OUH
Odense
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University of Southern Denmark

Antibiotics and gut microbiota among newborn infants

Sponsor-investigator
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Investigator
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Co-investigator
Nana Hyldig, PhD-fellow, University of Southern Denmark. Co-investigator for the project "Antibiotic prophylaxis for postpartum infections following caesarean section".

A nurse will be responsible for written and oral information and randomization. Responsible that the randomization during the caesarian section with antibiotics is carried out, collecting data from the pregnant women / parents, and material (blood and faeces) from the infants.

Contract Research Organization
The GCP unit
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Trial centres

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We, the undersigned, hereby certify that the trial will be carried out in accordance with the protocol, ICH-GCP guidelines and current statutory requirements/legislation.

Date: 19. September 2013 Date: 19. September 2013 Date: 19. September 2013

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Jan Stener Jørgensen Sponsor-Investigator Odense University Hospital	Gitte Zachariassen Investigator, responsible for the children part	Nana Hyldig co-investigator Odense University Hospital

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Introduction

This is a protocol combining a previous additional protocol with the study "Antibiotic prophylaxis and Intervention for postpartum infections following caesarean section" (Eudra-CT 2012-002068-29). The new protocol is a feasibility study on the original project and an additional / detailed protocol on "Antibiotics and gut microbiota among newborn infants".

Usually, surgical antibiotic prophylaxis is given before skin incision to reduce the risk of wound infection, but for caesarian section antibiotics is normally given after the birth to reduce the antibiotic exposure of the infant. As wound infection after caesarean section remains a clinically important problem, it is relevant to explore the relevance of this special precaution.

At birth, all mammals must rapidly adapt to intake of complex milk nutrients via the gut and simultaneously tolerate the invasion of billions of microbes. This requires rapid maturation of the digestive and immune functions to avoid gut disorders and infections. Full-term, breast-fed infants normally adapt well, but factors such as caesarean birth, high hygiene levels, antibiotics treatment and formula feeding may inhibit immune development both short and long term.
Birth by caesarean section in high-hygiene hospital environments, and widespread use of antibiotics, are factors that reduce gut microbiota density and diversity in the newborn for some time after birth (1, 2). On the other hand, high-hygiene environments and antibiotics are essential tools to combat infections, especially for the weakest newborn infants. Others have described a possible association between the impact of gut microbes in allergic diseases later in life (3).

Aim

Our hypothesis is that antibiotics given to the pregnant women prior to incision of the skin for a caesarean section will change the gut micobiota in newborn infants compared to antibiotics given to the mother after the child is born and the cord is clamped.

Materials and methods

The original study examines the effect of change in timing of prophylactic antibiotics on the rate of post-CS infections (endometritis, UTI and WI). This pilot study will be a feasibility study to the original study with focus on antibiotic and changes in the gut micobiota of newborn infants. The feasibility study will only include pregnant women

in Odense with a body mass index below 30, and planned cesarean section. The difference between the original study and the pilot study is shown in the table below.

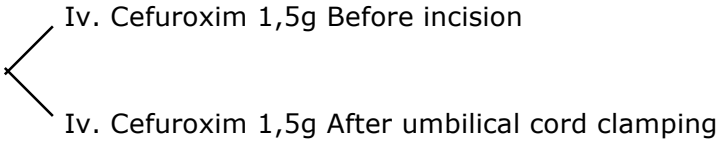
The original project	**The pilot study**
Double-blinded	Not blinded
2844 participants	40 participants
Elective + emergency CS	Elective CS
Sites: Odense, Hvidovre, Esbjerg	Site: Odense
All women, regardless of weight	BMI < 30

This part of the project is a workpackage (1.2b) in the international Neomune-project on infant gut microbiota (from University of Copenhagen, Prof. Gorm Greisen).

Intervention

The intervention is complete described in the original protocol.

We will compare a second-generation cephalosporin (iv cefuroxim 1,5g) administrated 15-60 minutes before incision versus after umbilical cord clamping, as per current practice. Exact timing will be registered in each case. Cefuroxim has been selected as it covers streptococci, staphylococci, and most enterobacteriaceae (4) and is standard procedure according to Danish National Guidelines (5).



As part of the study we will collect faecal content from infants of mothers treated with antibiotics (Cefuroxim) either before or after transection of the umbilical cord at caesarean delivery (e.g. with or without infant exposure to antibiotics)

Trial subjects

All women delivering a child by elective CS during the project period, at Department of Gynaecology and Obstetrics of OUH will be asked to participate. A project nurse will hand out written together with oral information about the project at the information meeting the day before the elective CS. The woman will be informed about her rights to bring a member of her family, a friend or an acquaintance with her to the

informative interview. If they accept to participate, they bring a signed written consent form when they arrive for the CS. It will be possible to call a doctor, who can answer questions before participation. Patients, who do not wish to participate, will receive iv. Cefuroxim 1,5g post umbilical cord clamping, as per current practice.

