1. General information

Title: To optimize antenatal management of women admitted with preterm Labor and intact membranes using multivariable prediction models: study protocol for a randomized control trial (OPTIM-PTL study)

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2. Rationale for the study

Preterm delivery is the most common complication in maternal and fetal medicine constituting around 6.5-9% of deliveries (1), with this prevalence increasing up to 12.5% in tertiary centers. Preterm delivery is the first cause of neonatal morbidity and mortality and the second cause in children under 5 years. The prevalence of these complications is inversely proportional to gestational age at delivery.

Despite the severe consequences of this clinical problem, the prediction of preterm delivery is poor. Clinical suspicion of preterm labor (PTL) based on symptoms (uterine contractions and cervical changes) has shown to have low predictive performance. Indeed, only around 10% of pregnant women with clinical suspicion of PTL will deliver within the following 7 days, and 70% will deliver above 37.0 weeks of gestation (1).

One of the most common etiologies involved in the origin of spontaneous preterm delivery (sPTD) is the occurrence of microbial invasion of the amniotic cavity (MIAC) and intra-amniotic inflammation (IAI) (2,3,4). Thus, almost 40% of pregnant women diagnosed with PTL before 32 weeks have MIAC and/or IAI (2). Women with MIAC and/or IAI have a short latency from the onset of symptoms to delivery (median (25th; 75th percentile) of 2 days (0; 6)) and an early gestational age at delivery (26.9 (25.2; 31.1) weeks). On the contrary, women without MIAC and without IAI have a median (25th; 75th percentile) latency to delivery of 50 days (20; 70) and a gestational age at delivery of 35.0 weeks (29.7; 38.3) (5). This group without MIAC and without IAI represents around 60% of hospital admissions for diagnoses of PTL.

It is a clinical challenge to individualize the management of women admitted for PTL differentiating those with low-risk (of sPTD within 7 days and of MIAC/IAI) from those with high-risk. The unnecessary over-treatment of women with a low-risk induces social (anxiety, medical leaves), medical (side-effects due to treatment) and economical costs (length of hospital stay, medical costs). Moreover, it has been reported that the impact of antenatal administration of steroids for fetal maturation on the short and long-term neurodevelopmental outcomes of neonates finally delivered nearly term is negative (6,7). Thus, in animal models and observational

cohort studies, cardiovascular and metabolic changes and neurodevelopmental impairment have been observed in adults who were antenatally exposed to steroids. Our group recently developed and validated prediction models of sPTD within 7 days and of MIAC in women with PTL from retrospective cohorts with good diagnostic performance (5). These prediction models include variables such as gestational age at admission, ultrasound cervical length, maternal C-reactive protein (CRP), amniotic fluid glucose and interleukin (IL)-6 concentrations that allow to rapid predict (in hours) whether a women admitted with a diagnosis of PTL has a low or high-risk (to delivery within 7 days or have MIAC) (5). Despite being promising, the effect to implement these prediction models into clinical practice has not been properly evaluated.

In contrast to the usual clinical studies, we hypothesized that the implementation of a predictive model of risk in women admitted with PTL targets two different groups: a low-risk group (in which standard management can be minimized) and a high-risk group (which would really benefit from standard management with antenatal steroids (8), magnesium sulphate (9) and, in case of MIAC and/or IAI, antibiotics (10)). Thus, the implementation of predictive models of risk would:

- optimize antenatal management without worsening perinatal outcomes (main hypothesis).
- be a cost-effective strategy.
- Early treatment of the group at high-risk of MIAC reduces neonatal morbidity in premature newborns < 30 weeks and maternal morbidity due to infection.

3. Bibliography

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4. Study design

4.1. Study population: Pregnant women with singleton gestations between 23.0-34.6 weeks admitted with a diagnosis of PTL (uterine contractions with intact amniotic membranes).

4.2. Type of study: Multicenter randomized clinical trial that aims to evaluate not a specific drug but rather an intervention based on the risk defined in predictive models. There will be two study arms: the intervention arm (optimization of standard management according to the risk obtained from predictive models) and the control arm. In the intervention arm, we will calculate the risk of delivery within 7 days and having MIAC using predictive models. Antenatal management will therefore be individualized according to this risk. In the control arm, antenatal management will follow the usual clinical protocols of each center.

4.3. Definitions of main outcomes:

4.3.1. Treatment administered (mainly number of antenatal steroid doses administered (low and high-risk group), but also tocolysis duration and antibiotic treatment).

4.3.2. Maternal length of hospital stay (days).

4.3.3. Maternal and neonatal infectious morbidity.

Other outcomes:

4.3.4. Gestational age at delivery (weeks).

4.3.5. Spontaneous delivery within 7 days after admission (yes/no), defined as the latency from admission to delivery less than or equal to 7 days. Women who delivered because of maternal or fetal indications will be consequently censored. Gestational age will be established according to crown-rump length at the first-trimester ultrasound scan.

4.3.6. MIAC (yes/no), defined as the presence of microorganisms in the amniotic fluid identified using aerobic/anaerobic/genital mycoplasma cultures or 16S rRNA gene sequencing.

