Selective Antibiotics when symptoms develop versus Universal antibiotic therapy for preterm Neonates At-risk of early-onset sepsis: a multicentric, randomized, controlled, non-inferiority trial (the SAUNA trial)

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PROJECT PROTOCOL

Title of the project:

Selective Antibiotics when symptoms develop versus Universal antibiotics for preterm Neonates At-risk of early-onset bacterial sepsis: a multicentric, randomized, controlled, non-inferiority trial (the SAUNA trial)

Objectives

- To determine whether antibiotics administered selectively to at-risk preterm neonates [<35 weeks gestation with prolonged rupture of membranes (ROM) or preterm premature rupture of membranes (pPROM)] when they develop signs of sepsis compared to administering antibiotics from 4 hours of life to all at-risk neonates is non-inferior with respect to the primary outcome of "all-cause mortality or any episode of culture-positive sepsis or severe sepsis" in the 1st 7 days after randomization.
- To determine whether neonates receiving selective antibiotics (as above) compared to those receiving antibiotics from 4 hours of life (as above) are superior with respect to the co-primary composite outcome of fewer "all-cause mortality within 7 days (i.e. 168 hours) after randomization, or cumulative intravenous antibiotic exposure exceeding 48 hours during those same 168 hours, summed across all antibiotic courses."
- To determine whether neonates receiving selective antibiotics (as above) compared to those receiving antibiotics from 4 hours of life (as above) are significantly different with respect to a wide range of secondary outcomes (listed under "Outcomes")

Summary of the proposed research project:

Background:

Sepsis is the major cause of neonatal mortality and early-onset neonatal sepsis (EONS) accounts for more than two-thirds of all cases of neonatal sepsis. Prolonged rupture of membranes (ROM) and preterm premature rupture of membranes (pPROM) are important risk factors of EONS. There is equipoise in the published literature whether antibiotics must be immediately initiated among all preterm neonates (<35 weeks gestation) delivered following prolonged ROM or pPROM who are asymptomatic at birth or whether antibiotics can be selectively administered if and when the at-risk neonates become symptomatic.

Research question

Among neonates <35 weeks gestation born with ROM >18 hours or pPROM and who are either asymptomatic or have no symptoms of sepsis at 4 hrs postnatally (P), is selectively administering antibiotics to neonates who later develop clinical sepsis [I] compared to administering antibiotics pre-emptively to all at-risk neonates [C] non-inferior with respect to the composite outcome of "all-cause mortality and/or culture-positive sepsis and/or severe sepsis" [0] within 7 days after randomization [T] by an absolute margin of 7% [E] in a randomized controlled trial (S)?

The trial will also have a composite superiority outcome: "all-cause mortality within 7 days (i.e. 168 hours) after randomization, or cumulative intravenous antibiotic exposure exceeding 48 hours during those same 168 hours, summed across all antibiotic courses". The anticipated absolute risk difference in the composite superiority outcome is 50%.

Methodology

At-risk inborn infants <35 weeks gestation as above will be enrolled in the study at 4 hours after obtaining parental consent. Eligible neonates will be randomly allocated to (a) receive antibiotics only if they develop symptoms of sepsis [criteria defined *a priori]* or have microbiological triggers to start antibiotics, or (b) start receiving antibiotics immediately. They will be monitored closely for the primary outcomes until 7 days after randomization and followed up for secondary outcomes until hospital exit (capped at 100 days) or day 30 of life, whichever is later.

Proposed outcome.

Primary outcome (non-inferiority): Composite of "all-cause mortality or culture-proven sepsis or an episode of severe sepsis" in the 1st 7 days after randomization. Co-primary outcome (superiority): Composite of “all-cause mortality within 7 days (i.e. 168 hours) after randomization, or cumulative intravenous antibiotic exposure exceeding 48 hours during those same 168 hours, summed across all antibiotic courses.”

