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)

NCT number. To be allotted

Date: 2nd April 2024

PROJECT PROTOCOL

Title of the project:

Selective **A**ntibiotics when symptoms develop versus **U**niversal antibiotics for preterm Neonates **A**t-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 (PROM) or preterm premature rupture of membranes (pPROM)] when they develop signs of sepsis compared to administering antibiotics from birth to all at-risk neonates is non-inferior with respect to the primary outcome of "mortality or any episode of culture-positive sepsis or severe sepsis" in the 1st week of life
- To determine whether neonates receiving selective antibiotics (as above) compared to those receiving antibiotics from birth (as above) are superior with respect to the co-primary outcome of fewer antibiotic courses of 48 hours duration or more in the 1st week of life
- To determine whether neonates receiving selective antibiotics (as above) compared to those receiving antibiotics from birth (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 (PROM) 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 PROM 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 PROM >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 "mortality and/or culture-positive sepsis and/or severe sepsis" [O] within 7 days after enrolment [T] by an absolute margin of 7% [E] in a randomized controlled trial (S)?

The trial will also have a superiority outcome: "need for antibiotic treatment lasting greater than 48 hours within 7 days after enrolment". The absolute superiority margin will be 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 further antibiotics only if they develop symptoms of sepsis [criteria defined *a priori*] or (b) start receiving antibiotics immediately. They will be monitored closely for the primary outcomes until 7 days after birth and followed up until discharge or day 30 of life.

Proposed outcome.

Primary outcome (non-inferiority): Composite of "mortality or blood culture proven sepsis or episode of severe sepsis" in the 1st 7 days. Co-primary outcome: Need for antibiotic treatment for greater than 48 hours in the 1st 7 days.

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 PROM 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 FiO_2 >35% at 6-8 hours OR need for CPAP £6 cms and FiO_2 £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).

Randomisation:

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 ($28^{0/7}$-$31^{6/7}$ weeks and $32^{0/7}$-$34^{6/7}$ 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 www.randomization.org. 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 enrolment and randomization	• ***No antibiotics started***.	• ***Start administering antibiotics***.
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.

** Contaminant defined as onset of growth beyond 72 hours or growth of aerobic spore bearers or Propionibacterium spp or Corynebacterium spp or diphtheroids or CONS in asymptomatic neonate*

*** "Symptoms of sepsis" in the 1st week of life that merit antibiotics will be clearly defined. A standard operating procedure (SOP) with clusters of clinical signs and the threshold above which they will be considered clinical signs of sepsis, will be made after evolving a consensus across all the participating centres. The research staff will closely monitor the administration of antibiotics and will ensure adherence to the protocols.*

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 1st 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 discharge 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 discharge 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 discharge 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 1st 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 outcome will be need for intravenous antibiotics for ≥ 48 hours within the 1st 7 days of life.

Secondary outcomes

- Individual components of the composite outcome
    - All-cause Mortality within 1st 7 days of life
    - Blood culture positive sepsis of any severity within 1st 7 days of life
    - Episode of severe sepsis within 1st 7 days of life
- Outcomes within the 1st 72 hours
    - Composite of mortality/blood culture positive sepsis/severe sepsis
    - individual components of composite outcome within 1st 72 hours
- Outcomes during hospital stay
    - Composite of mortality/blood 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/blood culture positive sepsis/severe sepsis
    - Individual components of composite outcome during 1st 30 days

    - Necrotizing enterocolitis, stage II-III by modified Bell's staging criteria
- Cause-specific mortality
    - Sepsis-related mortality within 1st 72 hours of life, 7 days, during hospital stay and during 1st 30 days of life
- Episodes of clinical EONS (as per definition from a repertoire of clinical signs with normal lab parameters)
    - clinical sepsis within 1st 72 hours of life, 7 days, during hospital stay and during 1st 30 days of life
- Episode of Probable EONS [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 EONS within 72 hours of life [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 of life, during 1st 7 days of life, 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 of life
    - Cumulative duration of antibiotic therapy during 1st 72 hrs
    - 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 of life 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. A detailed AE chart with definitions of the AE and criteria for classifying the level of severity has been made. These have been adapted from the Common Terminology Criteria for Adverse Events (CTCAE) version 5.
  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].

Sample size calculation

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 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%. Accounting for losses to follow up, approximately 1500 patients will be required, with 750 in each arm.

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.

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. Sample size would 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.

Statistical analysis plan

Categorical variables will be described as frequencies and percentages, normally distributed numerical variables as mean (standard deviation) and variables with skewed distributions as median (1st, 3rd quartile). Dichotomous variables will be compared between the 2 groups by chi-square test and Fisher's exact test as appropriate. Normally distributed numerical variables will be compared by unpaired student's t-test. Variables with skewed distributions will be compared by Mann-Whitney U test. Magnitude of the effect size will be expressed as unadjusted odds ratios (95% confidence interval). Missing data will be replaced by a process of multiple imputation.

As it is a noninferiority trial, the primary analysis will be as per protocol and the secondary analysis will be as per intention-to-treat.

Multi-variable logistic regression analyses will be performed with the primary outcome as the outcome variables, and the group of randomization as the primary predictor variable, adjusted for the following baseline covariates decided *a priori:* gestation strata, neonate received no doses of antibiotics versus received one dose of antibiotics prior to enrolment, center, duration of maternal intrapartum antibiotic prophylaxis (0-4 h vs >=4 h), was completely asymptomatic until enrolment versus had any transient symptoms prior to enrolment, was on CPAP at enrolment versus no CPAP at enrolment, Apgar score (<7 at 5 min vs. >=7), HVS culture positive for pathogenic bacteria or not, placental histology suggestive of infection or not. Variables will be checked for multicollinearity using Eigenvalues and Variance Inflation Factor. Variables that are not multicollinear will be forced into the model. No software-based variable selection algorithm will be used.

Subgroup analyses will also be performed for all the covariates mentioned above under multivariable logistic regression analysis.