Efficacy of intravenous paracetamol compared to placebo in the prevention of patent ductus arteriosus in very preterm newborns

END OF TERM PROJECT
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1. Abstract

BACKGROUND: Patent ductus arteriosus (PDA) is the most common cardiac disease in preterm newborns. Cyclooxygenase inhibitors, mainly indomethacin and ibuprofen, have been largely used in the prevention of this pathology. However, this therapeutic approach has lately been abandoned since more adverse effects are being reported. Besides that, since 2011, paracetamol is gaining more and more importance in the treatment of PDA.

OBJECTIVE: The purpose of the present study is to determine the efficacy of paracetamol in the prevention of PDA in comparison to placebo in very preterm newborns. Safety of paracetamol will also be assessed.

DESIGN AND SETTING: A triple-blinded randomized trial will be performed among the Neonatal Intensive Care Units (NICUs) of Hospital universitari Dr. Josep Trueta in Girona and Hospital universitari Vall d'Hebron in Barcelona.

PARTICIPANTS, INTERVENTION AND METHOD: A total of 336 prematures under 32 weeks of gestation will be randomized into one of the two treatment groups. One group will be receiving paracetamol (loading dose of 20 mg/kg followed by a maintenance dose of 7.5 mg/kg/6h) while the other group will receive placebo. Follow-up echocardiographies will be held on day three and seven after beginning of treatment, as well as on month one and three, to assess the presence or not of PDA, which will be the main outcome. Physical exploration, blood analysis and transfontanellar echographies will be held regularly to evaluate drug adverse effects and comorbidities, to assess secondary outcomes.

KEY WORDS: Patent ductus arteriosus • Prevention • Preterm • Paracetamol • Intravenous
2. List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
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<td>BPD</td>
<td>Bronchopulmonary dysplasia</td>
</tr>
<tr>
<td>CPD</td>
<td>Chronic pulmonary disease</td>
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<tr>
<td>DA</td>
<td>Ductus arterious</td>
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<tr>
<td>GA</td>
<td>Gestational age</td>
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<tr>
<td>hs-PDA</td>
<td>Hemodynamically significant patent arterious ductus</td>
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<tr>
<td>HUDJT</td>
<td>Hospital universitari Doctor Josep Trueta</td>
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<tr>
<td>HUVH</td>
<td>Hospital universitari Vall d'Hebron</td>
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<tr>
<td>IVH</td>
<td>Intraventricular hemorrhage</td>
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<tr>
<td>NEC</td>
<td>Necrotizing enterocolitis</td>
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<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>NSAID</td>
<td>Non-Steroid Anti-Inflammatory Drug</td>
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<tr>
<td>NT-proBNP</td>
<td>N-terminal pro-B-type natriuretic peptide</td>
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<tr>
<td>PDA</td>
<td>Patent ductus arterious</td>
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<tr>
<td>PGE2</td>
<td>Prostaglandin</td>
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<tr>
<td>PGI2</td>
<td>Prostacyclin</td>
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<td>RDS</td>
<td>Respiratory distress syndrome</td>
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3. Introduction

3.1. Background

3.1.1. Fetal to neonatal circulatory transition

In the fetus, oxygen and metabolite supplies are provided by the placenta through a low-resistance circulation. Oxygenated blood comes from the placenta to the fetus through the umbilical vein. Thus, since lungs don’t provide gas exchange, pulmonary vessels are vasoconstricted, keeping the blood out of the pulmonary circulation and leading just enough blood for the growing needs of the lungs. This is maintained thanks to three cardiovascular structures or shunts that are unique to the fetus: the foramen ovale, ductus venosus and ductus arteriosus (please see Image 1).\(^{1-3}\)

The foramen ovale allows that most of the blood that enters the right atrium flow into the left atrium instead of into the right ventricle. From the left atrium, the oxygenated blood is pumped into the left ventricle and into the aorta.\(^ {1,2}\)

The ductus venosus is a narrow, trumpet-shaped vessel in the fetal liver that connects the umbilical vein directly to the caudal inferior vena cava. It shunts oxygenated and nutrient-rich umbilical venous blood from the placenta to the brain and myocardium, bypassing the fetal liver.\(^ {4}\)

The ductus arteriosus is one of the most important vessels of the fetal circulation, since it shunts about 90% of the right ventricular stroke volume (which constitutes two-thirds of the combined cardiac output of the fetal lamb) towards the descending aorta, protecting lungs of circulatory overload.\(^ {3,5}\)

At birth, a dramatic change in the circulatory pattern occurs. The lungs inflate, which lowers resistance in the lung arteries, making that blood from the right ventricle preferentially flows into the lungs. The increase in blood flow into and out of the lungs increases pressure in the left atrium. This causes a one-way flap on the left side of the foramen ovale, called the septum primum, to press against the opening, effectively
separating the two atria. This also further increases blood flow to the lungs as blood entering the right atrium can no longer bypass the right ventricle.\textsuperscript{(2)}

\begin{center}
\textbf{Image 1}: Fetal circulation. Adapted from Stanford Children's Health web page.\textsuperscript{(6)}
\end{center}

This rising blood-flow in pulmonary circulation together with an increase in systemic vascular resistances (due to the fact that the placenta is not providing blood-flow any more) leads to systemic vascular resistances that are higher than pulmonary vascular resistances, and this leads to a reversion in the ductus circulation. The following days, the high arterial pO2 concentrations (together with other molecular changes later explained) constrict and finally close the ductus arteriosus, that then turns into a fibrous band without lumen called ligamentum arteriosum.\textsuperscript{(1,7)}

\subsection*{3.1.2. Patent ductus arteriosus}

Ductus arteriosus (DA) is a vascular structure derived from the 6th aortic arch that connects the proximal descendent artery with the principal pulmonary artery. The
aortic end of the ductus is usually distal to the origin of the left subclavian artery, and the ductus enters the pulmonary artery at its bifurcation (please see Image 2).\(^{(7,8)}\)

The ductus arteriosus is essential during fetal life, when most of the pulmonary arterial blood is shunted into the aorta through it. It spontaneously closes after birth in most of the full-term newborns. However, this doesn’t occur that spontaneously in premature newborns.\(^{(8,9)}\)

![Image 2: Heart diagram with patent ductus arteriosus.\(^{(10)}\)]

**Epidemiology and risk factors:**

Patent ductus arteriosus (PDA) is the most frequent form of congenital heart disease in preterm\(^1\) infants (particularly in those under 1000 g birth weight).\(^{(10,11)}\)

The delay in the DA closure is inversely proportional to gestational age and weight at birth. The incidence vary from 20-30% in prematures under 32 weeks of gestation to 60% in those prematures less than 28 weeks of gestation.\(^{(8,12-14)}\) Related to weight at birth, clinically apparent PDA occurs in 30% to 45% of premature infants with birth

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\(^1\) According to WHO\(^{(56)}\), preterm is defined as babies born alive before 37 weeks of pregnancy are completed. There are subcategories of preterm birth, based on gestational age: extremely preterm (<28 weeks), very preterm (28 to <32 weeks) and moderate to late preterm (32 to <37 weeks).
weights under 1750 g, and it reaches 80% in those prematures under 1000 g birth weight.\textsuperscript{11,15}

Female patients with patent ductus arteriosus (PDA) outnumber males 2:1.\textsuperscript{7,9}

Although PDA is usually sporadic, it can be associated with:\textsuperscript{7-9,13}
- caucasian race;
- lack of exposure to antenatal betamethasone;
- maternal rubella infection during early pregnancy, particularly in the first four weeks;
- genetic conditions, such as trisomy 13, trisomy 18, trisomy 21, Carpenter’s syndrome, Holt-Oram syndrome or incontinentia pigmenti;\textsuperscript{6}
- surfactant deficit (in prematures with respiratory distress syndrome) and followed surfactant administration therapy in the preterm neonates with respiratory distress syndrome (due to the decrease in the pulmonary vascular resistance, it can prevent natural PDA closure in up to 90% of the cases);\textsuperscript{15}
- prenatal administration of magnesium sulfate;
- excessive administration of liquids;
- pharmacologic inhibition of prostaglandin synthesis by indomethacin in human pregnancy (in utero constriction produces ischemic hypoxia, increased nitric oxide production and smooth muscle cell death within the ductus wall, that will make the ductus resistant to the constrictive effects of postnatal indomethacin preventing the ductus from constricting after birth);\textsuperscript{13}
- prematurity (since the smooth muscle in the wall of the preterm ductus is less responsive to high Po2 and therefore less likely to constrict after birth).

