PROTOCOL TITLE
(Version n.2 April, 2 2018)

CORRELATION BETWEEN LEVELS OF FETAL HEMOGLOBIN AND TRANFUSION OF RED CELLS FROM CORD BLOOD

PROTOCOL APPROVAL

1. INTRODUCTION

During the first 12 weeks, pregnancy is carried out without significant blood flow from mother to the fetus. Later, fetal circulation in the developing tissues occurs at low oxygen pressure: in fetal blood, rarely pO2 is higher than 30 mm/Hg. Therefore, from conception to delivery, the development of mammals occurs in hypoxia [1,2]. During delivery, infants are to cope with what could be defined as *"hyperoxic challenge".* The oxidative stress, that is the imbalance between oxidants and antioxidants in favor of the formers, affects tissues by producing free radicals. Extremely low birth weight infants (ELBW, birth weight lower than 1000 grams) are particularly sensitive to the oxidative stress, because of the immaturity of the antioxidant metabolic systems as well as the existence of frequent comorbidities, especially infections that, on their turn, increase the oxidative stress [3]. All the pathologies caused by the oxidative damage are defined as "oxidative stress-related diseases in newborns" [4,5]. These disorders, that are generally developed at the same time by the patient, include bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), periventricular leukomalacia (PVL) and, at least partially, intraventricular hemorrhage (IVH) [4,5]. EPICure (Extremely Premature Infant Cure) studies carried out in the United Kingdom showed that among premature infants born between the 22nd and the 26th gestational week, and with a postmentrual age of 26 weeks, 68% of those who survived developed BPD, 21% were affected by ROP at least in one eye and 13% presented serious alterations during brain ultrasound scans [6,7]. Most recent papers described red blood cell transfusions as a main risk factor for the development of these pathologies [8-14] and the overall survival [14-16]. There are various factors explaining the correlation between the number of transfusions and the seriousness of the pathologies due to the oxidative damage, such as the immune modulatory effect (the term "transfusion-related immunomodulation" is used for the transfusion-related immunosuppression syndrome) [17,18] or the inflammation caused by "micro particles" that are released during the storage of red blood cells [19-21]. However, it is increasingly evident the role played by adult hemoglobin (HbA) that, due to its lower affinity to oxygen than fetal hemoglobin, greatly contributes to the oxidative stress [13,22,23]. The harmful role of HbA in preterm has clearly emerged in a recent prospective study, which showed that the incidence of ROP was inversely related to patients' fetal hemoglobin (HbF) levels, with a higher incidence among those showing the lowest levels [13]. In these patients, the level of HbF was obviously inversely proportional to that of HbA [13]. The current neonatal transfusion guidelines, by the Italian Society of Neonatology and the Italian Society of Transfusion Medicine, recommend the use of fresh leukoreduced red cell concentrates and the irradiation of blood components [24].

However, it seems that at present these precautions are not able to reduce the incidence of the oxidative damage-related pathologies [25, 26].

Nonetheless, transfusions of red cell concentrates continue to be a life-saving therapy and they are fundamental to treat the anemia of preterm infants. Anemia in preterm infants, compared to the physiological anemia in full-term newborns, is characterized by a faster and more serious decline in the levels of hemoglobin (Hb) and hematocrit (Hct). The repeated venous samplings for laboratory tests, the lack of nutrients, the insufficient production of endogenous erythropoietin, associated to the concurrent infections and to the possible blood losses, further exacerbate the anemia of prematurity [27-32]. In spite of the numerous precautions, such as the increasingly reduced volumes of samplings, the reduction of transfusion limits and the widespread use of iron and erythropoietin, the transfusion of 20 ml/kg of red cell concentrates is inevitable in almost 90% of ELBW patients [30-32]. Since in ELBW preterm infants anemia is particularly relevant during the first weeks of life, they all receive most transfusions before the 32nd week of PMA [14-16, 25].

