Maternal age (years)[a]	31.5 (5.0)	31.6 (4.8)
Maternal BMI, pre-gestational (kg/m^2)[a]	24.7 (3.4)	22.7 (3.2)
Singleton	20 (91%)	20 (100 %)
Primiparous	9 (41%)	7 (35%)
Multiple gestations	2 (9%)	0 (0%)
Previous caesarean section	10 (45%)	10 (50%)
Diabetes mellitus	2 (9%)	0 (0%)
Hypertension	0 (0%)	2 (10%)
Preeclampsia	0 (0%)	1 (5%)
Smoking during pregnancy[c]	1 (5%)	4 (20%)
Time between antibiotic administration and skin incision (minutes)[b]	23.8 (28.0 to 20.0)	-6.8 (-4.5 to -8.0)
Time between cord clamping and antibiotic administration (minutes)[b]	-29,6 (-33.0 to -26.0)	0.8 (0.0 to 1.0)
Estimated blood loss (mL)[b]	411.1 (250 to 600)	454.5 (265 to 535)
Operative time (minutes)[b]	32.6 (24.0 to 39.0)	31.9 (23.5 to 42.5)
Duration of hospital stay (days)[b]	3.9 (3.0 to 4.0)	3.3 (3.0 to 4.0)
Readmission	0 (0%)	1 (5%)
Infant characteristics	(n = 24)	(n = 20)
Birthweight (g)[a]	3480 (514)	3701 (529)
Gestational age (weeks)[a]	38.6 (0.7)	38.4 (0.5)
Apgar at 5 minutes[a]	10 (10)	10 (10)
Suspected sepsis	0 (0%)	0 (0%)
NICU admission	1[d] (4%)	0 (0%)

a, mean (standard deviation); b, median (25 to 75 percentile); BMI, Body Mass Index; c, self-reported; NICU, Neonatal Intensive Care Unit; d, one child was admitted to the NICU due to mother with gestational diabetes mellitus and was hospitalised for three days.

Table 2. Maternal Infectious Morbidity

	Prior to skin incision (n=22)	After cord clamping (n= 20)	Relative risk (95% CI)	P-value
Total infectious morbidity	**2 (9.1%)**	**7 (35%)**	**0.26 (0.06–1.12)**	**0.062**
Endometritis	0 (0%)	1 (5.0%)	NA	
Wound infection	0 (0%)	0 (0%)	NA	
Urinary tract infection	1 (4.6%)	4 (20%)	0.22 (0.03–1.87)	0.174
Mastitis	1 (4.6%)	2 (10%)	0.46 (0.05–4.64)	0.598

CI, confidence interval; NA, not applicable