In full-term newborns, PDA is usually associated with an anatomical defect of the ductus or heart.\textsuperscript{7,9}

\textit{Physiopathology:}

Fetal patency of the DA and its spontaneous closure after birth is the result of an equilibrate and complex interaction between oxygen, neuro-humeral local and
circulant factors and the special characteristics of the smooth muscle of the ductal wall:

- Histologically, the DA has a tunica media poor in elastic fibers and rich in smooth muscular fibers arranged in a helical shape, which allow the DA to contract and dilate.
- Oxygen: elevated oxygen pressure produces ductal closure, while hypoxemia produces relaxation.
- Prostaglandins and prostacyclins: they are found in high concentrations in the fetus, and produce vasodilatation.

In the utero, the DA stays open since the ductus itself produces several vasodilators that maintain ductus patency, such as prostaglandins, that are the dominant vasodilators that oppose the ductus constriction in the later part of gestation; and also due to the low in utero oxygen tension.

In full-term infants, obliteration of the ductus arteriosus takes place after birth through a process of vasoconstriction and anatomic remodeling. Several events promote this ductal constriction in the full-term newborn after delivery:

1) postnatal increase in arterial PaO2,
2) decrease in blood pressure within the ductus lumen (due to the postnatal decrease in pulmonary vascular resistance),
3) decrease in circulating prostaglandin E2 (due to the loss of placental prostaglandin production and the increase in prostaglandin removal by the lung),
4) decrease in the number of prostaglandin E2 receptors in the ductus wall, losing its ability to respond to prostaglandin E2.

Nevertheless, premature infants frequently fail to close their ductus arteriosus, leading to a systemic-to-pulmonary shunt, as the vascular resistance falls.

Ductus arteriosus patency is determined by the balance between dilating and constricting forces. The factors known to play a prominent role in ductus arteriosus regulation involve those that promote constriction (oxygen, endothelin, calcium channels, catecholamines Rho kinase) and those that oppose it (intraluminal pressure,
prostaglandins, nitric oxide, carbon monoxide, potassium channels, cyclic adenosine and guanosine monophosphate). The main mechanisms responsible for continued patency are related to the inability of the ductus arteriosus in immature infants to respond normally to an increased oxygen tension and to changes in prostaglandin concentrations.

After birth, there is a sudden increase in oxygen pressure that inhibits potassium dependent calcium channels in the ductal smooth muscles. This increases the intracellular concentrations of calcium, which leads to a DA constriction. PGE2 and PGI2 levels abruptly decrease. The muscular fibers of the tunica media contract, decreasing the lumen irrigation and producing ischemia of the internal wall, which produces the definitive closure of the DA.

Gestational age has a marked effect on the rate of ductus closure after birth. In contrast with the full-term ductus, the premature ductus is less likely to constrict after birth due to several mechanisms:

1) Premature infants have elevated circulation concentrations of prostaglandin E2, which may also play a significant role in maintaining ductal patency during the first days after birth. This is due to the decreased ability of the premature lung to clear circulating prostaglandin E2. Moreover, in the preterm newborn, circulating concentrations of prostaglandin E2 can reach the pharmacologic range during episodes of bacteremia and necrotizing enterocolitis. Thus, these situations are often associated with reopening of a previously constricted DA.

2) Premature newborns have less number of muscular fibers, a decreased intrinsical tone of the ductal wall and the subendothelial tissue; leading to a weaker contractile capacity.

3) The less close to full-term the newborn is, the less sensibility it will present to high oxygen pressure and more sensibility of the DA to the vasodilating effects of the PGE2.

All mentioned above is what facilitates the failure of the DA closure in premature newborns.
**Clinical manifestations:**

Small PDA are usually asymptomatic, associated with normal peripheral pulses; while large shunts are usually associated with multiple clinical manifestations, and can result in heart failure. Clinical signs usually appear from day 4-5 onward, being the silent ductal shunt the norm until the latter half of the first postnatal week; so most large PDAs will become clinically apparent, but the diagnosis (if clinical) will be made late. The most frequent clinical sign is the presence of an ejection systolic murmur, that is better heard in the left infraclavicular region and superior paraesternal limit. Other frequent clinical signs and symptoms are worsening blood gas, high pulmonary blood pressure (due to runoff of blood into the pulmonary artery during diastole), low peripheral diastolic blood pressure together with increase in differential tensions (wide pulses) and bounding peripheral arterial pulses, hyperdynamic precordial impulse, cutaneous vasoconstriction, tachypnea, increasing respiratory distress, higher oxygen requirements, hypercapnia, hepatomegaly, metabolic acidosis, oliguria, retardation of physical growth and feeding intolerance. Heart size is normal when the ductus is small, but enlarged in cases with large communication. In these cases, the apical impulse is prominent.

These signs, together with evidence of an interrupted improvement or worsening of the respiratory status, are the clinical criteria of hs-PDA. The most specific clinical signs for the detection of a PDA are the heart murmur and hyperdynamic precordial impulse; and the sign with higher sensibility is the worsening in respiratory pathology.

**Diagnosis:**

Clinical signs of PDA (systolic murmur, hyperdynamic precordial impulse, worsening respiratory status...) usually appear later than echocardiographic signs and are less sensitive in determining the degree of left-to-right shunt. Moreover, all the signs and symptoms mentioned before have a range of different causes, and often, the only way
to know whether they are being caused by the PDA is closing it and seeing if the symptoms disappear.\(^{(18)}\)

In general, the chest x-ray (which can show cardiomegaly and pulmonary congestion symptoms or can also be normal) and electrocardiogram are not useful in diagnosing a PDA in a newborn.\(^{(8,13)}\) But still, they can often be altered in those PDA which are still patent in adulthood.\(^{(7)}\)

Biomarkers to diagnose PDA are currently being investigated. Recently, some studies\(^{(12,15,21-25)}\) have concluded that elevated plasma concentrations of brain natriuretic peptide (BNP) or N-terminal pro-B-type natriuretic peptide (NT-proBNP) correlate with the presence of a moderate-sized left-to-right PDA shunt and thus, they can be an alternative to repeated echocardiography in the diagnosis and management of PDA in preterm infants, although cannot replace echocardiography. Moreover, these same studies found that levels of the above mentioned biomarkers also decrease once the ductus is being treated and starts to close, being a good predictor of treatment response.\(^{(13)}\) However, other studies\(^{(25,26)}\) have concluded that these biomarkers are not useful since they can only be used in subspecific groups of patients, for example, they can’t be used in newborns with sepsis, in whom NT-proBNP levels are already high. Other biomarkers in study are cardiac troponin T and atrial natriuretic peptide.\(^{(18)}\)

**Echocardiography:**

Echocardiography is the gold-standard for the diagnosis of PDA.\(^{(18,21,22)}\) It plays the most important role in PDA diagnosis due to the fact that clinical signs in PDA are not always related with the size of the shunt. Moreover, sometimes the pulmonary vascular resistances are elevated and thus the pressure differences between aorta and pulmonary arteries, and the blood flow velocity can not be enough to produce murmur. Also a murmur can be heard but not due to the presence of PDA, but because of a mild tricuspid insufficiency or a stenosis of the pulmonary branches, for example.\(^{(8)}\)

It is known that echocardiography should be done in those newborns with clinical signs of PDA.\(^{(8)}\) However, it is not well known when should it be done in non-symptomatic
newborns. The actual clinical guides about PDA of the Spanish association of neonatology\(^8\) conclude that echocardiography should be used as a screening test in the third day of life in premature newborns of 30 weeks of gestation or less and also in those newborns that weight less than 1000 grams and who are receiving respiratory support with mechanical ventilation.

