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Decontamination of Two Umbilical Cord Blood Grafts Prior to Autologous Administration

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Abstract

Umbilical cord blood (UCB) is proving to be a valuable resource of stem cells and currently is being used for a variety of oncological and hematological pathologies and metabolic disorders; in addition, new clinical trials are showing promising results in certain neurological, autoimmune and developmental disorders. More recently the Food and Drug Administration – FDA - has granted approval for the clinical use of cellular therapies with UCB-based products and new therapeutic utilizations are being studied for regenerative medicine; all these developments will increase the utilization of “off-the-shelf” UCB units.

As a drawback, contamination of UCB grafts is a significant occurrence (upwards of 5% in most analyses), and even though it consists mainly of non-pathogenic bacteria it can raise serious questions regarding intravenous UCB administration, especially in patients who are not receiving coincidental antibiotic coverage.

Here we report the successful decontamination of two UCB grafts prior to administration without compromising the viability of the stem cells administered, and propose to apply the same principle and procedure to any contaminated graft.

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Introduction

Since the first successful administration of stem cells from UCB in 1988[1][2], banking of cord blood in public and family stem cell banks, together with bone marrow donor registries, has greatly attenuated the difficulty of finding stem cell donors for transplantation so that more than 40.000 UCB grafts were administered since [3]. Being harvested much easier than bone marrow and having better graft-versus leukemia and graft-versus-host characteristics, UCB has also shown some important limitations in transplant procedures: a smaller number of total nucleated cells (TNCs), stem cells and delayed engraftment, which often prompts the use of two or more UCB grafts; and also microbiological contamination of the graft.

The UCB advantage is likely due to the presence of young pluripotent stem cells which have not been exposed to post-natal environmental and infectious factors suspected to induce modification of the immune system, and the relative T cell immaturity from UCB can translate into better immunologic tolerance and modulation of existing T cells in the recipient [4]. This is in line with the less stringent matching requirements for UCB donors and the observed better graft-versus-host (GVHD) properties for UCB and the graft-versus-leukemia effect observed in children [5][6][7]. UCB has T cells with lower cytotoxic activity and higher activation-induced apoptosis compared to T cells from peripheral blood [8] and the different cytokine profiles (ex. IL-7, IL-15, IL-12, etc.) are the probable factors influencing the activity and fate of T cells from administered UCB grafts.

UCB is also successfully used for treating metabolic disorders (Hurler and Sanfilippo syndromes, Krabbe disease) in early childhood with 75% being disease-free at 5 years post-transplant [9]; and outside the field of transplantation was successfully administered for neurological disorders (cerebral palsy, neuron motor disease) and developmental disorders – autistic spectrum disorders (ASD), developments which will most likely be followed by increased UCB utilization.

The usefulness of a UCB graft for transplantation is typically judged by its ability to engraft before day 45 posttransplantation and is evaluated before cryopreservation by the generation of colony-forming units (CFUs) at 2 weeks [3]; after decryogenation evaluation with an aldehyde dehydrogenase test was proposed[10]. In order to predict the UCB quality prior to harvesting – verified afterwards by the number of CD34+ and CFUs – it was observed that a younger gestational age (34-37 weeks) was a common positive predictor in some studies while the importance of other factors differed [11][12]. Shorter interval (<10 hrs) to processing, higher birthweight (>3500 g), larger collection volumes (>80 ml) and Caucasian race correlated with better UCB grafts [11]; while cesarean delivery and male babies had better predictive value than the UCB volume in another study [12]. In this context, it's interesting to mention that male babies are also more prone to ASD.

A larger UCB collection volume was also associated with a lower rate of contamination in more than 10.000 UCB samples harvested between 2010 and 2020 in a German bank, with 12% of UCBs of less than 60 mL and 6% of UCBs with volumes of more than 60 mL were contaminated, yielding an overall contamination rate of 8% and a correlation coefficient r = -0.9 between UCB volume and the presence of contamination [13]. The same study showed that cesarean deliveries had a much lower contamination rate (1.4%) than vaginal deliveries (9.7%). Also shown was a difference between contaminating bacteria, so that Bacteroides species was practically absent in C-section deliveries; Propionibacterium acnes (40%) being much more frequent in C-section than vaginal, and the rest of the bacteria being higher in percentage terms in vaginal: Staphylococcus (35%), Bacteroides (23%), Enterococcus (22%) and Escherichia (14%) vs cesarean section – where only Staphylococcus (34%) was comparable [13].

The contamination rates of UCB vary substantially among stem cell banks (between 1-15%) – however, a common characteristic is that its rate decreased from the 1990s to 2010, most likely due to better practices and standards; this fact alongside the above data (ex. difference between cesarean vs vaginal deliveries) allow us to infer that most instances of contamination of the UCB grafts occur during collection of cord blood at birth and less during the cryopreservation procedures [14][15][16][17][18]. Another aspect is the detection of contamination, which is influenced by the testing methods used (ex: 10 ml anaerobic culture bottles have more positive results than 1 ml bottles, plasma vs culture medium, needle gauge, etc [16][19][20][21][22][23]. Most contaminations involve bacteria normally populating the skin or mucosa Stafilcococcus, Peptostreptococcus, Streptococcus anginosus, Propionibacerium, etc) which are not normally pathogenic [16][17][19][24] but also Bacteroides, Pseudomonas, Actinobacter, Clostridium, Bacillus which can be more problematic {m/18, 24/}.