Compared to the adult donor blood, cord blood taken from placenta and umbilical vessels has some important characteristics in terms of neonatal transfusions. As a matter of fact, cord red cells contain HbF, which is more physiological than HbA in preterm infants because of its higher affinity to oxygen. Moreover, the immune and infection profile of cord blood is absolutely “naïve”, as shown by the years of experience with hematopoietic transplants [33]. All these characteristics are a valid reason for using it when transfusing preterm infants. The use of autologous or allogeneic umbilical cord blood as an alternative to the transfusion of adult red cells was greatly confirmed by literature to the point that cord blood red cell concentrates were included by the Italian National Blood Center in the blood components acknowledged by the UNI 10529:1996 norm that regulates their traceability (umbilical cord blood red cell concentrates, UCB-RBC, blood component 73). These units can be irradiated like those obtained from adult donors. In a recent study, we tested the ability of the UNICATT Cord Blood Bank to cover the transfusion needs of low birth weight infants who were admitted to the Neonatal Intensive Care Unit (NICU) of our hospital with red cell concentrates from cord blood [46]. The study included patients who were born before the 30th week of gestation and/or with a birth weight lower than 1,500 g and who needed transfusions during the first 28 days of life. After the first transfusion event, each patient was assigned to the treatment arm (umbilical cord blood-packed red cells, UCB-RBC) or to the control arm (adult donor red cell concentrates, A-RBC), depending on the availability of UCB-RBC of the same blood type (ABO-Rh). From March, 1 to February, 28 2014, 20 patients out of the 63 that were hospitalized in the NICU were included in our study. Out of 20 infants, 9 were transfused with UCB-RBC and 11

with A-RBC. Overall, we transfused 23 UCB-RBC units and 27 A-RBC units. We observed that the two types of blood components led to a similar increase in the hematocrit levels and that the patients that were transfused with UCB-RBC or A-RBC received a similar number of transfusions at similar time intervals. There were no adverse events nor transfusion reactions and no CMV infections were recorded. In this limited number of patients, with an average follow-up of 57 days, the incidence of prematurity-related pathologies appeared to be globally comparable [46]. However, it was interesting to notice that the only two patients who did not develop ROP were only transfused with UCB-RBC [47]. More recently, a prospective study was carried out to show that the incidence of ROP was related to the levels of HbF in patients: the lower the levels, the greater the incidence [13]. Among those patients, the level of HbF was obviously inversely proportional to that of HbA. Therefore, even if it was a limited number of patients, the absence of ROP among the patients who were transfused with umbilical cord blood indirectly confirms the potentially damaging effect of adult blood transfusions [46, 47]. Our feasibility study also demonstrated that with current resources we are able to cover 50% of these patients' transfusion needs [46].

At global level, the implementation of a program for the transfusion of cord blood in a preterm infant could have an important clinical impact. Additionally, the introduction of this transfusion strategy could be an advantage in the rationalization of resources when using blood components. Actually, according to the criteria of cellularity established by the Italian Bone Marrow Donor Registry (IBMDR), less than 10% of cord blood units are cryopreserved and listed for transplants [48].

2. OBJECTIVES OF THE STUDY

The objective of the study is to investigate the correlation between the levels of HbF and the use of umbilical cord blood red cell concentrate transfusions in ELBW preterm infants and/or infants who were born before the 30th week of gestational age (GA). Since transfusions are one of the main mechanisms to reduce HbF, they will be carried out with umbilical cord blood red cell concentrates (UCB-RBC). Our objective is to demonstrate that UCB-RBC transfusions, until the 32nd week of PMA, guarantee average levels of HbF that are higher than in adult-RBC (A-RBC) transfusions. The following outcomes were defined:

Primary outcome:

- Average levels of HbF at 32nd week of PMA in patients receiving different transfusion support.