Echocardiographic examination of the PDA should include: determine whether there is ductal patency, inform about the internal diameter of the ductus and the direction and pattern\(^2\) of its flow, assess the loading volume of the heart and examine the peripheral circulation effects.\(^{14,19}\) Since pharmacological ductal closure can be fatal in duct-dependent cardiac abnormalities, normal structure of the heart should be ensured by echocardiography before treating the ductus.\(^{14,}\)

Two-dimensional echocardiography and color Doppler flow mapping are helpful in assessing the ductus patency, the ductus lumen size (magnitude), direction and velocity of the shunt, significance of the shunt and impact of the shunt on the systemic and pulmonary circulation.\(^{13,18}\) Shuntal magnitude depends on length, diameter and morphology of the ductus.\(^{14}\) The PDA can then be classified as large, moderate, small or “silent” (when it doesn’t have clinical expressions and is incidentally diagnosed by an echocardiography done for any other reason).\(^7\)

Although the magnitude of the shunt flow plays a significant role in creating neonatal morbidity, equally important factors are the duration of exposure to the shunt and the infant’s ability to compensate for the shunt. For example, the same magnitude left-to-right PDA shunt may be clinically “silent” when present within the first 24 hours after delivery, whereas it may be associated with signs of congestive failure if it persists for 7 to 10 days.\(^{13}\)

Echocardiographic criteria for hs-PDA are (although there is not a clear consensus):\(^{8,13,20,27,28}\)
- ductal diameter higher than 1.4-1.5 mm/kg (measured in 2D, higher than 2 mm/kg if assessed by Doppler color),

\(^2\) PDA echocardiographic patterns are: pulmonary hypertension pattern, growing pattern, pulsatile pattern and closing pattern.
- presence of retrograde flow in more of the 30% of the diastole in descending aorta,
- left atrium distention (left atrial/aortic root (LA/Ao) ratio of 1.4 or greater),
- diastolic diameter of the left ventricle above p95,
- pulsatile pattern ductal flow,
- transductal Doppler velocity lower than 2 m/s.

**Prognosis and complications:**

Newborns with small PDAs may live a normal time lag with few or no cardiac symptoms, but late manifestations may occur. In those with large PDAs, cardiac failure most often occurs in early infancy. Therefore, the clinical consequences of the patent ductus arteriosus (PDA) are related to the degree of left-to-right shunt through the PDA with its associated changes in blood flow to the organs such as lungs, kidney and intestine.\(^9,13\)

Despite the ability of the left ventricle to increase its output in the face of a left-to-right ductal shunt, blood flow distribution is significantly rearranged. The ductal shunt diverts part of the aortic blood flow towards the pulmonary circulation, resulting in pulmonary vascular overload and decreased blood flow to systemic organs. This redistribution of systemic blood flow occurs even with small shunts. Blood flow to the skin, bone and skeletal muscle is most likely to be affected by the left-to-right shunt. The next most likely organs to be affected are the gastrointestinal tract, kidneys and brain. These organs receive decreased blood flow due to a combination of decreased perfusion pressure (caused by a drop in diastolic pressure) and localized vasoconstriction and may even experience significant hypoperfusion before there are any signs of left ventricular compromise.\(^11,24\)

Therefore, PDA can lead to several complications. The main diseases related to PDA are: 1) chronic pulmonary disease (CPD) (also known as bronchopulmonary dysplasia, BPD), as it is well-known that a big left-to-right shunt could affect in the mechanics of pulmonary function decreasing the dynamic compliance and leading to an increase in
the respiratory assistance requirements; 2) necrotizing enterocolitis (NEC) as the ductal shunt decreases the diastolic blood flow and the flow velocity to intestines, which can result in ischemia and it increases the risk of NEC, and 3) intraventricular hemorrhage (IVH) due to the increase in cerebral blood flow.\(^{(8,10,12,15,17,23,27)}\)

Decreased renal perfusion can lead to a decrease in glomerular filtration rate and subsequent renal failure.\(^{(10,13,17)}\) PDA can also lead to retinopathy of prematurity\(^{(27)}\) and to heart failure and pulmonary hypertension, which involves a higher risk of developing pulmonary vascular disease. A wide-open PDA exposes the pulmonary microvasculature to systemic blood pressure and increased pulmonary blood flow, and therefore, early severe pulmonary hemorrhages frequently occur when large PDA shunt produces a sudden increase in pulmonary blood flow.\(^{(12,13,17,23)}\) Infective endarteritis may be seen at any age. More rare complications include: aneurysmal dilatation of the pulmonary artery or the ductus, calcification of the ductus, noninfective thrombosis of the ductus with pulmonary or systemic embolization, and paradoxical emboli. Pulmonary hypertension (Eisenmenger syndrome) usually develops in patients with a large PDA who do not undergo ductal closure.\(^{(9,11,13,15,17,23)}\) Feeding difficulties have also been related to PDA.\(^{(10,23)}\) Prolonged exposure to PDA has been associated with a 4- to 7-fold increase in death.\(^{(10)}\)

**Treatment:**

a) **Prevention:**

Ductus prevention should be started prenatally, with the administration of steroids to the mother. Once the child is born, the physician should be careful with fluidotherapy, not to exceed liquid administration.\(^{(8)}\)

The actual clinical guides about PDA of the Spanish association of neonatology\(^{(8)}\) agree with many clinical trials, studies and reviews\(^{(10,23,29-33)}\) that prophylactic treatment with indomethacin is useful in reducing the incidence of PDA, and also in reducing the need of surgical closure, reducing the incidence of pulmonary hemorrhage and reducing the incidence of IVH grade III-IV. And all these without increasing the ECN, sepsis or
excessive clinical hemorrhage rates. Similar results have been shown with prophylactic treatment with ibuprofen. However, since these drugs have many side effects, they are not nowadays recommended.\(^{(34)}\)

b) **Conservative treatment:**

Conservative treatment consists of:

- liquid restriction (although few evidence) avoiding extreme fluid restriction,\(^{(8,10,13)}\)
- adjustments in mechanical ventilation: shorten inspiratory times, increase the end-of-expiration positive pressure and the maximum inspiration pressure, considering permissive hypercapnia,\(^{(8,10,13)}\)
- avoid the administration of diuretics such as furosemide (since it stimulates renal synthesis of PGE2),\(^{(10,13,20)}\)
- uphold the hematocrit above 35%;\(^{(20)}\)

It is indicated as only treatment in those premature newborns with an echocardiographically small PAD who weight less than 1000 grams and who are not receiving mechanical ventilation, in which the PDA do not worsen the respiratory distress syndrome and who shown no apneas.\(^{(8)}\) However, it is thought by some\(^{(13)}\) that these therapies usually put off, rather than prevent, the ultimate need for PDA closure.

c) **Pharmacological treatment:**

Pharmacological treatment of PDA is indicated when the Doppler echocardiography gives the diagnosis of a medium to large PDA, without having to wait for haemodynamic or respiratory deterioration to start (especially in those newborns who weight less than 1000 grams, in which is common the presence of “silent” ductus).\(^{(8)}\)

The main pharmacological drugs used in the treatment of PDA are indomethacin and ibuprofen, that are inhibitors of the cyclooxgenase enzyme and have an effect on
ductal closure by inhibiting prostaglandin synthesis.\(^{(8,29)}\) Effectivity of 70-80% with this drugs has been reported.\(^{(28)}\)

- **Indomethacin.** Indomethacin is a cyclooxygenase inhibitor in use since 1976 as the standard treatment to pharmacologically close the PDA. Between 70% and 90% of the newborns will respond to these drug, decreasing the response rate as we get to lower gestational ages, reaching responses rates of 60% in premature of 26 weeks of gestation or less. Reopening rate is around 20% and 35% of the initially responding PDA.\(^{(8)}\)

Indomethacin is contraindicated in those newborns with oliguria (< 0.5 cc/K/h) in the previous 8 hours, serum creatinine > 1.8 mg/dl, platelets < 60000, active bleeding, active IVH evidence or NEC suspect.\(^{(8)}\)

The Spanish association of pediatrics\(^{(8)}\) recommend three intravenous doses of 0.2 mg/kg every 12-24 hours, administered in a period of time of 20-30 minutes.

- **Ibuprofen.** Ibuprofen is a non-steroid anti-inflammatory drug (NSAID) as useful as indomethacin in the pharmacological closing of the ductus. Moreover, NSAIDs reduce the incidence of oliguria in comparison to indomethacin.\(^{(8)}\)

The Spanish association of pediatrics\(^{(8)}\) recommend three intravenous doses of 5 mg/kg every 24 hours, administered in a period of time of 15 minutes.