Materials and Method

Informed consent was obtained from the parents prior to the preparation and administration of UCB grafts, which was done as part of the CORDUS clinical trial, registered on clinicaltrials.gov with NCT04007224 and approved by The National Committee on Bioethics IS/4/12.02.2020.

An important aspect of decontamination is the killing of bacteria without affecting the viability of stem cells, and we have obtained that by using antibiogram-based antibiotic treatment of the UCB graft and verified it by testing for sterility of decontaminated graft via Bactalert cultures and the viability of stem cells from UCB post-decontamination with Trypan Blue staining and flowcytometry testing with 7-amino-actinomycin D (7-AAD).
We based our approach on well-established facts: i) antibiotics act selectively on bacterial protein synthesis (16, 30 and/or 50 S ribosomal sub-units) inhibiting bacterial wall formation and do not alter mammalian or human cells, which lack such organelles [25][26][27][28] ii) it is current practice in laboratories to protect human cell cultures against bacterial contamination by adding a mixture of penicillin and streptomycin (P&S), which was shown to be much better tolerated than amphotericin B or gentamycin for exposures of 24 hours and more [29][30][31].

We have added a very small amount of antibiotic to the UCB graft after decryogenation and the addition of dextrane

and albumin according to the Rubinstein protocol [32]. The antibiotic dose was calculated such that the concentration achieved in the bag was up to 10 times the minimal inhibitory concentration (MIC) which kills 100% of the respective bacterial isolates (MIC100) as per antibiogram and literature and confirmed by clinical practice.

Adding the antibiotic early after decryogenation allowed it to act on the contaminating bacteria before the graft was administered, so that in most likelihood they were killed before infusion (confirmed by bacterial cultures); at the same time, the relatively short exposure time - between 1-2 hours – at relatively high antibiotic concentration is having minimal impact on the stem cells (confirmed by viability tests).

Viability and cell count were performed on the resulting graft before (Trypan Blue) or after (flow cytometry) UCB graft administration, and bacterial cultures were performed from the UCB graft wash (centrifugation supernatant) with results being read in the Bactalert system 1 week after inoculation. Patients also received the respective antibiotic either orally 3 days before and 3 days after UCB administration, or in one dose of intravenous antibiotic prior to UCB administration, which was preceded by administration of the Duke University premedication according to the respective protocol [33].

Results

Both contaminated UCB grafts (presented below as A and B) were used for autologous administration in two children with ASD and without prior immune suppression; one of the kids was previously treated twice with autologous bone marrow stem cells.

Each case represents a different scenario – one in which the antibiogram was performed on the identified bacteria before cryopreservation, and the other in which the antibiogram was performed after cryogenation, on a portion of the decryogenated graft.

In both cases the cultures performed on the graft after decontamination for the bacteria which were initially reported were negative, and the viability of stem cells in both grafts was very good.

A. First contaminated UCB graft had an initial harvested volume of 113.9 ml which was reduced after treatment with hydroxyethylstarch so that the final graft volume was approximately 25 ml, placed in a dual cryo bag of 20 + 5 ml, with 10% DMSO, 1% dextran. TNC 790 x 10^6 WBC (TNCd corrected for nRBC) 470 x 10^6; CD45 viability 99% and a CD34+ number of 1.1 x 10^6

The cord blood tested negative for Anti-Hbc, Anti-HCV, Anti-HIV 1/2, but prior to cryogenation the bacterial test was positive for Propionibacterium acnes, and the antibiogram performed with three antibiotics showed that it was sensitive to Penicillin G and clindamycin but resistant to metronidazole.

The patient recorded body weight was 32 kg, blood group A II and Rh+, and blood testing prior to administration showed normal values for complete blood count, erythrocyte sedimentation rate, liver and kidney function tests, lactate dehydrogenase, sodium, potassium, serum protein electrophoresis, IL - 6; abnormal values for ferritin 5.2 ng/mL (lower limit normal 13.7 ng/mL), TNF-alpha 10,5 pg/mL (ULN 8.1), serum albumin 53.8% (LLN 55.8%).

The patient tested negative for IgEs to penicillin and was started on clindamycin 300 mg PO q12 h 3 days prior to the procedure, and also 3 days after. Additional HLA-A, HLA-B, and HLA-DRB1 typing of both patient's peripheral blood and a contiguous segment attached to the UCB graft confirmed the identity of the autologous graft.
Clindamycin 0.1 mg was added to the UCB (25 mL) after decryogenation and after adding dextrane and albumin according to the Rubinstein protocol and before the first centrifugation. Ensuing cell viability was good, exceeding 90% for TNCs.