Inclusion Criteria

- Age ≥ 18 year
- Women, who can read and understand Danish
- A gestational age ≥ completed 28 weeks of gestation
- Rupture of membranes and active labour (uterine contractions) is allowed.
- BMI < 30

Exclusion Criteria

- Hypersensitivity to Cefuroxim or to any other cephalosporin antibiotics
- Previous immediate and/or severe hypersensitivity reaction to penicillin or any other beta-lactam antibiotic.
- Systemic exposure to any antibiotic agent within 1 week before delivery Antibiotic indicated due to PROM, fever, GBS or other indications at the time of caesarean section.
- Women being immunologically incompetent (e.g. HIV positive)
- Very sick newborn infants transferred to a NICU and treated with antibiotics will be excluded from the study

Randomization

A computerized random-number generator will be prepared by Odense Patient data Explorative Network. The participants will be randomized 1:1. No stratification will be used. Since the study is non-blinded a randomization code will not be needed.

Blinding

The pilot study is a non-blinded RCT because we only want to collect blood samples from infants delivered by mothers in the intervention group.

Project medication

Cefuroxim is a second-generation cephalosporin. The summery product characteristics (SPC) of cefuroxim is described at the Danish Health and Medicines Authority's webpage (4).

The woman will be given iv Cefuroxim 1,5g, dissolved in 100 ml NaCl before incision or after umbilical cord clamping. Because Cefuroxim is shelf-medication we will not keep an overall medical recording. Batch No. will be recorded at an individual level.

Adverse reactions/Adverse events

Adverse Events

The definition of an adverse event (AE) is any untoward medical occurrence in a patient or clinical trial subject to whom a medicinal product has been administered and which does not necessarily have a causal relationship with this treatment. A serious event (SAE) is any untoward medical occurrence effect that at any dose results in: death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

Monitoring AE

All protocol-specific AEs are handled and reported in accordance with relevant regulations and are included in the final clinical report. Reporting and collection of AEs begins at the time of antibiotic administration and ends at discharge from the hospital. The infants will be followed by a nurse prior to hospital discharge, and if any problems that possibly could be related to the time of antibiotic administration are observed a paediatrician will examine the child.

Recording of AE

All information about adverse events must be recorded in the case report form (CRF). E.g. all deviations from the normal admissions cycle (complications, treatment initiatives and extra bed days). An exception is any incidence that can be related to CS or birth, e.g. low haemoglobin due to bleeding, decrease in blood pressure associated with the administration of iv syntocinon or due to uterine atonty, breathing difficulties caused by high-acting anaesthetic, shoulder pain occurring in connection with the operation (occurs when blood and amniotic fluid flows into the peritoneum), nausea or vomiting (anaesthesia), fatigue, wound pain, breast tenderness, emotional labile, crying labile. These incidences will not be treated as adverse events.

All adverse events must be followed until the end of the event, stabilization, or until it is determined that the project drug or participation in the project has not been the cause of the incident.

Reporting of SAE

Information on all serious adverse events must be collected and recorded on the SAE form. All SAEs must be reported to co-investigator (Nana Hyldig, 65415156 / 20647630), investigator (Gitte Zachariassen 21360426) or sponsor-investigator (Jan Stener Jørgensen, 65412346) within the next working day after the investigator becomes aware of the SAE. An exception to this reporting is serious incidents that, according to the trial protocol, do not require to be reported immediately. Reporting must be followed-up by a detailed report in writing, and in both the immediate report and the subsequent report, the investigator must identify the trial subjects with a personal code number.

The following serious incidents will not be handled as an SAE

- Hospitalization due to problems with the child
- Re-hospitalization due to infection

Adverse Reaction

The definition of an adverse reaction (AR) is all untoward and unintended responses to an investigational medicinal product related to any dose administered. An unexpected AR is an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

The half-life of cefuroxim is about 70 minutes after intravenous injection. The elimination is approximately five times the half-life, corresponding to the majority of cefuroxim being eliminated within 6 hours after the administration. ARs are therefore expected to occur during the period of hospitalization.

Since prophylactic cefuroxim is standard practice for CS, the study differs from this practice by administering cefuroxim prior to cord clamping, thereby exposing the infant via placenta to one dose as well. Cefuroxim is generally considered safe to use during pregnancy and early life, but it has been debated, whether antibiotic exposure in early life alters the normal colonizing flora of the infant in a way, which contributes to a risk of later development of allergic illnesses (6). This is still an unresolved issue, but the risk of one dose is considered low, and the American College of Obstetricians and Gynecologists recommends using antibiotic prophylaxis prior to cord clamping (7). Maternal adverse reactions to cefuroxim and infants admission to special care will be registered during hospital stay.