- **Paracetamol (acetaminophen).** Paracetamol has been found to be effective on ductal closure in about 26 studies.\(^{(35-37)}\) It is a peroxidase inhibitor, and thus, its effects on the ductal closure are related to its ability to inhibit the peroxidase component of prostaglandin synthesis (see Figure 1).\(^{(29,38)}\)

Prostaglandin synthase is composed of a cyclooxygenase and a peroxidase subset. First, arachidonic acid undergoes oxygenation (by the cyclooxygenase subset of the prostaglandin synthase) and prostaglandin \(G_2\) is formed. Next, the
prostaglandin G₂ turns into prostaglandin H₂ when it gets in contact with the peroxidase subset of the prostaglandin synthase.²⁹

**Figure 1**: Prostaglandin synthesis. Adapted from DuBois.³⁹

In comparison with indomethacin and ibuprofen, paracetamol has several advantages: 1) it has no peripheral vasoconstrictive effects; 2) at the doses used, it has minimal and usually well-tolerated side effects;³⁶,⁴⁰ 3) it can be effective in closing PDAs in those babies who did not respond to indomethacin and ibuprofen.¹⁰,²⁹ Moreover, it can be administered to those newborns with contraindications to cyclooxygenase inhibitors.¹⁰ Adverse effects, such as hypotension, hepatic alterations (mainly in overdosage), general discomfort and vomiting, rash, fever, thrombocytopenia, leukopenia, neutropenia or hypersensitivity reactions are very rare.⁴¹,⁴²

With little differences between them, most authors’ recommended dosage is 7.5 mg/kg/6-8h administered in a 15-minutes infusion for 3 to 6 days.²⁰,³⁶,⁴¹-⁴⁷

d) **Interventional or invasive treatment:**

Interventional treatment is reserved to those cases in which the pharmacological treatment is contraindicated or has failed, understanding pharmacological treatment
failure as the permanence of hs-PDA after the administration of two cycles of indomethacin or ibuprofen.\(^8\)

Interventional options include transcatheter closure with a coil (treatment of choice), Amplatzer ductus occluder and surgery by open thoracotomy or by thoracoscopy and PDA ligation.\(^{(7,11,13)}\)

The adverse effects of the surgical treatment can be classified in: 1) reversible complications, such as pneumothorax, infection and hemorrhage, and 2) irreversible complications, such as vocal cords palsy and quilotorax.\(^{(8,10)}\) Ductus recanalization is very rare after PDA surgery.\(^8\)

Since the fatality rate of interventional or surgical treatment is less than 1\% and the long-term complications of a not treated PDA entail greater risks, closure of the ductus is indicated in all asymptomatic patients, preferably before their first year of life.\(^{(7,9)}\)

Symptoms of heart failure, such as pulse and blood pressure, quickly disappear once the DA has been closed. Radiographically, signs of cardiac enlargement and pulmonary circulation overloading disappear over a period of several months.\(^9\)
3.2. Justification

Patent ductus arteriosus is a very frequent pathology in the newborns in our environment. According to Catalonia Institute for Statistics, 4918 prematures were born in Catalonia in the last 10 years\(^\text{(48)}\). Catalunya has a rate of 7-8% of prematurity from all newborns. As said before, the incidence of PDA range from 30% to 80% of the prematures, varying depending on their gestational age and weight at birth\(^\text{(8,11-15)}\)

PDA has been recognized as a cause of significant morbidity and mortality in premature infants.\(^\text{(17,24,27,30,49)}\) In fact, prolonged exposure to PDA has been associated with a 4- to 7-fold increase in death.\(^\text{(10)}\)

Debate continues regarding the optimal time to treat PDA.\(^\text{(10,18,30,49)}\) However, it is known that delays in treatment instauration are associated with lower success rates\(^\text{(49)}\) and that early treatment of a hemodynamically significant PDA can reduce symptoms, morbidity, hospital stay, ventilator support and the need for surgery.\(^\text{(15,17)}\)

The benefit of prophylactic intervention has been found to be useful in reducing the morbidity risk and incidence of PDA.\(^\text{(23)}\) However, since the actual drugs used for PDA treatment have many side effects, prophylaxis has been lately abandoned. Nowadays, prophylactic treatment is still being used by some clinicians but only in high-risk subpopulations (<800-1000 g).\(^\text{(10)}\)

Of course, prophylactic treatment may lead to prematures whose PDA closes spontaneously to unnecessary exposure to the treatment, but it will also prevent those prematures whose PDA wouldn’t have closed from the effects of ductus-induced circulatory disturbances, which can persist even after the closure of the ductus, and turn fatal.\(^\text{(7,10)}\)

Thus, prophylactic treatment could be reconsidered if there was a drug which closed the ductus without considerable side effects. An precisely, since more potentially life threatening adverse effects and contraindications of indomethacin are being found, alternative drugs, such as paracetamol, are being studied.\(^\text{(30)}\)
Paracetamol is a peroxidase inhibitor, and thus, it inhibits prostaglandin synthesis in the central nervous system.\(^{(50)}\) Many studies\(^{(35-37)}\) have proved its efficiency in closing the PDA with less adverse effects than indomethacin or ibuprofen. So, the question is: if paracetamol is proving efficiency in closing PDAs with less side effects, and prophylactic treatment, although efficient, was abandoned due to side effects of cyclooxygenase inhibitors, why not to examine whether paracetamol can be a good prophylactic treatment in the prevention of PDA? This is the aim of this study.

Two studies\(^{(43,45)}\) have been already realized to determine whether prophylactic treatment with paracetamol is useful in reducing PDA's incidence in premature infants. However, they had several limitations and concluded more research was needed, including large randomized trials like the one we suggest. One of the studies was a retrospective analysis of a cohort before and after paracetamol was introduced in clinical practice for the treatment of pain and discomfort during respiratory therapy\(^{(43)}\), and the other one, although it was a randomized trial, it only included 48 newborns, and thus, they concluded that the study had insufficient power to confirm efficacy in decreasing the incidence of hs-PDA\(^{(45)}\).
4. Hypothesis and objectives

4.1. Hypothesis

Principal hypothesis:

Prophylactic treatment with paracetamol reduces the incidence of PDA in very preterm infants (<32 weeks) versus placebo.

Secondary hypothesis:

Prophylactic treatment with paracetamol decreases morbimortality at day 90 after birth and is well tolerated.

4.2. Objectives

Principal objective:

To determine whether prophylactic treatment with paracetamol in very preterm newborns decreases the incidence of PDA versus placebo.

Secondary objectives:

To determine whether prophylactic treatment with paracetamol in very preterm newborns decreases morbimortality at day 90 after birth.

To evaluate the side effects of paracetamol in very preterm newborns.
5. Study design and methodology

5.1. Study design

A randomized, triple-blinded trial will be performed in the neonatal intensive care units (NICUs) of the Hospital universitari Dr. Josep Trueta (HUDJT) and Hospital universitari Vall d’Hebron (HUVH).

5.2. Population of study

Definition of the population

The population of study will be premature neonates with gestational ages under 32 weeks who have been born in or referred to Hospital universitari Doctor Josep Trueta in Girona or Hospital universitari Vall d’Hebron in Barcelona, and are being followed up in the NICU service.

Inclusion and exclusion criteria

Inclusion criteria
- Neonates with gestational ages under 32 weeks who have been admitted to the NICU service.
- Neonates who survive the first 24 hours of life.
- Patients whom parents or legals tutors agree with their participation in the trial.

Exclusion criteria
- Having a severe hepatic or renal illness which contraindicates the treatment with paracetamol.
- Having lethal malformations.
- Having heart malformations.
- Having chromosomal abnormalities.
- Having an underlying septic shock.
5.3. Sample and sampling method

Sample size determination:

To determine the study’s sample size we used online GRANMO* Calculator. Our aim is to compare the incidence of PDA between those newborns who have received paracetamol prophylactic treatment and those who have been given placebo. Thus, we estimated that the incidence of PDA in the group receiving placebo would be similar to the actual incidence of PDA (40%, considering that most prematures are not extremely preterm and we do not include in our trial late preterms) and we hypothesized that the incidence of PDA in the group treated with paracetamol would be, according to previous studies, of 25%.

With an alpha risk set at 0.05 and a beta risk set at 0.2 in a two-tailed test, 336 subjects are needed to be able to detect as statistically significant a difference between the two groups, that for the placebo group it is expected to be of 0.4 and 0.25 for the group treated with paracetamol; expecting a follow-up loosing rate of 10%.

Recruitment strategies:

According to Catalonia Institute for Statistics, there were a total of 436 premature newborns in Girona and 3.253 in Barcelona in 2015. Data for 2016 is still provisional, but a total of 399 premature newborns in Girona and 3.043 in Barcelona have been already registered. These rates include all premature infants (late preterm infants, very low preterm infants and extremely preterm infants). Approximately 70 prematures of less of 32 weeks of gestation are admitted each year in Hospital universitari Dr Josep Trueta in Girona (HUDJT) and 100 in Hospital universitari Vall d'Hebron (HUVH).