Detailed research plan:

Methodology

Study design: multi-centric, 2-armed, randomized, active-controlled, stratified, block randomized, non-inferiority trial with outcome assessment blinded. Before the start of the study, all centres will share the empiric obstetric antibiotic policy for mothers delivering with prolonged ROM or pPROM and empiric neonatal antibiotic policy for suspected EONS followed in their centres and the basis for the policy. Centres will be permitted to follow their own written down policies as these are likely to be based on local antibiotic sensitivity patterns.

Screening criteria: All mothers and newly born infants will be screened if delivery occurs at a gestational age of $26^{0/7}$ to $34^{6/7}$ weeks with prolonged rupture of membranes >18 hours or pre-labour rupture of membranes. Placental and umbilical cord histopathology, high vaginal swab culture and amniotic fluid culture (by amniocentesis) are not standards of care in all the participating centres, but if any of these tests is performed, its result will be considered. For neonates requiring any form of respiratory support, a chest x-ray will be obtained prior to 4 hours after birth.

Inclusion criteria:

- Gestational age of $26^{0/7}$ to $34^{6/7}$ weeks
- Chronological age 4 hours
- Have any one or both of the following risk factors of EONS:
  - Prolonged rupture of membranes >18 hours
  - Pre-labour rupture of membranes [as all subjects will be preterm, this is effectively pPROM]
- Are either asymptomatic or have no signs attributable to sepsis at 4 hours. This will be defined as *absence of the following* clinical signs or need for interventions mentioned below:
  1. Apnea (Standard definition) requiring intervention at any time until enrolment.
  2. Need for a fluid bolus or inotropic support at any time until enrolment.
  3. Seizures or seizure-like activity at any time until enrolment.
  4. Upper GI bleed in the absence of a history of ante-partum hemorrhage at any time until enrolment.
  5. Pus from any site at any time until enrolment.
  6. Need for CPAP >6 cms of water with Fi02>35% at 4 hours OR need for CPAP $\leq$6 cms and FiO_2 $\leq$35% but with increasing requirement of support
  7. Chest Xray (if performed) with radiological features of pneumonia.
  8. Need for intubation and mechanical ventilation.
  9. Temperature >37.5°C or <36°C, unexplained by environmental causes
  10. Feed intolerance [bilious or bloodstained vomiting (or gastric residuals) or visibly distended abdomen or >50% of the previous feed volume as gastric residuals]
  11. Lethargy or unarousability
  12. Sclerema

Exclusion criteria

Subjects will be excluded if they have any 1 of the following:

1. Life-threatening congenital malformation
2. Severe perinatal asphyxia (Apgar score <5 at 10 minutes or cord pH <7.0)
3. Clinical chorioamnionitis# [see definition below]
4. Foul-smelling liquor

5. Multiple gestation
6. Received a dose of antibiotics
7. Positive amniotic fluid culture (if performed and available prior to randomization)
8. Treating neonatologist unwilling to enroll the patient in the trial on the grounds that the patient needs antibiotics.

#Fever: (maternal temperature -≥38.0°C) PLUS any 2 of the following: (a) maternal tachycardia (HR >100 bpm), (b) baseline fetal tachycardia (FHR >160 bpm for 10 minutes or longer, excluding accelerations, decelerations and periods of marked variability; OR an FHR >160 bpm during and after at least 3 consecutive contractions], (c) purulent fluid from the cervical os, (d) uterine tenderness, (e) maternal WBC count ≥15,000/microlitre in the absence of corticosteroids.

Informed consent and enrolment

Parents of eligible neonates would be approached for possible participation in the study. A parent information sheet would be provided to them and they would be given the opportunity to ask questions and clear their doubts. Neonates will be enrolled after obtaining written informed consent. The research nurses and project scientist will be responsible for enrolling and assigning participants to interventions.

Baseline characteristics:

The following baseline characteristics will be recorded:

Maternal: age, educational status, parity, duration of rupture of membranes, duration of labour, spontaneous premature onset of labour, placental or umbilical cord inflammation on pathology (if tested), high vaginal swab or placental or amniotic fluid culture (If tested), Intrapartum antibiotics if administered (names, duration before delivery), mode of delivery, duration of labour (if any) prior to Cesarean section.