The well-known side effects to cefuroxim are listed at the Danish Health and Medicines Authority's webpage (4). As only a single dose of iv Cefuroxim is administered, it is not considered likely that the most common undesirable effects such as thrombophlebitis and pain following iv injection, skin rashes, urticaria, pruritus,

gastrointestinal disturbances, diarrhoea, nausea and vomiting will occur. Because the mentioned side effects could equally well be caused by anaesthesia or related to hormones due to pregnancy and childbirth, they will only be collected if the woman spontaneously expresses them. Women who previous had immediate and /or severe hypersensitivity reaction to penicillin or any beta-lactam antibiotics are excluded from the study. If a woman during surgery develops anaphylactic shock there is, in the department, a standard operating procedure for how this condition should be treated.

Reporting of serious unexpected adverse reactions (SUSARs)

The summery of product characteristics (SPC) describes serious side effects. The SPC is available from the Danish Health and Medicines Authority's webpage (4). In this protocol the SPC version used is updated the 21.05.12. The sponsor shall assess whether an AR is unexpected. A SUSAR is a serious adverse reaction (SAR) of which the relationship with the drug cannot be rejected and of which the nature or severity is not consistent with the SPC. SUSARs must be reported to the Danish Medicines Agency and the Science Ethics Committee within seven days in case of death or if the SUSAR is life threatening. Other SUSARs must be reported within fifteen days.

Annual Safety Report

There will be an annual safety report throughout the duration of the clinical trial. The sponsor will provide the Danish Medicines Agency and the Science Ethics Committee with a list of all suspected SARs and SUSARs which have occurred during this period and a report on the trial subjects' safety.

Outcome

Maternal

The primary outcome is the incidence of post-CS infection (endometritis, UTI and WI) in each study group. The secondary outcomes are length of the primary and any secondary hospitalization, readmissions to hospital/contact to the general practitioner on suspicion of infection after CS and antibiotic treatment.

Infant

Concentration of Cefuroxim in blood samples during the first 24 hours of life, and fecal microbiota at the tenth day of life (+ 6 months if difference at 10 days).

Data collection

All the protocol required information on each trial subject are recorded in a paper CRF.

We will collect 40 (20+20) faecal samples. Faeces will be collected on day 10 from birth, frozen to -80 degrees Celsius for later analysis at University of Copenhagen, Department of Food Science.
If the pregnant woman was treated with antibiotics prior to umbilical cord clamping (20 women), blood samples from the umbilical cord, and two blood samples (each of 0,5 ml) from the infant (capillary blood) within 24 hours will be analyzed for content of cefuroxim. This part of the study - with only blood samples from infants exposed to antibiotics - will be possible since the feasibility study will be randomized but not blinded. On day 2.-3. all 40 infants will have 50 micro liters of blood taken for immunological analyses. This blood sample will be taken together with the routine PKU.
The parents will also be asked to collect fecal samples on day 10. The samples needs to be frozen immediately and will be picked up at the parents house on day 10 or shortly after. They will also be asked questions on day 10 (by telephone). At discharge they get the questionnaire about type of nutrition for the infant(s) since birth.
If gut microbiota is influenced by cefuroxim after 10 days, then also long term effect (6 months) will be investigated by collecting new fecal samples.
Both blood samples and fecal samples will be destroyed after analysis. A biobank will not be established.

Information about symptoms of infection after discharge will be obtained by a self-administered questionnaire sent electronically, using the Internet-based survey system SurveyXact, to all participants within 30 days post-CS[1]. If the participant does not answer the electronic questionnaire within one week, she will receive a paper questionnaire by mail.

Data on women and infants will be collected in the Medical Case Report Form and the Danish Medical Birth Registry (8), e.g.: maternal age at time of delivery, gestational age, birth weight, singleton birth, pre-pregnancy BMI, , dates of admission and discharge These variables are used for characterization of the two study groups and subsequently for adjustment for potential confounding factors in the analysis.

[1] According to the CDC's recommendation postoperative infections should by definition include those that is developed up to 30 days post procedure (see Appendix 1) (5).

Statistical analysis and sample size

In this pilot study we will include 40 pregnant women; 20 women / infants in each group who accept to participate and will complete delivering fecal samples. In this pilot study we expect to find a difference in microbiota from 10-25% of the infants (a p-value of 5 with a power of 80% making it necessary to evaluate 20+20 infants.