Thus, since the sample size in the study will include 336 very low and extremely preterm infants, we will consecutively recruit, during a period of two years, all the newborns admitted in HUDJT and HUVH’s NICUs who meet the inclusion requirements.
5.4. Variables and measurement methods

5.4.1. Independent variable

The independent variable will be early paracetamol administration. This variable will be managed as a categorical dichotomous variable (yes/no). Please see Intervention (point 5.5) for further information.

5.4.2. Dependent variables

*Principal dependent variable:*

The primary outcome variable will be the presence or not of PDA. PDA will be diagnosed by the visualization or not of the ductus in two successive echocardiographies realized by two different pediatric cardiologists who have received previous training in order to ensure variable’s validity and reliability. If they don’t agree with the absence or presence of a PDA, a third pediatric cardiologist will repeat the echocardiography and will conclude the presence or absence of diagnosis.

Echocardiographies to diagnose PDA will be realized to all newborns on day three and seven after initiation of treatment to determine the presence or not of PDA. Control echocardiographies will be realized on month one and three to ensure there is no ductus reopening. Presence or not of PDA will be determined by the last echocardiography realized.

*Secondary dependent variables:*

  a) *Morbimortality at day 90 after birth:*

Data in relation with morbimortality will be collected separately (survival and comorbidities).

Survival will be evaluated 90 days after birth. Cause of death will be classified in: cardiac cause, respiratory cause, gastrointestinal cause, septic cause or others.
Comorbidities will be studied until day 90 after birth, and they will be analyzed as dichotomic yes/no variables. Comorbidities that will be studied are:

- **Bronchopulmonary dysplasia (BPD).** It is a chronic pulmonary disease (CPD) that will be diagnosed when a premature newborn with respiratory problems continues needing extra oxygen (FiO₂ >21%) at 36 weeks or more of corrected gestational age.

- **Necrotizing enterocolitis (NEC).** NEC consists in death of intestinal tissue previously damaged. It will be diagnosed according to stage 2 and 3 from Bell’s Criteria.

<table>
<thead>
<tr>
<th>Bell's Staging</th>
<th>Clinical Findings</th>
<th>Radiographical Findings</th>
<th>Gastrointestinal Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Apnea, bradycardia, and temperature instability</td>
<td>Normal gas pattern or mild ileus</td>
<td>Mild abdominal distention, stool occult blood, gastric residuals</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>Apnea, bradycardia, and temperature instability</td>
<td>Ileus with dilated bowel loops and focal pneumatosis</td>
<td>Moderate abdominal distention, hematochezia, absent bowel sounds</td>
</tr>
<tr>
<td>Stage IIB</td>
<td>Metabolic acidosis and thrombocytopenia</td>
<td>Widespread pneumatosis, portal venous gas, ascites</td>
<td>Abdominal tenderness and edema</td>
</tr>
<tr>
<td>Stage IIIA</td>
<td>Mixed acidosis, coagulopathy, hypotension, oliguria</td>
<td>Moderate to severely dilated bowel loops, ascites, no free air</td>
<td>Abdominal wall edema, erythema, and induration</td>
</tr>
<tr>
<td>Stage IIIIB</td>
<td>Shock, worsening vital signs and laboratory values</td>
<td>Pneumoperitoneum</td>
<td>Bowel perforation</td>
</tr>
</tbody>
</table>

**Figure 2:** Bell’s Staging criteria for NEC. Adapted from Franklin *et al.*

- **Intraventricular hemorrhage:** It will be diagnosed by the presence of bleeding in a transfontanellar echography. Four transfontanellar echographies will be performed to the newborns as part of the routine testing while they are admitted in the NICU: two during the first week of life, one on day 15 and one at day 30 (one month) after birth.

b) **Paracetamol adverse reactions:**

Adverse reaction is defined by WHO as “a response to a drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis,
diagnosis, or therapy of disease, or for the modifications of physiological function”. All reactions observed in the newborns will be recorded in accordance with MedDRA (Medical Dictionary for Regulatory Activities) codes.

5.4.3. Covariates

Since the design of the present study is a randomized trial, it is expected that all covariates will be equally distributed in both treatment groups. However, the following data will be collected in case any mismatch is found when realizing the bivariant analysis.

a) *Data related to prenatal conditions and delivery process:*

*Type of delivery:* measured as a dichotomous variable (vaginal delivery / caesarean section).

*Prenatal maturation with steroids:* measured as a dichotomous variable (yes / no). It will be considered as “yes” the administration of either two intramuscular doses of betamethasone (12 mg) 24 hours apart or four intramuscular doses of dexamethasone (6 mg), one every 12 hours, in those women at risk of preterm delivery at 24 0/7 - 33 6/7 weeks of gestation.

*Maternal drug consume:* measured as a dichotomous variable (yes / no).

b) *Data related to the newborn:*

*Gender:* measured as a dichotomous variable (male / female).

*Ethnicity:* measured as a categorical nominal variable (White, Black or African American, Hispanic or Latino, Other).

*Gestational age at birth:* number of weeks and days from the first day of the last normal menstrual period to the day of delivery. This variable will be measured both as a continuous quantitative variable (weeks of gestation/days) and as a dichotomous
variable (extremely preterm (<28 weeks of gestation) / very preterm (28-31/6 weeks of gestation)).

**Small for gestational age**: said of those infants with a birth weight at 10th percentile or under for that gestational age. It will be measured as a dichotomous variable (yes / no).

**Weight at birth**: measured as a discrete quantitative variable (<1000 gr, 1000-1500 gr, >1500gr).

**Apgar score at 5 minutes under 7**: measured as a dichotomous variable (yes / no).

<table>
<thead>
<tr>
<th>Component</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>Absent</td>
<td>Slow (&lt;100 beats/min)</td>
<td>&gt;100 beats/min</td>
</tr>
<tr>
<td>Respirations</td>
<td>Absent</td>
<td>Weak cry, hypoventilation</td>
<td>Good, strong, cry</td>
</tr>
<tr>
<td>Muscle tone</td>
<td>Limp</td>
<td>Some flexion</td>
<td>Active motion</td>
</tr>
<tr>
<td>Reflex irritability</td>
<td>No response</td>
<td>Grimace</td>
<td>Cough or sneeze</td>
</tr>
<tr>
<td>Color</td>
<td>Blue or pale</td>
<td>Body pink, extremeties blue</td>
<td>Completely pink</td>
</tr>
</tbody>
</table>

**Figure 3**: Apgar score components and score definition. \(^{(54)}\)

### 5.5. Intervention

#### 5.5.1. Interventions, administration and duration

There will be two groups of treatment: one group will be receiving placebo and the other group will be receiving paracetamol.

Both treatments will be administered intravenously every 6 hours during five days. Treatment will be finished before if ductus closure is seen in the control echocardiography performed on day three from the first administrated dosage. They will be administered in the same NICU where the newborns are admitted.

Paracetamol dosage will consist on a loading dose of 20 mg/kg followed by a maintenance dose of 7.5 mg/kg (every 6 hours, as said before) diluted with 0.9% saline and administered in 15 minutes. This is the same dosage as the one used for pain therapy in neonates and actually indicated for PDA closure by most authors \(^{20,36,41,42,44,46,47,55}\) and also the one that has been used in the two previous similar studies. \(^{43,45}\)
5.5.2. Concomitant interventions that are allowed or prohibited

All needed interventions to garant the infant’s wellness will be allowed. If these contraindicate the use of paracetamol, the treatment will be stopped.

If renal or hepatic failure with increased risk of overdosage appear, treatment will be finalized.

Concomitant administration of oral anticoagulants, isoniazid, probenecid, salicilamida, phenobarbital and phenytoin is prohibited, since these inhibit paracetamol metabolism, increasing plasma concentrations of paracetamol and leading to toxicity.

5.5.3. Adherence assessment

Since the drugs will be administered intravenously in the NUCIs by the nurses, there will be no problems of adherence.

5.6. Follow-up plan

Once a newborn is admitted in the NICU, if he/she fulfills all the inclusion criteria, parents will be informed about the trial and will be asked to sign the information sheet and informed consent (which includes the compassionate use of paracetamol) (please see annexes 13.1 to 13.4) if they agree with their infant entering the trial. Later, two successive echocardiographies (realized by two different pediatric cardiologists) will be realized to this prematures to assess normal heart anatomy.