4.3.7. Cost analyses: costs have been calculated as the product of resource use and unit costs. Resource use during the study period will be documented. The following resource items will be collected: maternal and neonatal admissions, method of delivery, type of induction, outpatient visits, emergency visits, medication, surgical procedures, maternal stay length. Maternal admissions will be differentiated into three levels of care (intensive, medium, ward). Neonatal admissions will be divided into four levels of care (intensive, high, medium ward). Ward admissions of newborns have not been calculated; these costs have already been incorporated in costs of maternal ward admissions.

4.3.8. Maternal morbidity (yes/no) including intrapartum fever, endometritis, infection of surgical wound, sepsis, curettage, admission to ICU, hysterectomy, need for transfusion, maternal death.

4.3.9. Neonatal length of hospital stay (days).

4.3.10. Neonatal birthweight, height, cephalic, thoracic and abdominal perimeters, arm circumference.

4.3.11. Major neonatal outcomes (yes/no) are defined as the presence or one or more of the following outcomes: fetal or neonatal death, early onset sepsis**,** moderate/severe bronchopulmonary dysplasia, severe intraventricular hemorrhage, periventricular leukomalacia, surgical necrotizing enterocolitis, retinopathy requiring laser treatment.

5. **Formula of the predictive models**: Multivariable analysis by stepwise logistic regression will be used to identify independent factors associated with outcomes (development of predictive model). The models that can best predict spontaneous delivery within 7 days and MIAC will be constructed based on the final regression

model and the direction of effects. Goodness-of-fit models will be assessed by calculating Nagelkerke's R2.

The regression formula for spontaneous delivery within 7 days is: - 7.588 + 0.132 * gestational age at admission (weeks) - 0.051 * Ultrasound cervical length (mm) - 0.055 * amniotic fluid glucose (mg/dL) + 1.438 * amniotic fluid log (IL-6). R2=51.5%.
The regression formula for MIAC is = 1.034 + 0.169 * maternal CRP (mg/L) -0.158 * amniotic fluid glucose (mg/dL). R2=65.6%.

6. **Selection of participants**: Pregnant women with singleton gestations admitted with a diagnosis of PTL between 23.0-34.6 weeks will be eligible.

7. **Inclusion criteria:** singleton pregnancies admitted with a diagnosis of PTL between 23.0 and 34.6 weeks, not in labor at randomization and who do not meet exclusion criteria.

8. **Exclusion criteria:** maternal age < 18 years, multiple gestations, clinical chorioamnionitis at randomization (defined by the presence of fever ≥ 38ºC, fetal tachycardia (> 160 heart beat per minute > 10 minutes) and maternal white blood cells > 15000/mm^3 (not justified by the administration of antenatal steroids), cervical dilatation > 3 cm; major structural malformations of fetal complications that affect neurodevelopmental outcome, technical problems to perform amniocentesis (predictive models include information from amniotic fluid: glucose and IL-6 concentration).

9. **Intervention:** The initial management of women with PTL will follow the standard management of each center and include antenatal administration of steroids, tocolysis and magnesium sulphate (if suspicion of imminent delivery). After obtaining written informed consent from each woman, the patients will be randomly assigned to one of the two study arms in a 1: 1 ratio. Randomization will be stratified by gestational age (24.0-27.6; 28.0-31.6 and > 32.0 weeks of gestation)

and center. The gestational ages of the two groups will be chosen based on the severity of preterm delivery (< 28.0 weeks: extremely prematurity; 28.0-31.6 weeks: very preterm; 32.0-33.6 weeks: moderate to late preterm; 34.0-36.6 weeks). The randomization sequence will be computer-generated by a Clinical Research Organization (CRO) and will be implemented using a centralized controlled website randomization service and electronic clinical research data (eCRD). Recruiters or the trial coordinator will not have access to the randomization sequence. The allocation code number will be disclosed after the initials and gestational age of the woman are introduced into the system.

9.1. In the intervention arm, the management will be optimized according to the risk of the predictive model. The predictive model of MIAC includes maternal CRP and amniotic fluid glucose, and the predictive model of spontaneous preterm delivery within 7 days includes gestational age, cervical length, amniotic fluid glucose and IL-6. High risk will be defined when the risk is > 10% in the predictive model of spontaneous delivery in 7 days and > 20% in the predictive model of MIAC:

9.1.1. If *low-risk:* we will optimize the standard management reducing the dose of steroids (e.g not administering second doses), tocolysis or magnesium sulphate and facilitating discharge home.

9.1.2. If *high-risk*: we will follow the standard management of each center and we will treat with antibiotics:

9.1.2.1. If high-risk of MIAC, with broad-spectrum antibiotics (ampicillin 2g/6h ev + ceftriaxone 1g/12h ev + clarithromycin 500 mg/12h vo). In penicillin allergies: teicoplanin 600 mg/24h ev + aztreonam 1g/8h ev + clarithromycin 500 mg/12h vo). On confirmation of MIAC antibiotic treatment will be adjusted according to the virulence of the microorganisms isolated (if women have not delivered at time of the microbiological results). If the cultures are negative, antibiotic treatment will be discontinued.