Content of antibiotics in infant blood will be analyzed in order to calculate half life of Cefuroxim in newborn infants.
Molecular analysis (sequencing of the S16 region of bacterial DNA) allows determining the presence or absence of the large number of bacterial species that is normally present in the stools. Furthermore, sequencing of DNA that code for cefuroxim resistance will allow accessing the risks of selection of resistant organisms. As the study is randomized, the risk of confounding the indication for antibiotic treatment of the mother will not be relevant.

The odds ratio (OR) for post-CS infections will be estimated in relation to surgery and intervention. Crude and adjusted odds will be presented with a 95% confidence interval. Multivariable logistic regression analysis will be used to adjust for potential confounders. The analysis will be carried out on an intention to treat basis.

Ethical considerations

The gain of the drug trial is expected to be a reduction of post-CS infection (endometritis, UTI and WI). The gain must be evaluated against the side effects and risks, which is exposure of the child to antibiotics before birth. We will monitor the children closely to ensure that the trial does not influence negatively on the neonatal outcome.

The trial will be conducted according to protocol. Ordinary procedures for quality control and assurance are complied with §§ 3 and 4 of the Danish executive order on GCP, current guidelines of the Declaration of Helsinki, ICH-GCP and the applicable regulatory requirements. The law on Processing of Personal Data and Health Act protects information about trial participants. The project will be reported to the Data Protection Agency, the Science Ethics Committee and the Danish Health and Medicines Authority**.** In connection with monitoring, auditing and/or inspection by an scientific ethics committee, the Danish Medicines Agency or the GCP unit there will be authorized direct access to source data/documents (including patient files).

Information to the participants will be given orally and in written. Written consent will be obtained from all participants before inclusion. The written information will contain the data controller's name, the purpose of the project, and the information that it is voluntary to participate. Furthermore it will be stated that consent of participation can be withdrawn at any time, and that the project is notified to the Data Protection Agency. Participants are informed that they have the right to be informed about the results of the project. Any publication of data from the survey will be anonymous. Data will be stored for 5 years after completion of the project (§ 16 of the GCP-decree).

Time schedule

About 30 elective CS are carried out each month at Odense University Hospital.
We plan to enrol the first patient once the authorities have approved the study. The inclusion period is scheduled to last until we have 40 participants whom accepted to participate and have delivered fecal samples. We expect to complete the inclusion period approximately four months from the starting date (last patient last visit, LPLV). Microbiological analyzes and data processing is expected to be completed 6 months after the LPLV.

Financing and insurance

This pilotproject is a workpackage (1.2b) in the international Neomune-project on infant gut microbiota (from University of Copenhagen, Prof. Gorm Greisen) and is funded by the Neomune-project. Furthermore, the project has received 50.000 DKK in support from the Aase og Ejnar Danielsens Foundation

The participants are covered by the patient insurance scheme and the sponsor-investigator is covered by the hospital's mandatory insurance.

Perspective

The project is expected to result in the publication of at least one article in a peer-reviewed journal. Orders of authors are compiled as per Vancouver rules.

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Appendix

Appendix 1: Definition of hospital-acquired infection used in the Protocol

Urinary tract infection (UTI)*	Endometritis[#]	Postoperative wound infection (WI)*
One of the following: - A urine culture of $\geq 10^5$ cfu/ml plus one or more of the following clinical criteria: temperature > 38.0 °C, frequency, urgency, dysuria or suprapubic tenderness. - One or more of the before mentioned clinical criteria plus on or more of the following criteria: a) Positive dipstick for leukocytes and/or nitrate, b) Urine culture of $<10^5$ cfu/ml of a single uropathogen in patient being treated with appropriate antibiotics, c) Physicians´ clinical UTI diagnosis and prescription of antibiotic treatment.	Lower abdominal pain and/or flour/bleeding from the cervix and at least two of the following: - Temperature >38.0 °C. - Uterine tenderness. - Purulent drainage form orificium. - Endometrial pathology shown by gynaecologic examination or ultrasound. - Raised leukocyte count or CRP. - Positive cultures form the cervix or the uterus.	Superficial or deep wound infection occurring within 30 days after surgery. One or more of the following criteria fulfilled: - Purulent secretion form postoperative wound or drainage/incision, with or without laboratory confirmation. - Wound spontaneously dehisced or deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, tenderness, unless site is culture-negative. - Pathogen isolated form culture of fluid after wound closed primarily. - Detection of subfascial abscess found by direct examination, during reoperation or by histopathologic or radiologic examination. - Surgeon or physician's diagnosis of infection.

*) CDC-definition (Mangram AJ et al. Guideline for Prevention of Surgical Site Infection, 1999. Centres for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. Am J Infect Control. 1999 Apr;27(2):97-132.)

[#]) Definition from the Danish National Centre for Hospital Hygiene (CAS, The National Health Registry. Definitioner og kodning af Nosokomielle infektioner, Copenhagen; 1997)