Secondary outcomes:

- To show that UCB-RBC transfusions are not less effective than A-RBC transfusions, as for the post-transfusion increase in the hematocrit and the interval between two transfusions.
- To show the association between average levels of HbF during the inpatient stay and comorbidities (ROP, BPD, NEC, PVL and IVH) or deaths occurred within 36 weeks of PMA.

3. EXPERIMENTAL DESIGN

3.1 Study design. This study is a prospective non-randomized study to evaluate the correlation between the reduction in the level of HbF (based on the relation between HbF and HbA) before the 36th week of PMA and the type of transfusion (UCB-RBC or A-RBC).

3.2 Duration of the study. The study will have an overall duration of one year. The enrollment of patients will last 10 months. Considering the volume of activity of our NICU and the type of eligible patients, such a period of time was considered adequate to enroll the number of patients necessary to reach the established objectives. As for the transfusions (UCB-RBC or A-RBC), the intervention phase will last from the enrollment to the completion of the 32nd week of PMA, while the weekly monitoring of HbF will continue until the completion of the 36th week of PMA.

3.3 Early study termination. The study will be terminated with the occurrence of adverse events that can be risky for the patients or if the Proposer thinks it is necessary, based on the evaluation of safety data.

3.4 Study termination. The study will be considered over when the collected data make it possible to draw up the final report.

3.5 Study endpoints.

- HbF levels before and after each transfusion;
- Average HbF levels (at 32 weeks of PMA; at last control)
- Prevalence of ROP and/or BPD and/or NEC and whatever health problems (infectious pathologies, hemorrhages including IVH, kidney failure, thromboembolism, surgical complications, etc.) before the 36th week of PMA;
- Transfusion efficacy of UCB-RBC compared to A-RBC (all transfusions before the 36th week of PMA, evaluated based on a) average time interval between two subsequent transfusions and b) average increase in the post-transfusion capillary hematocrit (ΔHct).

4. TEST POPULATION

The study will include preterm infants who met the criteria of inclusion/exclusion and whose parents/tutors have given their informed consent. Based on the number of patients hospitalized every year in our NICU (roughly 150 patients/year) and the characteristics of the test population, we expect to achieve the sample size of 25 patients in 10 months.

4.1 Eligibility criteria

4.1.1 Inclusion criteria. All the patients who will be included in the study will have to meet the following inclusion criteria:

- Birth gestational age ≤ 30 weeks and/or birth weight ≤ 1000 grams;
- Need of transfusions before the 32nd week of PMA, according to the guidelines currently used by the NICU.

4.1.2 Exclusion criteria. None of the enrolled patients can be affected by one or more than one of the following pathologies:

- mother-fetus isoimmunization;
- hydrops fetalis;
- chromosomal anomalies and congenital malformations;
- acute hemorrhage at birth.

5. PROCEDURES

5.1 Enrollment. Cord blood donors will be enrolled according to the procedures implemented by the bank personnel of the UNICATT Cord Blood Bank. The medical personnel of the Neonatology ward will enroll patients when they are hospitalized in the NICU. The parents/tutors of the newborns who meet the inclusion criteria will be adequately informed about the study and will be asked to give their informed consent (Annex I and Annex I bis). Researchers will provide exhaustive information both on the aim of the study and the related procedures. Investigators will be in charge of providing all the necessary information, including that on the parents' right to withdraw their consent to participate in the study whenever they want and for whatever reason without affecting the newborn's treatment. A copy of the consent shall be provided to the parents/tutors and the researchers will keep another. Only the newborns whose parents have given their consent can be enrolled.

5.2 Enrollment communication to the Blood Transfusion Service. Once parents/tutors have given their informed consent, the researchers of the NICU will communicate the patient's

name to the Blood Transfusion Service. The researchers of the Blood Transfusion Service will keep an up-to-date list of the enrolled patients by reporting:

- date of the completion of the 32nd week of PMA (termination of A-RBC intervention phase);
- date of the completion of the 36th week of PMA (study termination).