Neonatal: gestational age, birth weight, sex, Apgar score at 5 minutes, age at enrolment, clinical signs and laboratory parameters, blood culture (at baseline sent for all eligible neonates).

Randomization:

Neonates will be randomized to 1 of the following groups:

1. Group 1: Intervention group (Selective antibiotic group)
2. Group 2: Comparison group (Universal antibiotic group)

A stratified, blocked randomization scheme with 1:1 allocation ratio will be adopted. Stratification will be performed for gestational age ($26^{0/7}$ to $31^{6/7}$ and $32^{0/7}$ to $34^{6/7}$ weeks), and for centre. Randomly varying, permuted, even-numbered blocks (Sizes 4, 6, 8) will be used. The randomization sequence will be generated from the website sealedenvelope.com. The statistician at the nodal center will generate the randomization sequence and will not be involved in patient recruitment.

Intervention groups:

In both groups 1 and 2, the choice of antibiotics will be as per the empirical antibiotic policy of the centre. The storage, dose, reconstitution, and mode of administration of antibiotics will be as per Neofax (Micromedex, June 2020) to make the processes uniform across centres. The duration of antibiotics will be as follows:

	Intervention arm	Control arm
Antibiotics immediately after randomization	• ***No antibiotics started***.	• ***Start administering antibiotics*** (for practical considerations a margin of up to 2 hours after randomization will be permissible)

Neonate remains asymptomatic over one week of observation, AND placental/cord histopathology (if sent) not suggestive of chorioamnionitis and/or amniotic fluid culture (if sent) sterile	• If pre-randomization blood culture reported positive with non-contaminant organism*, start antibiotics according to sensitivity report for 7-14 days. • If pre-randomization blood culture is sterile, no antibiotics	• If baseline blood culture reported positive with non-contaminant organism*, administer antibiotics (or change antibiotics, if required according to sensitivity report) for 7-14 days. • If pre-randomization blood culture sterile, stop ongoing antibiotics.
Neonate remains asymptomatic over one week of observation, BUT amniotic fluid culture positive (if sent), and/or placental histopathology (if sent) suggestive of chorioamnionitis	• If pre-randomization blood culture reported positive with non-contaminant organism*, start antibiotics according to sensitivity report for 7-14 days. • If pre-randomization blood culture is sterile, administer antibiotics for 5-7 days.	• If baseline blood culture reported positive with non-contaminant organism*, administer antibiotics (or change antibiotics, if required according to sensitivity report) for 7-14 days. • If pre-randomization blood culture sterile, continue ongoing antibiotics for 5-7 days.
Neonate becomes symptomatic** for sepsis within 1st week of life	• Start antibiotics. • If pre-randomization blood culture is sterile, send fresh blood culture for symptomatic episodes. If that is also sterile, administer antibiotics for 5-7 days. If blood culture for symptomatic episode is positive, treat accordingly for 7-14 days. • If pre-randomization blood culture positive report available around the time neonate becomes symptomatic, treat accordingly for 7-14 days.	• If pre-randomization blood culture is sterile, send fresh blood culture for symptomatic episodes. If that is also sterile, continue antibiotics for a cumulative duration of 5-7 days. If blood culture for symptomatic episodes is positive, treat for 7-14 days. • If pre-randomization blood culture positive report available around the time neonate becomes symptomatic, treat accordingly for 7-14 days.

*A blood-culture isolate will be classified as a contaminant when aerobic spore-forming organisms, Propionibacterium species, Corynebacterium species, diphtheroids, or coagulase-negative staphylococci are isolated from asymptomatic neonate. The blood culture isolate may be classified as a contaminant if growth begins after 72 hours or coagulase-negative staphylococci grow in a neonate with some symptoms, but these are interpreted in the context of NeoKISS criteria and the overall clinical picture.

**Clinical sepsis triggers use the standardized signs listed under the trial SOP. Antibiotic duration may be tailored to organism, site, meningitis, and clinical course within the algorithm's range.