9.1.2.2. If there is high-risk of delivery within 7 days but low-risk of MIAC, we will treat only with clarithromycin 500 mg/12 h vo during 5-7 days. Clarithromycin will be used due to the efficacy shown as anti-inflammatory treatment since the predictive model of delivery within 7 days includes the inflammatory marker IL-6.

9.2. In the control arm the standard management of each center will be followed. This standard management might include the administration of antibiotics in cases with clinical (fever), analytical or microbiological suspicion of infection. Differences regarding antibiotics in the intervention arm are that antibiotics will be administered (in the intervention arm) in an early stage (at randomization, in a subclinical stage and before microbiological results).

The same study variables will be collected in both groups.

10. **Duration of the study**: We plan study duration of 3 years.

11. **Sample size calculation:** Sample size has been calculated to reduce antenatal steroids doses in the intervention arm. The prevalence of women at low-risk who deliver within 7 days or who have MIAC is around 60% (project leader data). Moreover, the rate of pregnant women diagnosed with PTL who receive at least 1 course (2 doses) of antenatal steroids is around 90% in this low-risk group. To reduce the antenatal administration of steroids from 90% to 70% in the intervention arm, we would need 66 pregnant women per arm. Since the prevalence of this low-risk group might varies according to the center, we plan to include 102-156 women to find significant differences in the antenatal administration of steroids between the intervention- and control arm. In addition, we have also calculated the sample size needed to reduce the length of maternal hospital stay. The mean hospital stay in project leader's center is 3.5 days (SD 1.3-2). To reduce hospital stay to 1 day (SD 2) in the intervention arm we would need 70 women per arm. Since this length of stay might change according to the center, we estimated the inclusion of 108-156 women per arm to find significant

differences of 1 day in the length of hospital stay between the two arms. Therefore, taking into consideration 20% of patients lost to follow-up (missings), we estimate that a sample size of around 170 pregnant women per arm will be sufficient to detect differences in steroid administration and length of hospital stay. Error I is 5%, with a power of 80%. As a limitation we acknowledge that the sample size lacks statistical power to show differences in neonatal outcomes due to the low prevalence of these outcomes. Despite this limitation, we expect to find a tendency in our results.

12. **Statistical analysis**: A specific database will be created for the management and processing of the information, in which the participant's data will be entered in a coded form. As a general rule, qualitative variables will be described as absolute frequency and relative percentage and quantitative variables, as mean and median for the assessment of the central tendency, and standard deviation (SD) and interquartile range (IQR) for the assessment of dispersion. In the case of ordinal variables, the description of both forms will be evaluated. Univariate analysis: for the comparison of two qualitative variables, the Chi-square test or Fisher's exact test will be used. When the variables are quantitative, the Student's t-test for independent samples or the Mann-Whitney U test will be used if the applicability criteria are not met. Multivariate analysis will be performed by means of multiple linear regression (continuous variables) or logistic (categorical variables) controlling the possible confounding factors. For statistical analysis, values of $p \leq 0.05$ will be considered as statistically significant. The data will be analysed with the SPSS program (version 20.0, IMB or newer) and STATA for MAC (version 15.1 or newer StataCorp LP).

13. **Data analysis plan:**
    - Creation of electronic clinical research data (eCRD) including online randomization (stratified by center and by gestational age at randomization): January 2021-Mars 2021.

- Study initiation: April 2021.
- Recruitment and follow-up including the inclusion of eligible women; randomization process; antenatal management according to the study arm, follow-up until delivery and coding data: April 2021-April 2023.
- Monitoring visits to audit the trial. It is planned to monitor each center once a year including the study initiation visit and the study close-out visit (total number of 4 visits each center): April 2021-June 2023.
- Interim analysis plan: An interim analysis plan of results will be performed by an independent Data Monitoring Committee (DMC) at mid-term of the trial: April 2022.
- Last patient included and randomized: April 2023.
- Study close-out: June 2023.
- Final statistical analysis: September 2023.
- Preparation manuscript for publication in a high-impact journal: December 2023-February 2024.

14. Ethics and dissemination: ethical and safety considerations and any dissemination plan (publications, data deposition and curation) should be covered here.

The trial is registered in the European Union drug regulating authorities clinical trials database (EUDRACT) (2020-005202-26). It has been submitted for evaluation to the Spanish Agency of Medicines and Medical Devices (AEMPS) as a low intervention trial and the local Ethical Committee of the Hospital Clínic (code HCB/2020/1356).

The study has been registered in REeC.aemps.es

The study will be performed in accordance with the relevant parts of the International Conference of Harmonization Guidelines for Good Clinical Practices, the World Medical Association Declaration of Helsinki (Brasil, October 2013), in accordance with all national, state, and local laws of the pertinent regulatory authorities (RD 1090/2015).

The Investigator shall provide a copy of the signed and dated informed consent to the patient and the original shall be maintained in the patient's study files. Patients who do not sign the consent form will not be permitted to participate in the study. The trial will comply with the general data protection regulation (UE 2016/679).

15. Publication rules

Results will be published even if they are negative following article 2 (RD 1090/2015).