5.3 Description of the blood component Umbilical Cord Blood red cell concentrates. The study will be based on the transfusion of umbilical cord blood units taken from the pregnant women who are part of the Cord Blood Bank donors and who gave their informed consent on the use of the donated unit for research purposes.

5.4 The collection of cord blood will be carried out in the birth centers that are part of the Cord Blood Bank, according to the procedures used by the Bank itself. The collected units will be adequately conserved at low temperatures (2-6°C) and fractionated within 24 hours. The units that are not eligible for transplant because of low cellularity (total nucleated cells <1,200 x 10^6), with a volume >60 ml excluding the anticoagulant (CPD, Citrate, Phosphate, Dextrose), without clots and/or hemolysis, will be considered eligible for fractionation to obtain UCB-RBC. Eligible UCB units will be fractionated by an automatic blood component processing system (the UNICATT Cord Blood bankl will use Compomat®G4 by Fresenius HemoCare GmbH, D-61346, Bad Homburg, Germany). Red cell concentrates will be resuspended in a preservative solution containing adenosine, glucose and mannitol (SAGM, ratio red cell: SAGM = 2:1) and filtered with the filter Purecell® NEO Neonatal High Efficiency Leukocyte Reduction Filter (Pall, East Hills, NY, USA) to remove leukocytes. Blood cultures for bacteria and fungi will be set up. Only the units that tested microbiologically negative will be considered eligible for transfusion (blood cultures, molecular and serological tests for HCV, HBV, HIV and syphilis). UCB-RBC units will be stored at a temperature of 2-6°C for 14 days, in order to allow the γ-irradiation (^{137}Cs) when they are assigned to the patient.

5.5 Transfusion request. Units will be requested according to the procedures established by the Blood Transfusion Service, based on the current legislation and in accordance with the guidelines reported in Annex III. When a blood request arrives, the hematologist on duty at the Blood Transfusion Service will check if the patient's name is included among those enrolled in the study and the date of completion of the 32nd week of PMA; then he/ she will identify ABO-Rh(D) units to transfuse (UCB-RBC if available, otherwise A-RBC). The transfusion dose will be determined regardless of the type of blood component being used and will be established according to the criteria generally implemented in the NICU. The UCB-

RBC and A-RBC units will be transfused within six hours in order to reduce functional alterations of red cells and the risk of microbial contamination.

5.6 Monitoring of transfusion need. All the transfusions carried out in all the patients before the 36th week of PMA will be recorded. Before and after every transfusion, HbF, HbA and capillary arterial-blood gas will also be tested. Blood samples for CBC and/or reticulocytes will only be taken if clinically necessary and not for study-related reasons.

5.7 Monitoring of HbF and HbA levels. Three capillary blood samples will be taken every week for dosing HbF and HbA. If other tests for routine assistance, or for blood units cross-match, are carried out, part of the blood sample will be recovered for dosing HbF and HbA. The dosage will be carried out on samples that were stored at the Hematology Service of the Foundation, through HPLC on lysate samples. This procedure needs very low sample volumes (50-100 µL).

5.8 Definition of the prematurity-related pathologies (ROP, BPD, NEC and IVH). ROP (grade I-IV) will be diagnosed based on the criteria of the "International Committee for the Classification of Retinopathy of Premature" [49]; NEC will be diagnosed based on the criteria of Bell [50]; BPD is diagnosed when an infant continues to need additional oxygen after 28 days of age, at the 36th week of PMA [51]; the diagnosis of IVH will be based on the criteria of Papile [52].

5.9 Recording of clinical data and laboratory parameters. For each patient, the following parameters will be recorded on specific case report forms*:* GA and birth weight, sex, ethnicity, twin birth, PMA, respiratory support, additional oxygen, length of the hospitalization in the NICU, number of days under antibiotic therapy and PMA when leaving the hospital. All the causes of mortality and morbidity occurred within the 36th week of PMA will be recorded (infectious episodes, hemorrhagic events, kidney failure, thromboembolism, surgical complications, etc.). All the possible adverse events occurred within that date would also be recorded (for example, the unexpected adverse events, including all the alterations of the laboratory parameters, regardless of their seriousness). All the hematological (Hb, HbF% and HbA%, Hct) and transfusion data (date, number of transfusions, type of transfusion, volumes of transfused RBC) will also be recorded. For all those patients who were discharged before the completion of the 36th week of PMA, the clinical evaluation, the determination of HbF on capillary blood and the collection of data will be carried out at ambulatory level.