Concealment of allocation:

Allocation will be concealed using serially numbered opaque sealed envelopes. Envelopes will bear slips of paper mentioning the group of allocation. Investigators will open the envelopes only after the name of the enrolled patient has been written outside the envelope.

Blinding:

Project staff, nurses and resident doctors looking after the neonate will not be blinded. The assessment of the primary outcome will be performed by a blinded adjudicator, who is not involved in the recruitment and monitoring of subjects. A part of the case report form (CRF) containing relevant details of all episodes of sickness in the 1" week of life will be detached

from the main form and will be sent to the blinded adjudicator. This part will be linked to the main form only by a unique identification number. No patient identifiers or allocation group will be mentioned on the part sent to the blinded adjudicator.

Monitoring:

All enrolled subjects will be monitored for the following until hospital exit (capped at 100 days) or day 30 of life, whichever is later:

a. Clinical signs of sepsis including date of onset: As mentioned earlier, a standard operating procedure (SOP) with clusters of clinical signs and the threshold for designating clinical signs of sepsis, will be made after evolving a consensus across all the participating centres.
b. In case the subject develops clinical signs of sepsis during the 1st week of life, the following investigations will be mandatorily performed:
    - complete blood counts (CBC) [including total leukocyte count (TLC), absolute neutrophil count (ANC)],
    - C-reactive protein (CRP),
    - Procalcitonin (if facilities available),
    - Chest x-ray (if respiratory signs present),
    - blood culture and
    - cerebro-spinal fluid (CSF) examination (including, but not limited to, TLC, ANC, glucose, protein, Gram stain and culture).

    The results of these investigations will be recorded. Age-appropriate nomograms of CBC, CRP and Procalcitonin will be used to identify abnormal values. Investigations for clinical signs beyond the 1st week until hospital exit will be left to the discretion of the treating team.
    c. If antibiotics are started at any time during hospital stay, the name of antibiotics, start and end date will be recorded for each course of antibiotics.
    d. If antibiotics are changed at any time during hospital stay, name of antibiotics, start and end date will be recorded for each course of antibiotics.
    e. Need for the following during any episode of clinical sepsis during hospital stay:
        - Fluid boluses
        - Inotropes
        - CPAP
        - Mechanical ventilation
        - Blood product transfusion
        - Exchange transfusion

    f. Duration of hospital stay
    g. Mortality [if so, date and whether cause of death ascribed to sepsis]

    An asymptomatic neonate who is otherwise fit to be discharged home, can be discharged, with instructions to report back to the research team in case of any symptoms. The research team will make a follow-up telephonic call on day 7 of life, and weekly thereafter until day 30 of life.

Co-Interventions

Co-interventions after randomization until hospital exit will be recorded. An SOP of management of all common neonatal conditions will be evolved as a consensus between the participating centres before the start of the trial. This will be based upon the NNF clinical practice guidelines and the ICMR standard treatment workflows. There are no specific co-interventions permitted or prohibited during the trial.

Outcomes

Primary (Non-inferiority outcome)

Composite of all-cause mortality and/or any episode of culture-positive sepsis and/or severe sepsis* within the 1' 7 days after randomization

**Severe sepsis will be defined as:*

- *Clinical signs of sepsis AND*
- *Either a positive blood culture or laboratory evidence of sepsis (either CRP OR Procalcitonin above the age-appropriate cut-off value OR any two of the CBC parameters outside the age-appropriate ranges OR chest x-ray suggestive of pneumonia) AND*
- *One or more of the following indices of severity:*
    - *Need for intubation and mechanical ventilation*
    - *Need for inotropes >10 mic/kg/min dopamine or >10 mic/kg/min dobutamine or adrenaline > 0.05 mic/kg/min*
    - *Need for exchange transfusion*
    - *Need for platelet concentrates or FFP*
    - *Meningitis (defined as either CSF culture positive or Gram stain positive or Cell count >25/microlitre or glucose <25 mg/dl or protein >180 mg/dl)*

Co-primary outcome

The co-primary superiority outcome will be the composite of " all-cause mortality within 7 days (i.e. 168 hours) after randomization, or cumulative intravenous antibiotic exposure exceeding 48 hours during those same 168 hours, summed across all antibiotic courses".