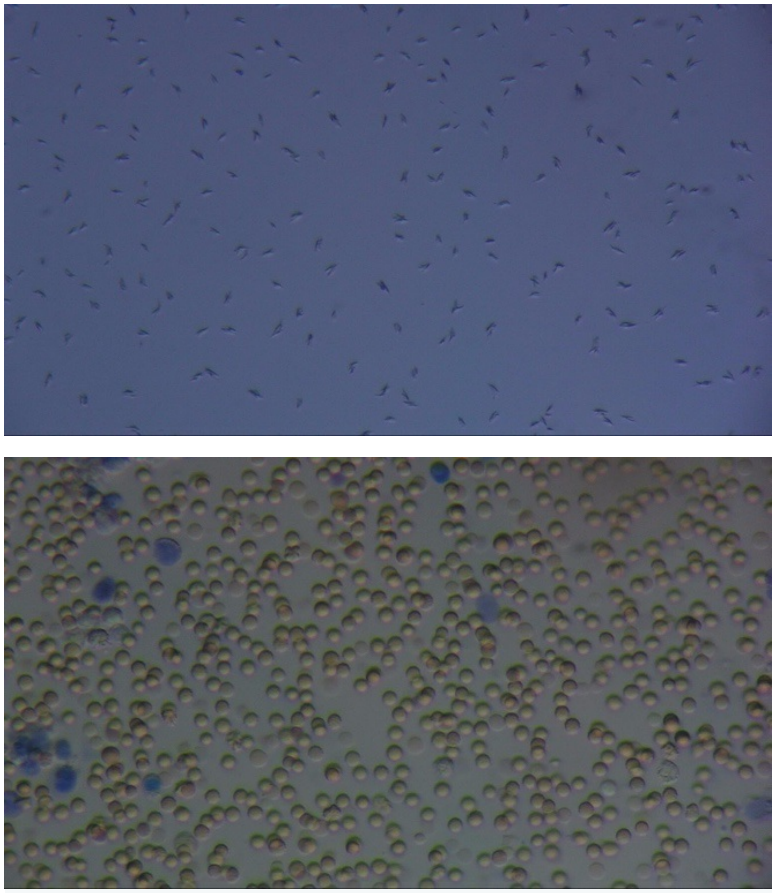

Figure 1. A. Trypan Blue, live RBCs, dead cells (blue) B. Trypan Blue, 200 x Mesenchymal stem cells

Bactalert cultures – both aerobic and anaerobic – were performed from the decryogenated UCB and were negative at 1 week and 10 days.

Overall, there were no adverse reactions observed acutely; patient's TNF-alpha (7,6 pg/mL) IL-6 (1.5 pg/mL) and ferritin (20.1 ng/mL) were normal at 1 month after administration, normal serum albumin 56.6%; and 6 months post-administration a quantifiable degree of progress was reported on the underlying ASD pathology.

B. The initial volume of UCB harvested was 116 ml, with WBCs 73.9 x 10^6/mL, viability 94%
Before cryogenation testing of the harvested UCB showed a positive aerobic culture and Staphylococcus milleri /

Streptococcus anginosus (*Sa*) was identified, but no antibiogram was performed at that time; anaerobic culture was negative.
Literature shows that *Sa* is susceptible to all third-generation parenteral cephalosporins except ceftazidime with 100% sensibility [34]. Minimum inhibitory concentrations (MIC50 and MIC90) for ceftriaxone are established at 0.25 and 0.5 mg/L, and a concentration of ceftriaxone above 1 mg/L ensures that 100% of isolates are killed, which corresponds to 0.001 mg/mL, 0.01 mg/10mL and 0.02 mg/20mL.

The patient presented with blood group B III Rh+ and a body weight of 23 kg; the identity of the autologous graft was confirmed via HLA-A, HLA-B, and HLA-DRB1 typing of both patient's peripheral blood and a cryotube from the UCB graft. Prior to UCB administration patient's blood testing showed normal liver function tests, sodium, potassium, calcium, ESR, ferritin and high hematocrit 41.7% (N <40%), platelets 535 x10^3/mm^3 (N < 450.000), CRP 0.104 mg/dL (N < 0.1) and alpha 2 globulins 13.3% (N < 11.8%); low creatinine 0.29 mg/dL (N > 0.31) and gamma-globulins 9.8% (N > 11.1%).
In this case, we used a different strategy which was possible given the very high number of TNCs (over 1.000 x 10^6); we "sacrificed" the smaller portion (5 mL) of the dual bag in order to perform two tests: most of the volume (4,5 mL) was combined with approximately 5 mL Dextrane 20% + albumin and 0.1 mg Ceftriaxone and the approximately 10 mL of decriogenated, treated cord blood was inoculated in a Bactalert aerobic bottle for 10 days; the remaining small amount (approximately 0,5 mL) was used to perform antibiogram testing.

The antibiogram showed the bacteria to be sensitive to 13 Antibiotics: Ampicillin, Oxacillin, Penicillin, Clindamycin, Daptomycin, Gentamycin, Amoxicillin+clavulanic acid, Ciprofloxacin, Moxifloxacin, Rifampin, Tetracycline, Mupirocin, Imipenem; and resistant to: Azithromycin, Erythromycin.
The Bactalert aerobic bottle inoculated with cord blood + Ceftriaxone 0.1 mg showed no growth after 1 week, so we proceeded with the UCB graft treatment.
On the day of administration, the remaining UCB (20 ml bag) was decryogenated according to the Rubinstein protocol, and after dextrane and albumin we added Ceftriaxone 0.2 mg, which is more than 10 times the concentration of antibiotic which kills all Sa strains (MIC100) [34].
Subsequent TNC for the administered graft was 850 x 10^6, with a viability of 85% on Trypan Blue and about 83% on flowcytometry.
We used 10 ml of supernatant from the decryogenated graft to inoculate another Bactalert aerobic bottle for culture, and the result came negative after 1 week of incubation.