6. STATISTICAL CONSIDERATIONS

6.1 Definition of analysis sets

- **Enrolled (E) set** or **Intent-to-treat (ITT) analysis.** It will comprise all the enrolled patients, regardless of the administration or not of RBC transfusions.
- **Treated (T) set.** This group will comprise all the patients who were at least administered one transfusion, regardless of the completion of the observation period (36th week of PMA). Excluded from this set are the enrolled patients who were not transfused.
- **Per-Protocol (PP) set.** This group will comprise all the transfused patients who completed the observation period (36th week of PMA) or who died after completing it.

6.2 Calculation of sample size. The main goal of the study is to correlate the average HbF levels to the type of transfusion (UCB-RBC or A-RBC). With a population of 25 patients that will be studied during the following 8 weeks and a mortality rate of 1.9 patients/month, we expect to have 196 weekly doses of HbF to correlate to 98 transfusions. The total expected number of transfusion from birth to the completion of the 36th week of PMA was calculated to be around four transfusions per patient, with an UCB-RBC:A-RBC ratio of 1:1. In fact, for those patients with birth-determined characteristics similar to those of the studied population, the average number of transfusions within the 36th week of PMA is around 4 transfusions/patient [25], while in our previous study the average number of transfusions was 3 ± 1.7 [46]. In accordance with the literary data, preterm infants have an expected HbF concentration between 85% and 90% at birth and between 70% and 75% at the 36th week of PMA [54]. We expect that for the newborns who will not be transfused the HbF levels will stay in this range (70%-90%), while for the patients who will be transfused with adult red cells, HbA will dilute HbF. Even though there are no literary data yet, if we know the volume of transfused red cells (20 ml/kg) and the patient's blood volume, we can also assume that, for every adult red cell transfusion, HbF will be reduced by 15-20% compared to the pre-transfusion values until reaching levels lower than 20% among those patients who were transfused more than 4 times with adult blood.

6.3 Statistical data analysis. The continuous variables will be expressed as mean (± SD) or as median (interquartile ranges, IQR); all deviations from normality will be detected with the Shapiro-Wilk test. The categorical variables will be expressed as n, (%, percentage). HbF levels in different type of transfusions will be compared in univariate analysis Mann-Whitney U test, end Kruskall Wallis test). Similarly, the transfusion efficacy analysis will be carried out based on a UCB-RBC and A-RBC non-inferiority study design by comparing the average time interval between two subsequent transfusions and the average post-transfusion capillary Ht

increase (ΔHt) (Mann-Whitney U Test). The transfusion sample included in the study makes it possible to detect a difference between groups that is equal to at least 1 standard deviation with type I error α =0.05 and type II error β = 0.2 and a medium effect size (0.6). The correlation between the type of transfusion and other variables (prematurity related pathologies) will be tested using Fisher's exact test. Secondarily, in order to evaluate the effect of HbF reduction on the onset of prematurity-related pathologies, the levels of HbF levels will be inserted in a multiple linear regression model, by expressing the correlation between the onset of prematurity-related pathologies and the variables in terms of odds ratios (ORs ± 90% confidence interval).

6.4 The primary outcome analysis will be tested on "E", "T" and "PP" sets. Transfusion efficacy outcome will be tested on "T" and "PP" sets.

7. DIRECT ACCESS TO DOCUMENTS AND DATA MANAGEMENT

The informed consents of both donor couples and enrolled patients' parents, as well as the data on the dosages on biological samples, will be available to the Ethical Committee. Nonetheless, the identity of the enrolled patients will be kept confidential and only the PI and his/her collaborators may have access to it.