For antibiotic treatment lasting greater than 48 hours, death is a competing outcome. If death is not included in the composite, participants who die before completing 48 hours of antibiotics would be classified as having received less than 48 hours of antibiotics. This would create an anomalous situation in which excess deaths in one arm could paradoxically favour that arm for the superiority outcome.

Secondary outcomes

- Individual components of the composite outcome
    - All-cause Mortality within 1st 7 days after randomization
    - Culture-positive sepsis within 1st 7 days after randomization
    - Episode of severe sepsis within 1st 7 days after randomization
- Outcomes within the 1st 72 hours after randomization
    - Composite of mortality/culture positive sepsis/severe sepsis
    - individual components of composite outcome within 1st 72 hours after randomization
- Outcomes during hospital stay
    - Composite of mortality/culture-positive sepsis/severe sepsis
    - individual components of composite outcome during hospital stay
    - Necrotizing enterocolitis, stage II-III by modified Bell's staging criteria
- Outcomes during 1st 30 days of life
    - Composite of mortality/culture positive sepsis/severe sepsis
    - Individual components of composite outcome during 1st 30 days of life
    - Necrotizing enterocolitis, stage II-III by modified Bell's staging criteria
- Cause-specific mortality
    - Sepsis-related mortality within 1st 72 hours after randomization, 7 days after randomization, during hospital stay and during 1st 30 days of life
- Episodes of clinical sepsis (as per definition from a repertoire of clinical signs with normal lab parameters)
    - clinical sepsis within 1st 72 hours after randomization, 7 days after randomization, during hospital stay and during 1st 30 days of life
- Episode of Probable sepsis [as per definition from a repertoire of clinical signs, AND with *abnormal* age-appropriate values of one or more of TLC, ANC, CRP, PCT, chest x-ray suggestive of pneumonia AND with sterile blood culture]
- Episode of asymptomatic proven sepsis within 72 hours after randomization [asymptomatic but baseline blood culture positive with non-contaminant organism]

- Need for sepsis workup ["Sepsis workup" defined as one or more of CBC, CRP, Procalcitonin, blood culture] during 1st 72 hours after randomization, during 1st 7 days after randomization, during hospital stay and during 1st 30 days of life
- Need for antibiotics during hospital stay
    - Cumulative duration of antibiotic therapy during 1st 7 days after randomization
    - Cumulative duration of antibiotic therapy during 1st 72 hrs after randomization
    - Cumulative duration of antibiotic therapy during hospital stay
- Duration of hospitalization
- Episodes of healthcare associated infection during hospital stay [Defined as any episode of culture positive sepsis with onset after 72 hours after randomization or any episode of culture-positive sepsis if baseline blood culture was sterile]
- Adverse effects (AE): An AE log will be maintained for all participants in the RCT. The log includes start and end date, severity, outcome, and action taken. Adverse events will be defined and graded according to the Neonatal Adverse Events Severity Scale (NAESS) version 1 (Salaets T, et al. Archives of Disease in Childhood – Fetal and Neonatal Edition, 2019 DOI: 10.1136/archdischild-2019-317399).

  SAEs will be reported to and independent safety monitoring committee appointed by the institutional ethics committee and to the DSMB. Adverse effects attributed to antibiotic therapy [WHO-UMC scale will be used for estimating causality].

The blinded adjudicator will adjudicate whether each episode of sepsis was culture-positive, probable or clinical sepsis, and whether mortality was attributable to sepsis.

Sample size calculation

The non-inferiority composite outcome (primary outcome) and the superiority composite outcome (co-primary outcome) will be tested hierarchically. Superiority will be tested only if non-inferiority has first been established. The primary non-inferiority hypothesis will be tested first using a one-sided significance level of 0.025. If non-inferiority is established, the co-primary superiority hypothesis will be tested at a two-sided significance level of 0.05. The prespecified sequential gatekeeping procedure controls the family-wise type-I error without dividing alpha between the two hypotheses.