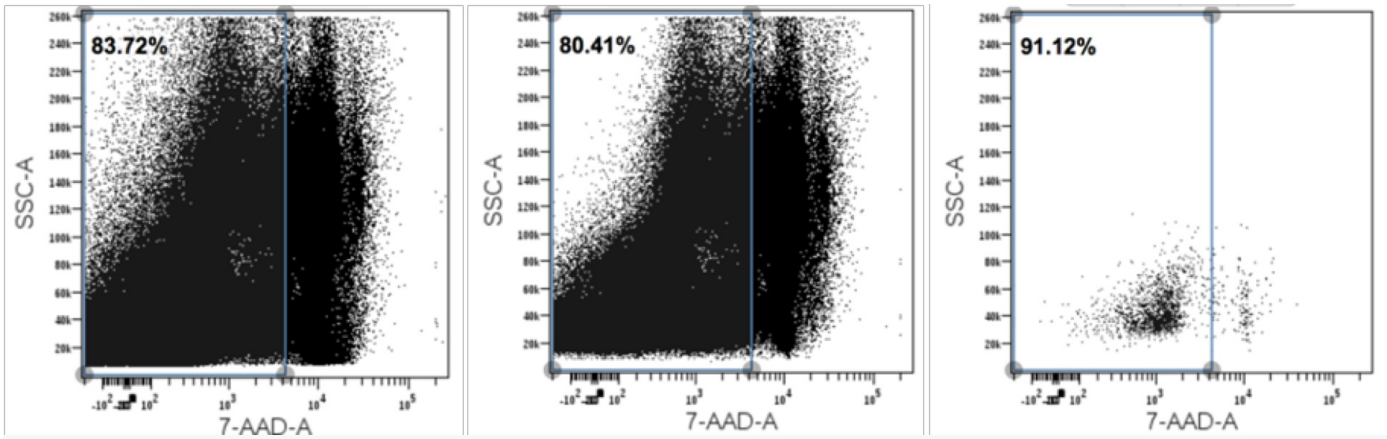

Figure 2. Flow cytometry dot plots depicting cell viability of UCB cells and fraction of viable CD45 + cells

Although the patient had a history of allergies including atopic dermatitis, there was no known antibiotic allergy and after premedication with antihistamine and hydrocortisone, Ceftriaxone 1000 mg was given iv in 100 ml normal saline in 30 minutes prior to administration of the UCB graft. During the administration, the patient had mild facial flushing which resolved spontaneously in a few minutes after occurrence without administering additional medication. For this patient behavioral progress was reported within days of administering the UCB, very early compared to other treated kids and we surmise that it was probably due to the high number of TNCs contained by the respective UCB graft.
Blood tests repeated at 1 year post UCB administration showed normal TNF-alpha (6.4 pg/mL), normal ESR (3 mm/h), normal neopterin (4.9 nmol/L) and improved value for alpha-2 globulin fraction (12.4% vs 13.3% initially) and the child continued to show cognitive and behavioral progress.

Discussion

Utilization of contaminated grafts in immune-suppressed patients was previously reported (considered safe because transplanted patients have wide spectrum antibiotic coverage while immunosuppressed), and while the vast majority of patients survived without serious side effects [35], there is at least one reported fatality following Pseudomonas cepacia inoculation [24], it is probably safer to eliminate the potentially infectious agent prior to intravenous administration while making sure that the viability of stem cells is not compromised. This step becomes more stringent in non-conditioned (non-immune-suppressed) patients treated with UCB for metabolic and neurologic conditions (cerebral palsy, autistic spectrum disease, etc) in whom antibiotic coverage is not administered (patients receiving stem cell treatments, not transplants).

Compared to treating cellular cultures with Penicillin + Streptomycin (P/S) to prevent bacterial contamination (in the absence of contamination), our procedure concerns cell cultures which are already contaminated. Treating the decryogenated UCB with antibiotics which were proven to be non-toxic (ex. excluding amphotericin B or gentamycin), for less than 2 hours, allows for good viability of stem cells while eliminating the contaminating bacteria.

Regarding the possible scenarios, for UCB grafts with low volume (<60 ml) we should employ rapid tests for detecting contamination, such as the ratio of nicotinic acid/nicotinamide [36] or bacterial identification by 16 S sequencing [37], and very importantly before cryogenation an antibiogram should be performed for each contaminated graft, because it gives prompt and important information and spares graft material.

Another possible path for contaminated grafts for which no antibiograms were done prior to cryogenation, in order to preserve as much as possible the cryogenated graft material, we can only perform an antibiogram from the segment attached to the UCB graft or from a cryotube (without performing blood cultures for verification). Even in the absence of negative bacterial culture before administration, we are reasonably assured that no live bacteria will be infused considering that we can achieve high antibiotic concentrations (more than MIC100) in the administered graft.