8. ETHICAL ASPECTS

The study will be carried out according to the principles of Good Clinical Practice and the Declaration of Helsinki (Annex VI).

9. AUTHORIZATION OF THE ETHICAL COMMITTEE

Before beginning the study, the experimental design, consents and the related information material will be submitted to the Ethical Committee to approve it. Only after the Committee's approval will the study begin.

10. STUDY-RELATED RISKS AND BENEFITS

Due to the type of the patients, the volume of blood necessary to carry out the expected tests is a critical element. For this reason, the study was designed not to entail any more blood samples than those already taken to assist the patient. The HbF will be determined by using the capillary blood residue of other routine tests. As for the UCB-RBC transfusion, the immune

and infection risks are lower than A-RBC transfusions. Screening tests, adult and cord red cells production and storage techniques are comparable; the units of cord blood, unlike those of adult blood, are only released after checking the negativity of the microbial cultures. This procedure is due to the methods of cord blood collection and, if it guarantees sterility on the one hand, on the other, it prevents it from being transfused before five days have passed (i.e. the days necessary to detect any microbial contamination). This means that fetal blood transfusions could be less fresh than adult blood transfusions, even though a recent randomized study has not proven any remarkable differences in the safety and efficacy of the transfusions that were conserved for ± 7 days [25]. Among the expected benefits of UCB-RBC transfusions is a lower risk of developing prematurity-related pathologies and the certainty that donors are CMV-negative.

This study could have a strong impact on the National Italian Health Service. In fact, it is only the first of many studies to test if the prevalence and seriousness of the prematurity-related pathologies can be reduced by applying an innovative transfusion approach, the aim of which is that of maintaining HbF at physiological levels during various weeks after birth. As far as we know, this approach has never been studied. It is also important to underline that the strategy of transfusing cord blood also has an economic impact since it is based on unused existing economic resources. Nowadays, Italian public Cord Blood Banks collect a very high number of CB units that cannot be used for transplants: in 2016, 14,661 CB units were collected but only 983 (6.7%) of them were listed for transplants.

The results of this study could be the assumption of many other randomized studies that aim to determine the negative impact of blood components on preterm infants and especially to establish if it can be a consequence of HbF reduction.

11. ADVERSE EVENTS

This single institution prospective study on the correlation between HbF and UCB-RBC or A-RBC transfusions is the first of its kind and all the adverse events occurring within the observation period will have to be carefully recorded. The researchers shall classify the adverse events (any signs or symptoms or diseases, including altered, unexpected or negative laboratory tests that are temporally related with the transfusion, regardless of their possible relation or not) as for:

- Seriousness (death, life-threatening, etc.);
- Intensity (severe, moderate, mild);

- Possible relation with the transfusion (not related, possibly correlated, probably related, definitely related);
- Outcome (resolved, resolved with sequelae, ongoing/continuing treatment, death).

12. SENSITIVE DATA AND BIOLOGICAL SAMPLES COLLECTION AND STORAGE

Data storage. The study PI will be in charge of storing an accurate recording of the transfusion procedures and clinical and laboratory data of the enrolled patients, as well as keeping the consents signed by the parents/tutors. Clinical data will be stored in electronic format for the following 10 years after the study termination. They will also have to include the recording of any adverse events that should occur during the study. There will also be specific consents for the treatment of personal data (Annex I bis).

Biological sample storage. In accordance with the Ethical Committee recommendations and pursuant to the authorization of the Italian Data Protection Authority to process generic data (February 22, 2007), samples will be identified anonymously and by using a numerical code. Only the physicians who are part of the study can trace the sample identity and the patient's clinical data through the number. The study PI will be in charge of storing the biological samples in the Cell Processing Laboratory of the Blood Transfusion Service. All the collected samples will only be used for the purposes of the study.