Sample size calculation for the noninferiority outcome: An estimate of the primary outcome was made from the results of the DeNIS study (14). If there is truly no difference in the primary outcome between the intervention and standard treatment groups (20% in both groups), then 1374 patients (687 participants per arm) are required to have 90% power that the upper limit of a one-sided 97.5% confidence interval will exclude a difference in favour of the standard treatment by a noninferiority margin of more than 7%. This does not account for adjustment of sample size for the two planned interim analyses.

The two interim analyses will permit early stopping for non-inferiority using a Lan-DeMets O'Brien-Fleming alpha-spending approach, with information fractions of approximately 1/3, 2/3 and 1. The information inflation factor is approximately 1.0205. Thus, accounting for interim analyses, 1,374 x 1.0205 = approximately 1,402 participants. Assuming 6.5% loss to follow-up or unevaluable participants, the total sample size for the non-inferiority outcome will be 1,500.

Raw data from the DeNIS study shows that 1.6 % of all live births would be available for enrolling in the study. Thus, to get 1500 patients, we would have to screen 92,000 live births. If any given centre has approximately 5000 live births per annum, assuming that at least 6 months in the beginning and at the end are excluded for the run-in period of the trial and data analysis and writing respectively, then to get 92,000 live births available for screening over an effective recruitment period of 3.5 years in a 4-year study, we will need 9 centres.

Sample size calculation for the superiority outcome: *Standard-treatment assumption*: The incidence of the composite is likely to be close to 100% in the standard-treatment arm. In most centres, if a

participant remains asymptomatic, antibiotics are likely to continue until at least 48 hours, when the baseline blood culture is reported sterile. In a few instances, antibiotics may be stopped before 48 hours on the basis of a sterile blood culture reported by an automated blood-culture system. All participants whose baseline blood culture grows a pathogen, who develop clinical or probable sepsis within 7 days, or who die within 7 days before completing an antibiotic course would automatically meet the composite outcome. A conservative incidence of 95% was therefore assumed for this arm.
Intervention-arm assumption: It is difficult to estimate the incidence directly in a preterm cohort with pPROM or rupture of membranes greater than 18 hours, but without chorioamnionitis, that is not treated pre-emptively or prophylactically with antibiotics. Among preterm pPROM studies, the highest reported incidences of any clinical/laboratory infection were 43% and 42% (Turk J Pediatr 2011;53(2):180-186; and Int J Hematol 2021;113:199-206, respectively). An incidence of 45% was therefore assumed for the intervention arm.
Assuming 95% in the standard-treatment arm and 45% in the intervention, two-sided alpha of 0.05; power of 90%; allocation ratio of 1:1; the required total sample size is 32 (16 per arm). The information inflation factor of 1.0205 will have no material effect on the sample size requirement of 32. Assuming the same 6.5% loss to follow-up or unevaluable participants as for the noninferiority outcome above, the required sample size is 36 in total (18 per arm), after rounding and enforcing an even number. The sample size requirement for the noninferiority outcome is vastly greater that the sample required for the superiority outcome.

Data Safety Monitoring Board (DSMB) and Interim analysis

A DSMB will be constituted, which will be independent of the funding agency and the trial centres and will have no competing interests. The DSMB charter will be drawn up after the constitution. The DSMB will monitor serious adverse events and data accrued in the trial. 2 interim analyses will be performed. The first interim analysis after 33% of the planned sample size has been enrolled or 33% of the anticipated primary non-inferiority outcome events have occurred, whichever is earlier. The 2nd interim analysis after 66% of the planned sample size has been enrolled or 66% of the anticipated outcome events have occurred, whichever is earlier. A Lan-DeMets O'Brien-Fleming alpha-spending approach will be used, with information fractions of approximately 1/3, 2/3 and 1. Sample size may be recalculated at the 1st interim analysis. After any of the interim analyses, the DSMB can advise that the trial should be stopped for unequivocal benefit (noninferiority demonstrated), harm or futility. O'Brien Fleming stopping criteria will be used for the primary outcome. Pocock's stopping criteria will be used for harm.