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Sterility of cord blood units: Evaluation of the collection, preparation and conservation process with a view to establishing a bank of stem cells from cord blood at the Biological Resources Center of the Institut Pasteur de Côte d'Ivoire (2024)

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Abstract

Introduction: Cord blood stem cells are used as an alternative to bone marrow and peripheral blood cell transplantation. For this purpose, cord blood banks have been established worldwide for the collection and cryopreservation of cord blood units. These banks must implement rigorous protocols to ensure the microbiological safety of grafts. The aim of this study is to evaluate the process of selecting mothers and collecting and processing these samples developed for this purpose.

Methodology: Pregnant women were enrolled in the study on the basis of selection criteria. Maternal blood and cord blood samples were collected by the in utero method. Serological analyses of maternal blood for the detection of vertically transmitted infections and microbiological examinations of cord blood units were carried out.

Results: 31 women out of 46 (68.4%) were enrolled. All had negative serology for the targeted pathogens. Similarly, microbiology revealed no microbial contamination of the cord blood units.

Conclusion: This work demonstrates that the procedures for selecting mothers and processing implementations are effective in ensuring the microbiological safety of cord blood units and could be suggested in the context of banking cord blood stem cells at the Institut Pasteur de Côte d'Ivoire.

Keywords: Stem cells; Cord blood units; Cord blood stem cell bank; Microbiological safety; Institut Pasteur de Côte d'Ivoire

1. Introduction

Cell therapy, also called stem cell transplantation, is by definition the administration of living cells to patients to replace or repair damaged or dysfunctional organs or tissues [1]. It is mainly used in three types of treatments: In immunotherapy, in regenerative medicine where it is applied to pathologies involving cell destruction and degenerative diseases, and in hematopoietic supportive care in oncology [2]. Given their advantages, compared to bone marrow and peripheral blood [3], cord blood stem cells are used as an alternative to bone marrow and peripheral blood stem cell transplants. Thus, since the success of the first cord blood stem cell transplants in children with acute lymphoblastic

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leukemia [4]Fanconi anemia [5], these are used in research and clinically for the treatment of children and adults with a wide range of malignant and benign hematological and non-hematological pathologies. For this purpose, cord blood banks have been established worldwide for the collection and cryopreservation of umbilical cord blood stem cells called Cord Blood Units (CBUs), for allogeneic hematopoietic stem cell transplantation (HSCT), and more than 730,000 units have been stored [6].

Observations in recent years have shown that CBU transplantations may be the source of bacterial, viral, parasitic or fungal infections transmitted from the donor to the graft recipient [7], [8], [9], [10]. Therefore, the microbiological safety of CBUs is of primary importance for the graft effect.

Regulatory texts require donor selection based on history, clinical status and biological tests. These biological tests aim to detect markers of targeted infectious agents. The international standard Netcord FACT in its annex IV [11]defines the minimum criteria for infectious disease screening tests for cord blood and maternal blood samples, covering HIV1 and 2, hepatitis B and C, HTLV I and II, syphilis and CMV. The conditions of collection, preparation and storage of USC are also essential elements for microbiological safety. Indeed, contamination can come from various sources, including the environment, collection and processing procedures, as well as human handling [12], [13]. Previous studies have reported contamination rates ranging from 0.5% to 12% [14], [15], [16].

Therefore, to minimize these risks, it is essential to implement rigorous donor selection, disinfection and asepsis protocols at each stage of the process, from collection to cryopreservation, including handling and storage. In this context, the Institut Pasteur de Côte d'Ivoire (IPCI) has initiated a feasibility study to establish a cord blood stem cell bank and investigations have previously shown the acceptability of cord blood donation and banking for transplantation and research [17]. The present study aims to evaluate the maternal selection processes, as well as the CBU collection and preparation methods adopted in order to ensure the microbiological safety of CBU.

2. Material and methods

2.1. Type, framework and period of the study

A prospective descriptive study over a period of four months, from January to April 2024, was carried out. The study took place in the obstetrics departments of the Urban Community Health Training of Anonkoi Kouté in the commune of Abobo (Abidjan, Côte d'Ivoire) for the recruitment of mothers and the collection of cord blood. Health analyses on the collected cord blood samples were carried out at the IPCI in the departments of bacteriology-virology and parasitology-mycology.

2.2. Sterility conditions of the collection, treatment and storage process

Before the start of activities, sterility conditions were put in place at the different stages of the process, from collection to conservation of CBU:

2.2.1. Sterility condition for the collection of cord blood units

Collection kits were made available in the delivery room. These kits consisted of gloves, gowns, sterile compresses, a sterile field, a sterile blood bag containing Citrate Phosphate Dextrose (CPD), scissors and sterile forceps.