Then, depending on the group they are in, the prematures will be administered the loading dose of paracetamol or placebo, continued by the maintenance dosages during five days (or until day 3 if ductus has already closed). Echocardiographies will be performed on day 3 and 7 after birth to study ductus closure and later on month 1 and 3 to discard ductus reopening.
Physical exploration, blood analysis (which will include hemogram and coagulation, biochemical, renal and hepatic profiles), transfontanellar echocardiographies and other necessary tests (such as temperature and glucose measurement) will be realized as part of the routine neonatal NICU care.

<table>
<thead>
<tr>
<th>PROCEDURE</th>
<th>AGE (days/months of life)</th>
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<tbody>
<tr>
<td></td>
<td>Days</td>
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<td></td>
<td>1*</td>
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<td>7</td>
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<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Information sheet and informed consent</td>
<td>✓</td>
</tr>
<tr>
<td>Drug administration</td>
<td>✓</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>✓</td>
</tr>
</tbody>
</table>

* Day 1 → day of birth

Figure 4: Follow-up plan.
6. Data management and statistical analysis

There will be two or three different levels of data analysis:

A. UNIVARIATE DESCRIPTIVE ANALYSIS
A descriptive analysis of all the variables will be performed. Categorical variables will all be described as numbers (n) and percentages (%) and they will be represented with frequency tables and barcharts. The quantitative variable gestational age (GA) will be described as mean ± standard deviation (SD) and with median and interquartile range (p25 and p75), since GA will probably not follow a normal distribution. It will be represented in box-plots. Kolmogorov–Smirnov (KS) test will be used to determine whether this variable follows or not a normal distribution.

B. BIVARIATE ANALYSIS
The independent variable will be compared with the dependent ones. Contingency tables will be realized using Fisher’s exact test and correlation will be graphically expressed with barcharts. Chi-Squared Test ($\chi^2$) will be used to test the relationship between categorical variables. Log-linear regression will also be used to analyze the variables. The main outcome of the study will be assessed using risk ratio (RR).

A subgroup analysis of participants over and under 28 weeks of gestational age (<28 or 28-32/6) will be undertaken too.

C. MULTIVARIATE ANALYSIS
Although due to the aleatorizing design of the study it is not expected to occur, if any covariate were not distributed equally to both groups of treatment, these would then become potential confounders. If this occurred, a multiple linear regression with logarithmic transformation model would be performed.

To analyze all the above data, we will use intention-to-treat analysis. If any participant don’t show up in the monitoring visits, the last data collected will be the one analyzed. The level of statistical significance will be set at $p<0.05$. 

{ 32 }
7. Ethical considerations

Ethical problems related with this study are the age of the participants and that the study includes the administration of a drug not indicated yet for this disease. Since the illness studied is exclusive of premature newborns, it is mandatory that the present trial is executed in these population. And related with the compassionate use of a drug, it is the aim of this study to introduce a new authorization of these drug, which has been already proved to be safe in premature newborns, and already has an indication, for a different disease, in this population.

Before the study begins, his protocol will be submitted for approval to the reference Clinical Research Ethical Committee (“Comitè Ètic d’Investigació Clínica”, CEIC), that will be the “CEIC” of the “Hospital Universitari Doctor Josep Trueta”, where at least a pediatrician must be part of.

It will be carried out in accordance with the ethical principles established in the Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects (lastly updated in the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013).

Also, the project will be conducted in accordance with the “Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica”, and therefore, all parents or legal tutors of the patients included in the trial will have been fully informed and will have signed, voluntarily and beforehand, the informed consent.

The trial will likewise be conducted in accordance with the “Ley Orgánica 15/1999, de 13 de diciembre, de protección de datos de carácter personal”. Thus, all the data compilation resulting from the trial will be confidential and used only with the purpose of the research and always on condition of anonymity.

According to the “Real Decreto 1090/2015, de 4 de diciembre, por el que se regulan los ensayos clínicos con medicamentos, los Comités de Ética de la Investigación con medicamentos y el Registro Español de Estudios Clínicos”, the trial will be carried out following the Good Clinical Practices (GCP). Moreover, the research team will count
with experts in pediatrics, as it is determined due to the participation of minor child in the present trial. Also, and related to the realization of a trial on minor child, informed consent will be valid whenever one of the progenitors has signed it and with the express or tacit approval of the other, according to what is stated in the article 156 of the Spanish Civil Code. The protocol of the study will be submitted to the director of the "Agencia Española de Medicamentos y Productos Sanitarios" for their approval.

Additionally, the trial will be conducted in accordance with the “Real Decreto Legislativo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios", and thus, a specific insurance will be taken out for the usage of an investigational drug.

Finally, and according to the “Real Decreto 1015/2009, de 19 de junio, por el que se regula la disponibilidad de medicamentos en situaciones especiales”, a temporary authorisation will be requested to the “Agencia Española de Medicamentos y Productos Sanitarios” for the administration of a drug for compassionate use. Moreover, all adverse effects will be notified to the same immediately.

No conflicts of interest are related to this study.
8. Limitations of the study

Some of the limitations of the study are related to the PDA’s framework. Already well known are the limitations of diagnosing a PDA. Echocardiography is the gold-standard tool for the diagnosis and the one used in the study to diagnose PDA. However, echocardiographic criteria haven’t been yet established and, moreover, this medical test is highly operator dependent. To solve this problem, pediatric cardiologists will be trained in diagnosing PDA and two echocardiographies by two different pediatric cardiologists will be realized successively to each premature in order to make it the most unbiased possible. If the two pediatric cardiologists don’t agree with the presence or absence of PDA, a third pediatric cardiologist will repeat the echocardiography and determine the final verdict.

Another limitation related with PDA behaviour is its ability to close and reopen later on. To try to prevent mistakes, two control echocardiographies have been programmed on the first and third month after birth in order to ensure the perpetual of ductus closure.

Plasma half life of paracetamol can vary due to interpersonal pharmacokinetic variations, leading to variations in “real dosage”. To minimize this problem, we have excluded those newborns with renal failure who would need dosage adjustment and subsequent major altered plasma half life.

During the realization of the study, possible loss of patients, either by death or lack of attendance, may occur. Deaths will be analyzed as such. Lack of attendance is the one that can cause follow-up biases. However, since the participants are newborns and parents are usually well concerned about the need of medical care, lack of attendance will probably be rare. If this happens, the data will be analyzed using the last collected data.
9. Feasibility

9.1. Research team

The research team will be formed by the following:

- The main investigator (MI), who will be a neonatal pediatrician from NICU from HUDJT and will be in charge of the whole project. He/she will elaborate the trial protocol, coordinate the whole project, interpret the statistical analysis, elaborate the article and present the results.

- Neonatal pediatricians (NP), who will be in charge of the sample recruitment and early follow-up (while the premature is admitted in the NICU).

- Pediatric cardiologists (PC), who will be performing the echocardiographies.

- A clinical pharmacologist (CPh), who will aport knowledge and guidance during the administration of the drug, will take action towards data management and also towards legal and methodological issues of clinical trials.

9.2. Work plan

To carry out this study, all the necessary work will be held in four different stages:

Stage 0: Literature review and protocol design. This stage consists on the literature review, the protocol design and the proposal of the same to the Comitè Ètic d'Investigació Clínica (CEIC) for its evaluation and acceptance.

Stage 1: Trial execution. This stage consists on the realisation of the trial. It includes two coordinating meetings with all the staff, the echocardiographic training of the pediatric cardiologists and the recruitment of the sample and fulfillment of the clinical trial. This stage will last two years and five months.
Stage 2: Data analysis, interpretation of the results and article elaboration. Firstly, the statistician will analyse all the obtained data. Then, the results will be given to the main investigator for the interpretation of the results and the subsequent elaboration of the article. This stage will take 4 months.

Stage 3: Publication and dissemination of the results. In this stage, the main investigator will present the obtained results in a prestigious neonatology publication with the target of acceptance and publication of the article. The results will be also submitted to national and international congresses of neonatology. This final stage will last up to 3 months.
9.3. Chronogram

The starting date in the chronogram is August of 2017 for stage 0. Stage 1, if ethical approval has no delay, will start in December of 2017 and will last until May of 2020. Any adjustment of the beginning of stage 1 of the project can be made as long as the schedule previously defined is followed and its length is respected. Stage 3 will end in December of 2020, and along with this, the study.