These possible paths are summarized in Figure 3 below:

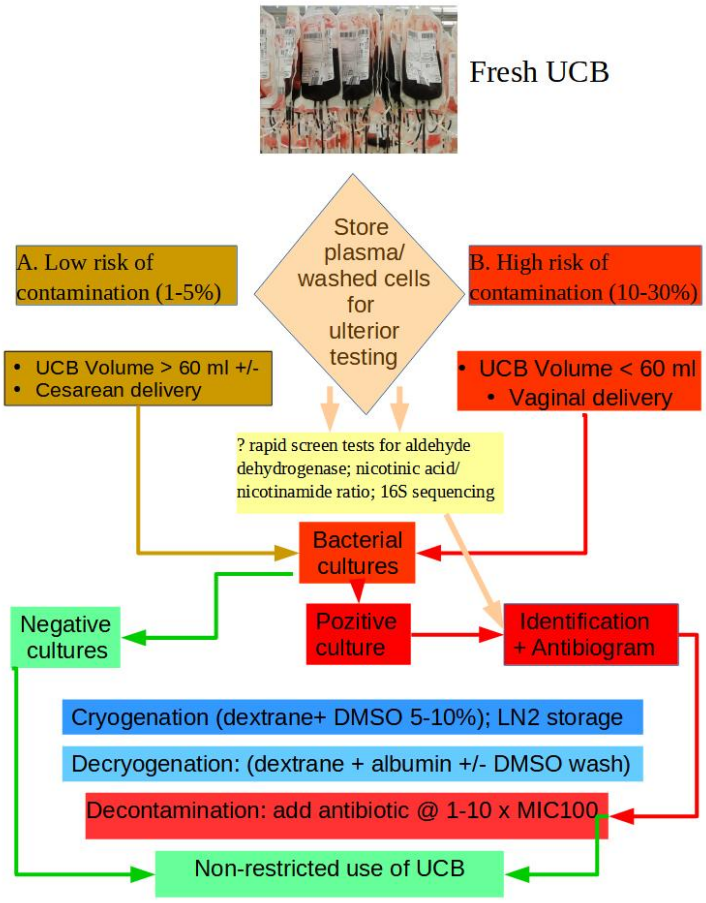

Figure 3. Flowchart for testing and decontamination of UCB grafts

Another aspect is that in some cases the bacteria which are present before cryopreservation will not be viable or grow/multiply after decryogenation [19][38][39], which raises the possibility that some UCB grafts contain inhibitory plasmatic factors [21], very likely T cells active against the respective bacteria which can eventually be expanded and used.

Expansion of viable and functional UCB stem cells ex-vivo using small molecules (nicotinamide, StemRegenin-1, UM171) was successfully demonstrated [6]. In this instance, the primary goal for administration of expanded UCB units was to address the shortcomings of UCB transplantation (delayed engraftment, higher rates of acute GVHD and infections with higher associated treatment-related mortality), while preserving the advantages of lower rates of relapse and chronic graft-versus-host disease, an objective which was safely accomplished in several clinical trials [40][41][42][43].

Expanded UCB was also used to lower the severity of viral infections after stem cell transplantation and improve the outcome of transplant-related viral infections [44] by expanding UCB stem cells in the presence of viruses such as Epstein-Barr and adenovirus, viruses for which cytotoxic T lymphocytes specific were demonstrated in the expanded grafts [45]. Future research in this direction may show whether this approach can be used outside transplantation procedures (with or without prior immune suppression) for other severe viral infections (Ebola, SARS-Cov2, HIV, etc) and perhaps in infections with bacteria resistant to multiple antibiotics.

Conclusion

Decontamination of UCB grafts which tested positive for bacteria can be successfully done prior to their administration by adding the antibiotic shown in the antibiogram without compromising the viability of the stem cells from the graft; it should be added in the first steps after decryogenation in order to allow time for the antibiotic to act before the administration of the graft.
If there is no antibiogram done prior to cryopreservation, it can be performed from a decryogenated portion of the graft prior to administration. High concentrations of the antibiotic, well above the established bactericidal concentrations for the respective species and which cannot be attained in vivo, can be obtained for a limited time – up to 2 hours - in the small volume of the decryogenated graft without producing important damage to the stem cells administered.

Future research may determine if expanding a UCB graft in certain conditions (Antigen + cytokines, etc) is a useful strategy for treating infections with difficult-to-treat viruses and bacteria.

Graft decontamination is especially needed in non-transplant administrations of stem cells, when patients are not usually administered antibiotics and the intravenous administration of a live bacteria is not a reasonable risk and an antibiotic concentration of 1-10 x MIC100 acting on the decryogenated graft for 1-2 hours is proposed as being effective for bacterial decontamination and safe for stem cells.

Declarations

- Informed consent was obtained prior to the described procedures from the parents of both children
- The authors declare no conflict of interest related to this article.
- All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for

publication.