13. COMMITTEE IN CHARGE OF THE STUDY

The Committee in charge of the study will be composed of the study proposer, 3 neonatologists (1 coordinator and 2 supervisors), 3 hematologists (1 coordinator and 2 supervisors) and 3 members not directly involved in the study. This Committee will be in charge of monitoring the general aspects of the study and its possible reviews other than supervising and collecting data. The Committee will also be in charge of recording the clinical events that determine the end-points as well as the adverse events. It also has the power to make the necessary changes for potential alterations and corrections. Finally, any abstracts or manuscripts to submit to conferences also need the Committee's approval before they are published. The Committee will meet every three months, from the enrollment of the first patient to the study termination.

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INFORMED CONSENT FOR PARENTS

Neonatal intensive Care Unit

CORRELATION BETWEEN FETAL HEMOGLOBIN LEVELS

AND TRANSFUSION OF RED CELLS FROM CORD BLOOD

Version 1, February 10 2018

INFORMATIVE SHEET

Dear Parent/Guardian/Legal Representative,In this University Hospital a medical-scientific research program, entitled "Correlation between fetal hemoglobin levels and transfusion of red blood cells from cord blood." is scheduled. This research takes place exclusively in this institution.To carry out this research, we need your collaboration, availability, and that of children who, like your child /protégé/person represented, meet the eligibility criteria for the evaluation that will be performed. However, before you accept or refuse to let your child/your protégé/person represented participate, please read carefully this document taking all the necessary time and ask us for information, especially if you do not understand or need to further clarifications. In addition, if you want, before deciding, you can ask your doctor for an opinion.

WHAT IS THE AIM OF THE STUDY

The aim of the study is to monitor the levels of fetal hemoglobin in relation to the transfusion support and to evaluate whether the reduction of fetal hemoglobin correlates with the development of the main pathologies of prematurity associated with oxidative stress.

Preterm neonates often need red blood cell transfusion within the first weeks of life and this may be responsible for a progressive reduction of fetal hemoglobin (which is present in the neonates in the first weeks of life) and an increase of adult hemoglobin (which is typical of the adult and, in this case, due to red blood cell transfusions). Since, in our Hospital, red blood cell from cord blood are available, this study will evaluate whether this type of transfusion strategy produces greater persistence of elevated fetal hemoglobin levels in the first weeks of life and, and whether it can be associated with the reduction of incidence of diseases related to prematurity.

If your child/protégé/person represented needs transfusions, he/she may receive red blood cells from cord blood or adult donor blood, depending on the availability of compatible cord blood units at the Blood Transfusion Service. There are no differences between these two type of products (cord and adult) in terms of preparations, screening test, validation and therapeutic efficacy. However, we expect that red cells from cord blood lead to a lower reduction in fetal hemoglobin levels.

During hospitalization, starting from the first transfusion event, the level of fetal hemoglobin will be monitored in your child's blood; the samples used will be those collected to perform other routine test

necessary for his/her assistance; if the samples are not available or usable, a capillary blood sampling will be performed.

WHAT THE PARTICIPATION OF HIS CHILD/HIS PROTECTED MEANS

If you decide to have your child/protégé/person represented participate in the study, the experimental design of this research requires that he/she will undergo the monitoring of the levels of fetal hemoglobin. Your child may receive red blood cells from cord blood (which contains fetal hemoglobin) or from adult donor blood (which contains adult hemoglobin), depending on availability of compatible cord blood units at the Blood Transfusion Service. These units are obtained from solidarity allogeneic donations of cord blood for hematopoietic stem cell transplantation, in accordance with the same criteria adopted in terms of donor suitability, preparation of the blood component, screening test, validation of the blood unit and therapeutic efficacy.The study will have an overall duration of one year. In these months, in all preterm infants who have specific characteristics at birth and whose parents have given their consent to participate in the study, determinations of the fetal hemoglobin levels will be performed up to the 36th week of post-menstrual age. Participation does not imply any additional costs for you and your child. Our institution will cover all the costs.

WHAT ARE THE RISKS FROM PARTICIPATION IN THIS STUDY

The participation of your child/protégé/person represented in the study does not involve any additional risk to what currently associated with transfusion therapy. Screening tests, storage and preparation of adult and cord blood are superimposable; cord blood units are subjected to some additional tests such as blood culture for microbial contamination.

WHAT ARE THE BENEFITS THAT YOUR CHILD/PROTÉGÉ/PERSON REPRESENTED CAN RECEIVE FROM PARTICIPATION IN THIS STUDY

Participation in this study will have the benefit of being able to contribute to the progress of neonatological medical knowledge in terms of understanding the mechanisms that underlie the complications of preterm birth and how to prevent them. Among the expected benefits, the transfusion of red blood cells from cord blood could reduce risk of developing diseases related to prematurity.

INVESTIGATIONS TO WHICH YOUR CHILD/PROTÉGÉ/PERSON REPRESENTED WILL BE SUBJECTED DURING THE STUDY

During hospitalization, from the moment your child/protégé/person represented needs transfusion support, and for each transfusion event, the levels of fetal and adult hemoglobin will be monitored in his/her blood. The samples used will be those collected to perform other tests required by normal assistance to the newborn. Only in the event that the samples are not available or are insufficient, a capillary blood sampling will be performed with a maximum of three determinations per week. All data (laboratory tests, blood and biochemical parameters and clinical events) concerning your child/your protégé/ person represented will be recorded.

WHAT HAPPENS IF YOU DECIDE NOT TO PARTICIPATE

You are free not to let your child/protégé/person represent participate in the study. In this case, however, he/she will receive all the standard assistance without any penalty.

WHAT HAPPENS IN THE EVENT OF DAMAGE

We inform you that this study is insured with the following company: XXXX, Policy number XXXXXXXXX, with effect from 00.00 AM on 26.11.2018. The policy is effective only for damages that occurred no later than 120 months from the end of the trial and for which a claim for compensation was made within 120 months of the end of the same.

INTERRUPTION OF THE STUDY

Your participation in this study is voluntary and you can withdraw your consent at any time.

INFORMATION ABOUT THE RESULTS OF THE STUDY

If you request it, at the end of the study, the general results of the study and in particular, those concerning your child/protégé/representative will be communicated to you.

ADDITIONAL INFORMATION

For additional information and communications during the study, the following staff will be available: XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX, Neonatal Intensive Care Unit and XXXXXXXXXXXXXXXXX, UNICATT Cord Blood Bank.

The study protocol, which has been proposed to you, has been drawn up in accordance with the European Standards of Good Clinical Practice, the current revision of the Helsinki Declaration and the Ethic Committee of this institution has approved it.

You can report any fact that you deem appropriate to highlight, in relation to the experimentation that concerns your child/protégé/person represented to the Ethic Committee of this institution.

CONSENT STATEMENT[1]

I, the undersigned: _____ (father)

I, the undersigned _____ (mother)

I declare that I received from _____
_____, MD

Exhaustive explanations regarding the request to participate in this study, as reported in the information sheet here attached, a copy of which has been delivered to me before.

[1]This declaration of consent must be signed and dated personally by the parent/guardian/legal representative of the subject and by the person who conducted the discussion on informed consent

I also declare that I was able to discuss these explanations, to have asked all the questions that I deemed necessary and to have received satisfactory answers, as well as to have had the opportunity to inform myself about the details of the study with a person I trust.

I therefore freely accept to let my child/protégé/representative participate in the study, having been able to fully understand the meaning of the request and having understood the risks and benefits that are involved.

I was also informed of my right to have free access to the documentation relating to the (clinical-scientific) experimentation and to the evaluation by the Ethic Committee.

_____	_____
Date	Signature of the physician who informed the parents
_____	_____
Date	Signature of the father
_____	_____
Date	Signature of the mother