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<tbody>
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<td>Literature review</td>
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<tr>
<td>Ethical approval</td>
<td>MI</td>
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<tr>
<td>StAGE 1</td>
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<td>Coordination meetings</td>
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<tr>
<td>Training</td>
<td>PC</td>
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<td>Sample recruitment</td>
<td>NP</td>
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<tr>
<td>Follow-up</td>
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<tr>
<td>StAGE 2</td>
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<tr>
<td>Statistical analysis</td>
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<tr>
<td>Interpretation of the statistics</td>
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<tr>
<td>StAGE 3</td>
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<tr>
<td>Results dissemination</td>
<td>MI</td>
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</tbody>
</table>

* MI main investigator     NP neonatal pediatrician     Lab laboratory staff
   PC pediatric cardiologist   CP clinical pharmacologist   Sta statistician

Figure 5: Chronogram.
10. Budget

Personnel recruitment and training:

A statistician will be contracted to realize all the data managing and statistical analysis and a monitoring and data collection assistant will be contracted to ensure the validity of the data.

Specialized professors will be contracted to deliver a training course on echocardiography. *Price has been estimated guided by the fees of the 12th European Echocardiography Course on Congenital Heart Disease (Prague, October 2017).

<table>
<thead>
<tr>
<th>PERSONNEL RECRUITMENT AND TRAINING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistician (30€/h, 40h/week, 4 weeks)</td>
</tr>
<tr>
<td>Monitoring and data collection assistant (30€/h, 8h/w, 24w)</td>
</tr>
<tr>
<td>Echocardiographic training * (650€) (x8 participants)</td>
</tr>
<tr>
<td><strong>Subtotal:</strong></td>
</tr>
</tbody>
</table>

Insurance policy:

As the present study is a clinical trial which include the off-label use of a drug, an insurance policy will be contracted.

<table>
<thead>
<tr>
<th>INSURANCE POLICY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial insurance policy</td>
</tr>
<tr>
<td><strong>Subtotal:</strong></td>
</tr>
</tbody>
</table>

Material:

To estimate the budget, fungible material for treatment administration has been taken into account. Echocardiographies have a cost of 0 € since the pediatric cardiologist who will be realizing them is part of the research team and the use of the computer itself entails no additional costs.
Results’ publication and diffusion:

**Price has been estimated guided by the fees of the “XXVI Congreso de Neonatología y Medicina Perinatal” (Zaragoza, September 2017).

***Price has been estimated guided by the fees of the 3rd European Spontaneous Preterm Birth Congress (Edinburgh, May 2018).

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<thead>
<tr>
<th>RESULTS’ PUBLICATION AND DIFFUSION</th>
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<tr>
<td>Publication (paper revision and publication)</td>
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<td>National Congress</td>
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<td>Inscription fee **</td>
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<td>Accommodation and diets</td>
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<td>International Congress</td>
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<td>Inscription fee ***</td>
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<td>Travel costs</td>
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<td>Subtotal:</td>
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**TOTAL BUDGET**

| Personnel recruitment and training | 15,760 € |
| Insurance policy | 25,000 € |
| Material | 500 € |
| Results’ publication and diffusion | 3,170 € |
| **TOTAL: | 44,430 € |
11. Impact of the study to the National Health System

Patent ductus arteriosus is the most frequent congenital form of heart disease in preterm babies, and is present in 30% of the very preterm infants and over 80% of the extremely preterm infants. PDA is an important disease since it can lead to many complications, such as intraventricular hemorrhage, necrotizing enterocolitis or bronchopulmonary dysplasia inter alia. All these complications will entail longer hospital stay, higher amount of tests and treatments received as well as higher risk of irreversible legacies at adulthood and even death.

PDA prevention has already been studied with positive results in decreasing the incidence of the disease and of many comorbidities, but with several adverse effects because of the drugs used.

The aim of this study is to decrease the incidence of PDA through the administration of a drug with minimum side effects, and thus, decrease the morbimortality in prematures related to this illness.

If the hypothesis is confirmed, a new pharmacological option for not curing, but preventing the onset of PDA will be settled. This will involve the prevention of many complications associated with PDA and, thus, the no longer necessity of repeated control visits, tests such echocardiographies and chest and abdominal x-rays, drugs with high adverse effects, surgeries... leading to a higher neonatal well-being and a saving in National Health economy.

To sum up, it the results of the study turn positive and statistically significative, we will have found a mean to prevent PDA and all its complications in both neonatal period and adulthood.
12. Bibliography


2. Everett AD. Fetal Circulation [Internet]. Johns Hopkins Children’s Hospital. [cited 2017 Sep 30]. Available from: http://www.pted.org/?id=sp/fetal1#


13. Annexes

13.1. Information sheet for parents or legal tutors (catalan version)

FULL D'INFORMACIÓ AL FAMILIAR RESPONSABLE O REPRESENTANT LEGAL

**Estudi:** Eficàcia del paracetamol endovenós comparat amb placebo en la prevenció del ductus arteriós permeable en gran prematurs.

Ens dirigim a vostè, com a pare/mare o tutor legal del recent nascut, per informar-lo de que s'està duent a terme un estudi amb l'objectiu d'avaluar l'eficàcia d'un medicament que pretén reduir el risc de desenvolupar ductus arteriós permeable, una alteració cardíaca força freqüent en nounats prematurs de menys de 32 setmanes de gestació.

Abans de prendre una decisió sobre la participació o no del recent nascut en l'estudi, és imprescindible que llegeixi i entengui el full informatiu que se li ha facilitat i que resolgui amb el doctor que li ha proposat entrar a l'estudi tots aquells dubtes que li puguin sorgir.

**Participació voluntària i interrupció de la participació en l'estudi:**

La participació a aquest estudi és totalment voluntària, i pot rebutjar entrar a l'estudi sense la necessitat de donar cap explicació. De la mateixa manera, si decideix entrar a l'estudi, té el dret de canviar d'opinió i retirar el seu consentiment en qualsevol moment si així ho desitja, sense que això alteri la relació del/de la pacient amb el seu metge ni es produeixi cap perjudici en la seva atenció.

**Descripció de l’estudi:**

El present estudi ha estat prèviament aprovat pel Comitè Ètic d'Investigació Clínica (CEIC) de l'Hospital universitari Dr. Josep Trueta (CEIC de referència).

L'estudi es duu a terme a les Unitats de Cures Intensives Neonatals (UCIN) de l'Hospital universitari Dr. Josep Trueta i de l'Hospital universitari Vall d'Hebron, i s'espera incloure una mostra d'almenys 336 prematurs de menys de 32 setmanes de gestació.
gestació. El grup d'investigació de l'estudi està format per pediatres especialitzats en neonatologia i cardiologia (formats especialment per a dur a terme aquest estudi) i per farmacòlegs clínics, amb la finalitat de aconseguir la màxima seguretat al llarg de tot el procés.

L'objectiu de l'estudi és demostrar l'eficàcia del paracetamol en la prevenció del ductus arteriós permeable, una patologia cardíaca força freqüent en grans prematurs (<32 setmanes de gestació) i encara més freqüent en prematurs extrems (<28 setmanes de gestació). Actualment aquest fàrmac s'utilitza com a una de les opcions de tractament en els prematurs que ja presenten la malaltia. Tot i així, estudis recents han plantejat la possibilitat de que, administrat al nadó de forma precoç, el paracetamol pot frenar el desenvolupament de la malaltia i evitar les possibles complicacions derivades d'aquesta.

Per a poder determinar si el paracetamol és efectiu, es fa un seguiment al nadó. Aquest seguiment consisteix en una ecocardiografia als tres i set dies des del moment de l'administració de la primera dosi de paracetamol i dues ecocardiografies de control al mes i als tres mesos.

**Grups de tractament:**

Al entrar a l'estudi, els recent nascuts són assignats aleatòriament a un dels dos grups de tractament. Un grup rep paracetamol intravenós, mentre que l'altre rep placebo.

**Beneficis:**

Amb els resultats d'aquest estudi s'espera poder demostrar la utilitat del paracetamol en la prevenció del ductus arteriós permeable amb la posterior implantació com a tractament profilàctic en grups de risc com son els grans prematurs i en especial els prematurs extrems.