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References

1. ^*Gluckman E, Devergié A, Bourdeau-Esperou H, Thierry D, Traineau R, Auerbach A, Broxmeyer HE. Transplantation of umbilical cord blood in Fanconi's anemia. Nouv Rev Fr Hematol (1978). 1990;32(6):423-5. PMID: 1983224.*
2. ^*Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989 Oct 26;321(17):1174-8. doi: 10.1056/NEJM198910263211707. PMID: 2571931.*
3. a, b*Ballen KK, Gluckman E, Broxmeyer HE (2013) Umbilical cord blood transplantation: the first 25 years and beyond. Blood 122: 491–498. 10.1182/blood-2013-02-453175*
4. ^*Ballen K. Update on umbilical cord blood transplantation. F1000Res. 2017; 6(24):1556. 10.12688/f1000research.11952.1*
5. ^*Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, Ciocci G, Carrier C, Stevens CE, Rubinstein P. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med. 1996 Jul 18;335(3):157-66. doi: 10.1056/NEJM199607183350303. PMID: 8657213.*
6. a, b*Sica RA, Terzioglu MK, Mahmud D, Mahmud N. Mechanistic Basis of ex Vivo Umbilical Cord Blood Stem Progenitor Cell Expansion. Stem Cell Rev Rep. 2020 Aug;16(4):628-638. doi: 10.1007/s12015-020-09981-w. PMID: 32424674.*
7. ^*Zhu X, Tang B, Sun Z. Umbilical cord blood transplantation: Still growing and improving. STEM CELLS Transl Med. 2021;10(S2):S62-S74. 10.1002/sctm.20-0495*
8. ^*Canto E, Rodriguez-Sanchez JL, Vidal S. Distinctive response of naive lymphocytes from cord blood to primary activation via TCR. J Leukoc Biol. 2003;74:998–1007.*
9. ^*Prasad VK, Mendizabal A, Parikh SH, et al.: Unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 pediatric patients from a single center: influence of cellular composition of the graft on transplantation outcomes. Blood. 2008;112(7):2979–89. 10.1182/blood-2008-03-140830*
10. ^*Shoulars K, Noldner P, Troy JD, Cheatham L, Parrish A, Page K, Gentry T, Balber AE, Kurtzberg J. Development and validation of a rapid, aldehyde dehydrogenase bright-based cord blood potency assay. Blood. 2016 May 12;127(19):2346-54. doi: 10.1182/blood-2015-08-666990. Epub 2016 Mar 11. PMID: 26968535; PMCID: PMC4865591.*

11. [a, b]*Page KM, Mendizabal A, Betz-Stablein B, Wease S, Shoulars K, Gentry T, Prasad VK, Sun J, Carter S, Balber AE, Kurtzberg J. Optimizing donor selection for public cord blood banking: influence of maternal, infant, and collection characteristics on cord blood unit quality. Transfusion. 2014 Feb;54(2):340-52. doi: 10.1111/trf.12257. Epub 2013 May 27. PMID: 23711284; PMCID: PMC3766489.*
12. [a, b]*Fumarola S, Lucarini A, Lucchetti G, Piroli L, Pierelli L. Predictors of cord blood unit cell content in a volume unrestricted large series collections: a chance for a fast and cheap multiparameter selection model. Stem Cell Res Ther. 2022 Jun 11;13(1):246. doi: 10.1186/s13287-022-02915-y. PMID: 35690786; PMCID: PMC9188136.*
13. [a, b]*Reuther S, Floegel K, Ceusters G, Albertini V, Baran J, Dempke W. Contamination Rate of Cryopreserved Umbilical Cord Blood Is Inversely Correlated with Volume of Sample Collected and Is also Dependent on Delivery Mode. Stem Cells Transl Med. 2022 Jun 22;11(6):604-612. doi: 10.1093/stcltm/szac020. PMID: 35486383; PMCID: PMC9216499*
14. [^]*Clark P, Trickett A, Stark D, Vowels M. Factors affecting microbial contamination rate of cord blood collected for transplantation. Transfusion. 2012 Aug;52(8):1770-7. doi: 10.1111/j.1537-2995.2011.03507.x. Epub 2011 Dec 30. PMID: 22211719.*
15. [^]*Zhu L, Lv H, Wang Y, Yang J, Ni B, Meng Z. Microbial screening of unrelated cord blood units in a Chinese cord blood bank. Transfus Med. 2013 Dec;23(6):438-41. doi: 10.1111/tme.12079. Epub 2013 Oct 10. PMID: 24118280.*
16. [a, b, c]*Honohan A, Olthuis H, Bernards AT, van Beckhoven JM, Brand A. Microbial contamination of cord blood stem cells. Vox Sang. 2002 Jan;82(1):32-8. doi: 10.1046/j.1423-0410.2002.00133.x. PMID: 11856465.*
17. [a, b]*Antoniewicz-Papis J, Lachert E, Rosiek A, et al.. Microbial contamination risk in hematopoietic stem cell products: retrospective analysis of 1996-2016 data. Acta Haematol Pol. 2020;51(3):29-33.*
18. [^]*Kamble R, Pant S, Selby GB, Kharfan-Dabaja MA, Sethi S, Kratochvil K, Kohrt N, Ozer H. Microbial contamination of hematopoietic progenitor cell grafts-incidence, clinical outcome, and cost-effectiveness: an analysis of 735 grafts. Transfusion. 2005 Jun;45(6):874-8. doi: 10.1111/j.1537-2995.2005.04178.x. PMID: 15934984.*
19. [a, b, c]*Li M, Lim D, Tang KF, Lim S, Boey K. Microbial Contamination in Umbilical Cord Blood: A Comparison Before and After Cryopreservation. Stem Cells Transl Med. 2018 Sep 5;7(Suppl Suppl 1):S2. doi: 10.1002/sctm.12354. PMCID: PMC6123798.*
20. [^]*Pasqua S, Vitale G, Pasquariello A, Douradinha B, Tuzzolino F, Cardinale F, Cusimano C, Di Bartolo C, Conaldi PG, D'Apolito D. Use of 27G needles improves sensitivity and performance of ATCC anaerobe reference microorganism detection in BacT/Alert system. Mol Ther Methods Clin Dev. 2021 Jan 26;20:542-550. doi: 10.1016/j.omtm.2021.01.010. PMID: 33665224; PMCID: PMC7890369.*
21. [a, b]*Girard M, Laforce-Lavoie A, de Grandmont MJ, et al.. Optimization of cord blood unit sterility testing: impact of dilution, analysis delay, and inhibitory substances. Transfusion. 2017;57(8):1956-1967.*
22. [^]*Ramirez-Arcos S, Kou Y, Yang L, Perkins H, Taha M, Halpenny M, Elmoazzen H. Validation of sterility testing of cord blood: challenges and results. Transfusion. 2015 Aug;55(8):1985-92. doi: 10.1111/trf.13050. Epub 2015 Mar 11. PMID: 25757514.*
23. [^]*Clark P, Trickett A, Chimenti M, Stark D. Optimization of microbial screening for cord blood. Transfusion. 2014 Mar;54(3):550-9. doi: 10.1111/trf.12352. Epub 2013 Jul 28. PMID: 23889674.*
24. [a, b]*Klein MA, Kadidlo D, McCullough J, McKenna DH, Burns LJ. Microbial contamination of hematopoietic stem cell*

products: incidence and clinical sequelae. Biol Blood Marrow Transplant. 2006 Nov;12(11):1142-9. doi: 10.1016/j.bbmt.2006.06.011. PMID: 17085307.

25. ^*Bohannon LK, Owens SD, Walker NJ, Carrade DD, Galuppo LD, Borjesson DL. The effects of therapeutic concentrations of gentamicin, amikacin and hyaluronic acid on cultured bone marrow-derived equine mesenchymal stem cells. Equine Vet J. 2013 Nov;45(6):732-6. doi: 10.1111/evj.12045. Epub 2013 Feb 28. PMID: 23448189.*
26. ^*Hövel H, Frieling KH. The use of doxycycline, mezlocillin and clotrimazole in cell culture media as contamination prophylaxis. Dev Biol Stand. 1987;66:23-8. PMID: 3582752.*
27. ^*Arenz S, Wilson DN. Bacterial Protein Synthesis as a Target for Antibiotic Inhibition. Cold Spring Harb Perspect Med. 2016 Sep 1;6(9):a025361. doi: 10.1101/cshperspect.a025361. PMID: 27481773; PMCID: PMC5008061.*
28. ^*Levin BR, McCall IC, Perrot V, Weiss H, Ovesepian A, Baquero F. A Numbers Game: Ribosome Densities, Bacterial Growth, and Antibiotic-Mediated Stasis and Death. mBio. 2017;8(1):e02253-16. Published 2017 Feb 7. doi:10.1128/mBio.02253-16*
29. ^*Okada M, Oka M, Yoneda Y. Effective culture conditions for the induction of pluripotent stem cells. Biochim Biophys Acta. 2010 Sep;1800(9):956-63. doi: 10.1016/j.bbagen.2010.04.004. Epub 2010 Apr 22. PMID: 20417254.*
30. ^*Onoda M, Shimizu M, Shinoda M, Tsuneoka K, Shikita M. Effects of various therapeutic agents on leukocytes in vivo and in vitro: competition between the cytotoxic drugs and colony stimulating factors (CSF) in the action on the proliferation of myeloid stem cells (CFU-C). J Pharmacobiodyn. 1980 Oct;3(10):493-9. doi: 10.1248/bpb1978.3.493. PMID: 6970806.*
31. ^*Hyun SW, Kim BR, Lin D, Hyun SA, Yoon SS, Seo JW. The effects of gentamicin and penicillin/streptomycin on the electrophysiology of human induced pluripotent stem cell-derived cardiomyocytes in manual patch clamp and multi-electrode array system. J Pharmacol Toxicol Methods. 2018 May-Jun;91:1-6. doi: 10.1016/j.vascn.2017.12.002. Epub 2017 Dec 27. PMID: 29288713.*
32. ^*Rubinstein P, Dobrila L, Rosenfield RE et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 1995; 92: 10119– 10122.*
33. ^*Dawson, G., Sun, J.M., Davlantis, K.S., Murias, M., Franz, L., Troy, J., Simmons, R., Sabatos-DeVito, M., Durham, R. and Kurtzberg, J. (2017), Autologous Cord Blood Infusions Are Safe and Feasible in Young Children with Autism Spectrum Disorder: Results of a Single-Center Phase I Open-Label Trial. STEM CELLS Translational Medicine, 6: 1332-1339. https://doi.org/10.1002/sctm.16-0474*
34. [a, b]*Aracil B., Gomez-Garces J.L., Alos J.I., A study of susceptibility of 100 clinical isolates belonging to the Streptococcus milleri group to 16 cephalosporins, Journal of Antimicrobial Chemotherapy, 1999 March,Vol 43, Issue 3, Pages 399–402, https://doi.org/10.1093/jac/43.3.399*
35. ^*Padley, D., Dietz, A. and Gastineau, D. (2007), Sterility testing of hematopoietic progenitor cell products: a single-institution series of culture-positive rates and successful infusion of culture-positive products. Transfusion, 47: 636-643. https://doi.org/10.1111/j.1537-2995.2007.01165.x*
36. ^*Huang J, Cui L, Natarajan M, Barone PW, Wolfrum JM, Lee YH, Rice SA, Springs SL. The ratio of nicotinic acid to nicotinamide as a microbial biomarker for assessing cell therapy product sterility. Mol Ther Methods Clin Dev. 2022 Apr 13;25:410-424. doi: 10.1016/j.omtm.2022.04.006. PMID: 35573051; PMCID: PMC9065052.*

37. ^*França L, Simões C, Taborda M, Diogo C, da Costa MS. Microbial Contaminants of Cord Blood Units Identified by 16S rRNA Sequencing and by API Test System, and Antibiotic Sensitivity Profiling. PLoS One. 2015 Oct 29;10(10):e0141152. doi: 10.1371/journal.pone.0141152. PMID: 26512991; PMCID: PMC4626235.*
38. ^*Schwella, N., Rick, O., Heuft, H.G., Miksits, K., Zimmermann, R., Zingsem, J., Eckstein, R. and Huhn, D. (1998), Bacterial Contamination of Autologous Bone Marrow: Reinfusion of Culture-Positive Grafts Does Not Result in Clinical Sequelae during the Posttransplantation Course. Vox Sanguinis, 74: 88-94. https://doi.org/10.1046/j.1423-0410.1998.7420088.x*
39. ^*Boey KPY, Zhu P, Tan H, et al. Effects of cryopreservation on gram-positive bacteria contaminants in umbilical cord blood, 2021, Transfusion Medicine, 10.1111/tme.12834, 32, 1, (82-87)*
40. ^*Cohen S, Roy J, Lachance S, Delisle JS, et al. Hematopoietic stem cell transplantation using single UM171-expanded cord blood: a single-arm, phase 1-2 safety and feasibility study. Lancet Haematol. 2020 Feb;7(2):e134-e145. doi: 10.1016/S2352-3026(19)30202-9. Epub 2019 Nov 6. PMID: 31704264.*
41. ^*Milano F, Thur LA, Blake J, Delaney C. Infusion of Non-HLA-Matched Off-the-Shelf Ex Vivo Expanded Cord Blood Progenitors in Patients Undergoing Cord Blood Transplantation: Result of a Phase II Clinical Trial. Front Cell Dev Biol. 2022 Apr 4;10:835793. doi: 10.3389/fcell.2022.835793. PMID: 35445027; PMCID: PMC9014214.*
42. ^*Bear AS, Hanley PJ, Bosque DM, Cruz CR, Kaur I, Liu H, Kebriaei P, Hosing C, Rezvani K, Oran B, De Lima MJ, Bollard CM, Shpall EJ. Low rate of infusional toxicity after expanded cord blood transplantation. Cytotherapy. 2014 Aug;16(8):1153-7. doi: 10.1016/j.jcyt.2013.12.011. Epub 2014 Feb 28. PMID: 24582458; PMCID: PMC4087060.*
43. ^*Shpall EJ, Quinones R, Giller R, et al. Transplantation of ex vivo expanded cord blood. Biol Blood Marrow Transplant. 2002;8(7):368-76. doi: 10.1053/bbmt.2002.v8.pm12171483. PMID: 12171483.*
44. ^*Abraham AA, John TD, Keller MD, Cruz CRN, Salem B, Roesch L, Liu H, Hoq F, Grilley BJ, Gee AP, Dave H, Jacobsohn DA, Krance RA, Shpall EJ, Martinez CA, Hanley PJ, Bollard CM. Safety and feasibility of virus-specific T cells derived from umbilical cord blood in cord blood transplant recipients. Blood Adv. 2019 Jul 23;3(14):2057-2068. doi: 10.1182/bloodadvances.2019000201. Erratum in: Blood Adv. 2019 Aug 27;3(16):2453. PMID: 31292125; PMCID: PMC6650740.*
45. ^*Hanley PJ, Lam S, Shpall EJ, Bollard CM. Expanding cytotoxic T lymphocytes from umbilical cord blood that target cytomegalovirus, Epstein-Barr virus, and adenovirus. J Vis Exp. 2012 May 7;(63):e3627. doi: 10.3791/3627. PMID: 22588077; PMCID: PMC3466930.*