2.2.2. Sterility conditions for the processing and storage of CBUs

All laboratory work was carried out under a Type II Class II microbiological safety cabinet, with strict sterility conditions. Disinfection protocols including hand washing and disinfection with alcoholic solutions, wearing of personal protective equipment (gloves, masks, sterile gowns), use of sterile single-use instruments when possible were rigorously followed.

2.3. Selection of donors (mothers/newborns)

The selection focused on pregnant women at term, aged 18 and over, who came for delivery and who gave their written consent for the study and who met the criteria set out below:

2.3.1. Personal and family history

The selection of mothers was based on the study of their prenatal consultation record and on the clinical examination in the delivery room. The selection criteria were as follows: (i) absence of family history of known hereditary diseases, (ii) history of negative serology for HIV1/2, hepatitis B and C, toxoplasmosis, syphilis.

2.3.2. Obstetric criteria and clinical status of mothers and newborns

- Gestational age (GA) ≥34 weeks of amenorrhea (WA),
- singleton pregnancy,
- Premature rupture of membranes <12h,
- Absence of maternal fever intrapartum,
- Newborn with an APGAR score (Appearance (color), Pulse, Grimace (on excitement), Activity (tone), Respiration) ≥7 at 1min,
- Absence of malformation of the newborn,
- Absence of complication occurring during delivery.

2.3.3. Criteria related to CBUs

CBUs meeting the following criteria were included in the study

- Time between CBUs collection and treatment < 48h,
- Non-hemolyzed CBUs,
- CBU volume > 40 ml.

2.4. Collection, processing and conservation of CBUs

2.4.1. Collection of cord blood samples

The samples were collected by the referring midwife, during the third stage of labor before delivery of the placenta (in utero method). The umbilical cord was clamped at two different locations (5 cm and 7 cm from the newborn) and cut between the forceps, within seconds of delivery. Cord blood was collected from the umbilical vein connected to the placenta using a 16-gauge needle, by gravity until delivery of the placenta. The collection was carried out in standard blood collection bags containing 63 ml of anticoagulant, namely citrate phosphate dextrose (CPD), previously reduced to a volume of 23 ml. During collection, the blood bag was gently shaken by hand, so that the anticoagulant mixed with the CBUs. After collection, the blood bag tubing was sealed and the collection needle removed. A 2 ml maternal blood sample was also collected after delivery for serological analysis. Subsequently, the samples were stored in a refrigerator at 4°C in the obstetrics department and then transported in triple packaging to the IPCI within 48 hours of collection, in a cooler refrigerated at 4°C to maintain the cold chain.

2.4.2. Sample processing

Handling of maternal blood samples and CBUs was performed under a type II class II microbiological safety cabinet, previously cleaned with 70% ethanol.

Processing of maternal blood samples

Maternal blood samples were centrifuged and plasma stored at -20°C until serological analyses were performed.

CBU Treatment

The volume of cord blood collected was calculated from the weight of the bag after collection minus the weight of the bag before collection. An aliquot of 0.5 ml of cord blood was taken upon receipt from the septum of the injection port of the CBUs' bag for sterility testing. The cord blood was then transferred to sterile 50 ml Falcon tubes **(Corning, Tewksbury, MA, USA)** and 4% human serum albumin was added to the cord blood in a volume ratio of 1: 5. The final product was centrifuged at 50 g for 5 minutes. The supernatant plasma and Buffy-Coat were transferred to a second sterile 50 ml Falcon tube and a second centrifugation at 400 g for 10 minutes was performed. Excess supernatant plasma was transferred to 5 ml cryovials and sedimented leukocytes were resuspended in the supernatant plasma to a total volume of 20 ml. At this stage, a 0.5 ml aliquot of cell suspension was collected for sterility control. Maternal blood samples were centrifuged and 2 ml plasma stored at -80°C until serological analyses and nucleic acid detection tests are carried out.

2.4.3. Cryopreservation of CBUs

Preparation before freezing.

The preparation of the freezing media was carried out according to the technique described by Boulanger et al. [18]. Under Type II Class II microbiological safety cabinet, a cryoprotectant solution was prepared from 100% Dimethyl sulfoxide (DMSO) in a macromolecular solution of human serum albumin (HSA4%), in a volume ratio of 1:5, to obtain a final concentration of 20% DMSO. An aliquot of 0.5 ml of the solution was taken for sterility control. The cell suspension was then mixed with the cryoprotectant solution to obtain a final concentration of 10% DMSO after volume-to-volume mixing.

Lowering the temperature and storing.

The tube suspensions were cooled in a temperature-controlled descent programmer, the Mini Digicool, according to a protocol using a classic set of cooling rates for the cryopreservation of hematopoietic stem cells: a 10-min equilibration at 4°C, followed by a cooling rate of 2°C min until -30°C, increased to 4°C min. Samples were cooled to -120°C and then transferred to liquid nitrogen (-196°C) for storage.

2.4.4. Thawing of CBUs.

CBUs were removed from the liquid nitrogen tank and immediately thawed by rapid immersion in a 37°C water bath for 2–3 minutes. A 0.5 mL aliquot was taken under Type II Class II microbiological safety cabinet for sterility control after thawing.

2.5. Sample analysis.

2.5.1. Serological analyses of maternal samples.

Serological tests for infectious diseases were performed on maternal blood collected on the day of delivery. These tests were carried out at the Bacterial and Viral Serology Unit of the IPCI using standard commercial tests and operational procedures for HIV 1 and 2, hepatitis B, hepatitis C, and Treponema pallidum (Table 1).

Table 1 Commercial tests for TTI detection used by USBV

Infectious diseases	**Screening tests**	**Principle**	**Antibody**	**Antigens**
Hepatitis B	Monolisa HBs Ag ULTRA Assay, BIO-RAD	ELISA		AgHBs
Hepatitis C	Monolisa ™ Anti-HCV PLUS Version 3, BIO-RAD	ELISA	Capsid, NS3, NS4	
Syphilis	RPR- carbon , SPINREACT	Non-treponemal slide agglutination test	Reagines	
HIV	Vironostika ® HIV Biomérieux uni-form II Plus O	ELISA	HIV-1(gp160) HIV-1 (ANT70, GpO) HIV-2 (gp36)	

2.5.2. Microbiological analyses of CBU.

Evaluation of sterility of cord blood units.

CBU samples were evaluated at several steps of the process: (i) within 24 to 48 h after collection, on cord whole blood samples, (ii) after preparation and before cryopreservation, on buffy coat suspensions, (iii) after 1 month of cryopreservation, on thawed buffy coat suspension. For each step, 0.5 ml of samples were subjected to an enrichment step in 5 ml of Brain Heart Infusion Broth (BHIB) in hemolysis tubes, and incubated at 35-37°C for 24 h. The inoculated broth cultures were subcultured in Petri dishes on fresh blood agar (**Trypticase soy agar enriched with sheep blood**) and on Müller Hinton agar for the detection of contaminating bacteria, and on Sabouraud agar for the detection of fungal contamination, using standard methods, then incubated at 35-37°C for a maximum of 5 days in an aerobic environment.

Internal quality controls.

Quality controls of BHIB, cryopreservation media (DMSO20%+HSA4%) and non-inoculated culture media were performed to ensure the absence of cross-contamination and the reliability of the results. All petri dishes were examined for colonies after 24 hours of incubation until the 5th day. The petri dishes containing Sabouraud agar were sent to the mycology unit of the IPCI for analysis.

2.6. Data analysis

Data were entered using Excel software and analyzed using XLSTAT software **(Addinsoft , Paris, France).** Results were expressed as percentages and frequencies for categorical variables, and as mean, maximum, minimum, and standard deviation for quantitative variables. The results of the mothers' selection criteria were reviewed to identify mothers to be included in the study. The results of serological tests and microbial cultures were reviewed to determine the absence of viral infections and microbial contamination at different stages of the process.

3. Results

3.1. Impact of donor (mother/newborn) and CBU selection criteria on CBU collection and storage.

During the study period, out of 46 potential donors, 15 (32.6%) donors were rejected. Among them, 11 (73.33%) were rejected before collection and 4 (26.67%) after collection of CBUs. The reasons for the non-inclusion of the 11 women are reported in Table 2.

Table 2 Causes of ineligibility/exclusion of USC donors and USC collections before and after collection

	Effective	**Frequency (%)**
Exclusion before sampling (N=11) 73.33%		
Maternal history (n=1)		
Maternal serological history	1	6.67
Obstetric criteria and clinical status of mothers and newborns (n=8)		
Intrapartum maternal fever	2	13.33
Water rupture ≥12 hours	2	13.33
Immediate placental abruption	2	13.33
Stillborn	1	6.67
Apgar <5 at 1 minute	1	6.67
Unsuccessful collection	2	13.33
Exclusion after sampling (N=4) 26.67		
Volume < 40 ml	2	13.33
Hemolysis	2	13.33
Total	**15**	**100**

3.2. Characteristics of the mothers included, and obstetric characteristics.

The mean age of the women included was between 27 and 28 years (standard deviation of 6 years), with an age range of 18 to 39 years. The mean gestational age was 39 weeks with a range of 37 to 44 weeks. The majority of the participants (64.51%) were multiparous, while 35.49% had never given birth. They were all in good apparent health and had no signs of infection after delivery. Among them, 2 (6.45%) had premature rupture of membranes, the duration of which did not exceed 12 hours.

3.3. Characteristics of newborns

The newborns were also in good apparent health with a mean APGAR score between 7 and 9 at 1 minute after birth.

3.3.1. Characteristics of CBU samples.

The mean time between delivery and collection was between 3 and 4 minutes. The time before processing of samples was between 14 h and 15 h on average with an interval ranging from 4 h to 30 h. The maximum time in our study between collection and processing of CBUs was 48 h after collection.

3.3.2. Sterility control.

Serological and molecular tests for the detection of markers of infectious agents were all negative for the 31 women included.

After collection and preparation of CBUs, each sample was inoculated into culture media and incubated at 37°C for 5 days. The following observations were made:

None of the 31 samples analyzed showed microbial growth in the culture media. Plates used for blood culture did not reveal any bacterial or fungal colonies, indicating the absence of microbial contamination at each stage of the collection, preparation and storage process.

Non-inoculated culture and cryopreservation media, used as controls, did not showed no growth, thus validating the integrity of the media and incubation conditions.

The detailed results are presented in Table 3.

Table 3 Results of USC sterility checks and internal quality controls

	Effective
Analysis of maternal blood samples	
Negative intrapartum maternal serologies (HIV 1, HIV2, Hepatitis B, Hepatitis C, Syphilis)	31 (100%)
Culture on USC.	
USC sterile after collection	31 (100%)
USC sterile after preparation and before freezing	31 (100%)
USC sterile after thawing	31 (100%)
USC sterile after collection	31 (100%)
Internal quality controls	
Culture on BCC	15 (100%)
Culture on agar plates	15 (100%)
Culture on conservation medium (DMSO 20% +HSA 4%)	15 (100%)

4. Discussions

The objective of this study was to evaluate the techniques adopted to ensure the microbiological safety of CBUs.

4.1. Selection criteria for mothers and CBUs

The selection criteria adopted allowed the exclusion of 15 mothers. The mothers included in the study were all healthy and without complications during pregnancy and delivery. This profile of mothers is more likely to give high-quality CBUs. This underlines the importance of a complete evaluation of the medical history of the participants.

4.2. Maternal serology

Screening for vertically transmitted infections from mother to child is an essential step in the CBU collection process. The results of this study, with a negative serology rate of 100% for targeted infections, are in compliance with regulatory

and normative requirements. These results also indicate that the established selection criteria for mothers are adequate. Previous studies have shown that early detection of mothers carrying viral infections is necessary to minimize the risks of transmission (19), [20].

4.3. CBU sterility during treatment

At each step of hematopoietic stem cell (HSC) collection and processing, there is a risk of product contamination. Contaminated HSCs can lead to infections when used in stem cell transplantation therapies [21]. In this study, no bacterial or fungal contamination was found in the processed and cryopreserved cord blood units. This result indicates good application of sterilization and asepsis protocols during collection and handling throughout the banking process.

4.4. Limitations of the study

Despite the promising results obtained in this study, some limitations must be taken into account for a critical interpretation of the data and their applicability on a broader scale.

4.4.1. Sample size and study population

The sample size (31 CBUs) might be too small to generalize the results to all CBU banks. Therefore, it would be relevant to conduct studies on a larger population in order to strengthen the representativeness of the results.

4.4.2. Infectious disease screening tests

Screening maternal blood for infectious diseases using serological tests may have limitations due to the window period, where an infection may not be detected due to a lack of antibodies. Netcord FACT further recommends that the required tests performed on maternal blood should be repeated on the CBUs if they are to be stored for a long period. Thus, detection of infectious agents directly in cord blood by more sensitive tests should be considered. Therefore, molecular techniques targeting microbial RNA or DNA would improve the ability to detect agents at the pre-seroconversion stage.

5. Conclusion

We conducted a feasibility study for the establishment of a CBUs bank at the IPCI. The objective was to evaluate the operational process of microbiological security of CBUs. The results of the study show that the procedures for selecting mothers, collecting and preparing cord blood units implemented are effective and safe, due to the absence of microbial contamination and negative results of maternal serologies. However, due to the kinetics of antibodies, there is a need to use more adapted methods, such as genomic screening, to ensure optimal safety of CBUs. Future studies involving larger sampling are also necessary to further evaluate these practices and their impact on the safety of CBUs grafts.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the National Committee for Ethics in Life and Health Sciences of Côte d'Ivoire under reference number 234-23/MSHPCMU/CNESVS-km. A study authorization was also obtained from the head of the selected center.

Statement of informed consent

Written consent was obtained from the women after being informed of the purpose of the study. They were also informed that their participation was completely voluntary and that they could withdraw from the study at any time without affecting their subsequent medical follow-up or that of their newborn. Data confidentiality and anonymity of the women were ensured by indirect identification using a subject code for research purposes.

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