**Riscos:**

Al ser un estudi en el que s'administren fàrmacs, és possible l'aparició de reaccions adverses, com ara reaccions irritatives a la zona d'injecció, vòmits, febre o alteracions de la sang (leucopènia, trombocitopènia). En el cas que apareguin, aquestes seran tractades segons la pauta clínica establerta, tenint sempre com a prioritat el benestar del pacient.
Responsabilitat i assegurança:

El doctor responsable d'aquesta investigació ha contractat una assegurança que cobreix els possibles danys ocasionats a les persones que hi participen d'acord amb la legislació vigent sobre assajos clínics amb medicaments en investigació.

Confidencialitat:

La informació mèdica i qualsevol altre informació recollida sobre el nadó durant l'estudi seran confidencials. En cap cas el nom del nadó o dels pares/tutors legals apareixerà en la publicació dels resultats. La seva privacitat està protegida i recollida en la Llei Orgànica 15/1999 sobre protecció de dades personals i el corresponent Reial decret nacional 1720/2007.

Compensació econòmica:

La participació en l'estudi no serà beneficiaria de cap compensació econòmica. En cas de generar-se qualsevol benefici dels coneixements obtinguts, aquests seran destinats a cobrir costos científics.

Consentiment informat:

En el cas que decidiu participar en l'assaig clínic, cal que firmeu el consentiment informat que se li proporcionarà per tal d'evidenciar que coneix les condicions de l'estudi i les accepta.
13.2. Information sheet for parents or legal tutors (spanish version)

HOJA DE INFORMACIÓN AL FAMILIAR RESPONSABLE O REPRESENTANTE LEGAL

**Estudio**: Eficacia del paracetamol endovenoso comparado con placebo en la prevención del ductus arterioso permeable en grandes prematuros.

Nos dirijimos a usted, como padre/madre o tutor legal del recién nacido, para informarle de que se está llevando a cabo un estudio con el objetivo de evaluar la eficacia de un medicamento que pretende reducir el riesgo de desarrollar ductus arterioso permeable, una alteración cardíaca bastante frecuente en neonatos prematuros de menos de 32 semanas de gestación.

Antes de tomar una decisión sobre la participación o no del recién nacido en el estudio, es imprescindible que lea i comprenda la presente hoja informativa que se le ha facilitado y resuelva con el doctor que le ha propuesto entrar en el estudio todas aquellas dudas que pueda tener.

**Participación voluntaria e interrupción de la participación en el estudio:**

La participación en este estudio es totalmente voluntaria, y puede rechazar entrar en el estudio sin la necesidad de dar ninguna explicación. De la misma manera, si decide entrar en el estudio, tiene el derecho de cambiar de opinión y retirar su consentimiento en cualquier momento si así lo desea, sin que esto altere la relación del paciente con su médico ni se produzca ningún perjuicio en la atención que va a recibir.

**Descripción del estudio:**

El presente estudio ha sido previamente aprobado por el Comité Ético de Investigación Clínica (CEIC) del Hospital universitario Dr. Josep Trueta (CEIC de referencia).

El estudio se lleva a cabo en las Unidades de Cuidados Intensivos Neonatales (UCIN) del Hospital universitario Dr. Josep Trueta i del Hospital universitario Vall d’Hebron, i se espera incluir una muestra de al menos 336 prematuros de menos de 32 semanas

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de gestación. El grupo de investigación del estudio está formado por pediatras especializados en neonatología y cardiología (formados especialmente para llevar a cabo este estudio) y por farmacólogos clínicos, con la finalidad de alcanzar la máxima seguridad a lo largo de todo el proceso.

El objetivo del estudio es demostrar la eficacia del paracetamol en la prevención del ductus arterioso permeable, una patología cardíaca frecuente en grandes prematuros (<32 semanas de gestación) y aún más en prematuros extremos (<28 semanas de gestación). Actualmente, este fármaco se utiliza como una de las opciones de tratamiento en aquellos prematuros que ya presentan la enfermedad. Sin embargo, estudios recientes han planteado la posibilidad de que, administrado al recién nacido de forma precoz, el paracetamol puede frenar el desarrollo de la enfermedad y evitar las posibles complicaciones derivadas de esta.

Para poder determinar si el paracetamol es efectivo, se hace un seguimiento al recién nacido. Este seguimiento consiste en una ecocardiografía a los tres y siete días desde la primera administración de paracetamol y dos ecocardiografías de control al mes y a los tres meses.

**Grupos de tratamiento:**

Al entrar a en el estudio, los recién nacidos son asignados aleatoriamente a uno de los dos grupos de tratamiento. Un grupo recibe paracetamol intravenosos, mientras que el otro recibe placebo.

**Beneficios:**

Con los resultados de este estudio se espera poder demostrar la utilidad del paracetamol en la prevención del ductus arterioso permeable con la posterior implantación como tratamiento profiláctico en grupos de riesgo como son los grandes prematuros y especialmente los prematuros extremos.

**Riesgos:**

Al tratarse de un estudio en el que se administran fármacos, es posible la aparición de reacciones adversos, como reacciones irritativas en la zona de inyección, vómitos, fiebre o alteraciones de la sangre (leucopenia, trombocitopenia). En el caso que aparezcan, estas serán tratadas según la pauta clínica establecida, primando siempre el bienestar del paciente.
Responsabilidad y seguro:

El doctor responsable de esta investigación ha contratado un seguro que cubre los posibles daños ocasionados a las personas que participan en este, de acuerdo con la legislación vigente en relación a los ensayos clínicos con medicamentos en investigación.

Confidencialidad:

La información médica y cualquier otra información recogida acerca del recién nacido durante el estudio serán confidenciales. En ningún caso el nombre del recién nacido o de los padres/tutores legales aparecerá en la publicación de los resultados. Su privacidad está protegida y recogida en la Ley Orgánica 15/1999 sobre la protección de datos personales y el correspondiente Real decreto nacional 1720/2007.

Compensación económica:

La participación en el estudio no será beneficiaria de ninguna compensación económica. En caso de generarse cualquier beneficio de los conocimientos obtenidos, estos serán destinados a cubrir costes científicos.

Consentimiento informado:

En caso que decida participar en este ensayo clínico, es necesario que firme el consentimiento informado que se le va a proporcionar para evidenciar que conoce las condiciones del estudio y que las acepta.
13.3. Informed consent for parents or legal tutors (catalan version)

**FULL DE CONSENTIMENT INFORMAT**

Estudi: Eficàcia del paracetamol endovenós comparat amb placebo en la prevenció del ductus arteriós permeable en gran premats.

Jo (nom i cognoms) ___________________________ en qualitat de (relació amb el participant) ___________________________ de (nom i cognoms del participant) ___________________________ afirmo que:

- he llegit i he entès el full d'informació sobre l'estudi;
- he resolt amb el metge responsable de l’estudi o altres metges implicats en l’estudi tots els dubtes que m’han sorgit;
- he tingut el temps necessari per pensar si vull o no participar en l’estudi;
- entenc que la participació a l’assaig clínic és voluntària i no remunerada;
- he entès que puc abandonar l’estudi en qualsevol moment, sense haver de donar explicacions i sense que aquesta decisió pugui afectar negativament en la relació metge-pacient;

i doncs, presto la meva conformitat a que (nom i cognoms del participant) ___________________________ partici en aquest assaig clínic.

Firma del pare/mare/tutor legal:  
Firma de l’investigador:

Nom i data:  
Nom i data:
13.4. Informed consent for parents or legal tutors (spanish version)

**HOJA DE CONSENTIMIENTO INFORMADO**

Estudio: Eficacia del paracetamol endovenoso comparado con placebo en la prevención del ductus arterioso permeable en grandes prematuros.

Yo (nombre y apellidos) ___________________________ en calidad de (relación con el participante) ___________________________ de (nombre y apellidos del participante) ___________________________ afirmo que:

- he leído y he comprendido la hoja de información sobre el estudio;
- he resuelto con el médico responsable del estudio o otros médicos implicados en el estudio todas mis dudas;
- he dispuesto del tiempo necesario para pensar si quiero o no participar en el estudio;
- entiendo que la participación en el ensayo clínico es voluntaria y no remunerada;
- he comprendido que puedo abandonar el estudio en cualquier momento, sin tener que dar explicaciones y sin que esta decisión pueda afectar negativamente en la relación médico-paciente;
- he entendido que el medicamento utilizado no está indicado actualmente en esta patología;

ante todo esto, presto mi conformidad para que (nombre y apellidos del participante) ___________________________ participe en este ensayo clínico.

Firma del padre/madre/tutor legal:                                                                                                                                                                                                 
Firma del investigador:

Nombre y fecha:                                                                                                       ...                                                                                                       Nombre y fecha: