Baby Cells

Paolo Rebulla

Department of Transfusion Medicine and Hematology
Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico
Via Francesco Sforza 35
20122 Milan, Italy

Email: paolo.rebulla@policlinico.mi.it

Keywords: placental blood; umbilical cord blood; platelets; red blood cells; platelet gel; plasma; neonatal transfusion.

Abstract

Over 90% of neonatal blood units, collected from the umbilical cord of full-term healthy newborns and generously donated to the community for the purpose of hematopoietic transplantation, do not contain a sufficient number of stem cells to perform this type of transplant and are consequently eliminated with hospital waste.
This article presents some data and proposes some reflections related to the following questions:

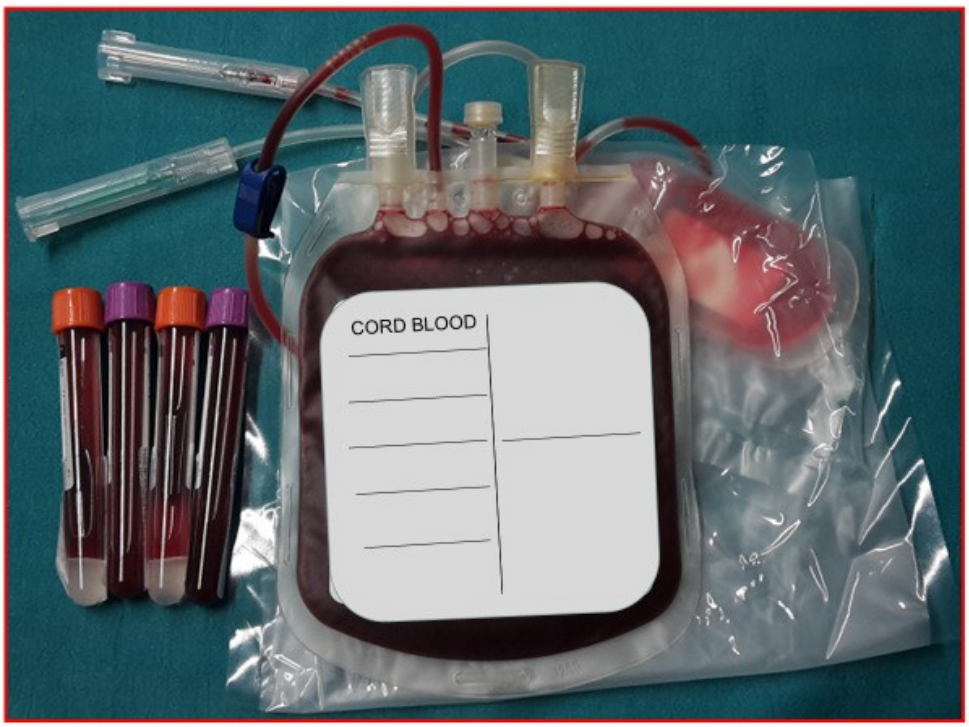
- How to improve the economic sustainability of public umbilical cord blood banks?
- Is it interesting to note that the placentas annually eliminated worldwide after deliveries (over 70 million) contain a neonatal blood volume corresponding to about 1/6 of the total volume of blood collected annually worldwide from adult blood donors (about 100 million whole blood donations)?
- What differentiates neonatal blood from adult blood?
- Can neonatal blood currently eliminated with placentas available in hospitals be a source of new therapeutic applications in addition to hematopoietic stem cell transplantation?
- What regulatory, organizational and infrastructural implementations are necessary to promote scientific studies and possible future developments of these new applications?

An Italian version of this article has been published in May 2019 by Biomedia s.r.l. in the online magazine 'Transfusion Medicine Network', available at www.invenadisapere.it

Introduction

The present article summarizes some scientific observations and personal considerations that have contributed to formulate the hypothesis that placental blood collected after birth from the umbilical cord of term infants (figure 1), whose current therapeutic use is limited to hematopoietic transplantation, may become a source of laboratory reagents and new allogeneic blood components with different or complementary biological characteristics and clinical efficacy compared to blood components traditionally obtained from the blood of adult donors.

Figure 1. Cord blood unit.



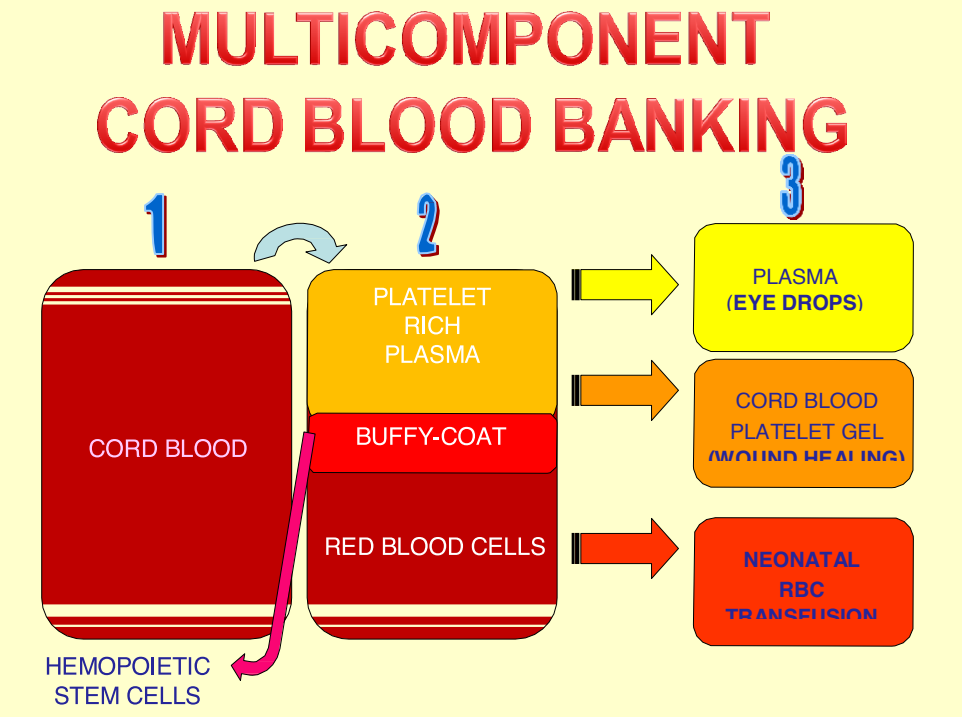
The purpose of the article is to stimulate the scientific community to carry out the necessary investigations to collect useful data to support, modify or reject this hypothesis. In particular, the need to involve specialists in transfusion medicine from the earliest stages of these investigations, and not only clinicians of different medical and surgical specialties, is evident in view of the significant organizational tasks that could arise if a new inventory of products obtained from placental blood were to be systematically manufactured, stored and distributed alongside to the traditional inventory of blood components and blood products prepared from the blood of adult donors.

The personal involvement of the author in the research and in the inventive process that led to the patent of a procedure to obtain platelet fractions from placental blood, owned by Foundation Ca' Granda Ospedale Maggiore Policlinico (Platelet fraction deriving from placental blood, US patent 8,501,170 B2), as well as in the foundation and scientific direction of the innovative start-up Episkey srl (www.episkey.eu), whose main purpose is the international promotion of 'Multicomponent Cord Blood Banking' (figure 2) and the industrial development of reagents and pharmaceuticals derived from placental blood, represent for the author the main disclosures related

to the arguments below.

MEDITALIA S.r.l
Sede legale - Stabilimento Di Produzione - Amministrazione
23030 Lovero Valtellino (SO) Italia - Via alla Piana, 1 - Tel. 0342 771070 - Fax 0342 771071 - info@meditaliasrl.com - www.meditaliasrl.com - info@pec.meditaliasrl.com
Ufficio Commerciale
20121 Milano (MI) Italia- via Giacomo Puccini, 3 – Tel 02 49538110
C.C.I.A.A. R.E.A. SO - 63876 - Cod. Fisc./P.IVA Reg. Imp. So 00846420149 - Capitale Sociale 628.258,00 i.v.

Figure 2. Multicomponent Cord Blood Banking.



Why are we interested in 'Baby Cells'?

The systematic collection, characterization and cryopreservation of placental blood donated for the purpose of hematopoietic transplantation by parents of full-term healthy newborns started in the early 90s of the last century, following some fundamental studies by Broxmeyer et al. (1), which showed the presence of a high number of hematopoietic stem cells in placental blood collected after delivery, and the pioneering cord blood transplant performed successfully for the first time in Paris by Eliane Gluckman in 1988 in a patient suffering from Fanconi anemia (2,3).

Today over 750,000 cord blood units donated to the community and stored at over 150 public banks are available worldwide. These units have so far allowed the execution of approximately 35,000 allogeneic hematopoietic transplants in patients suffering from oncohematologic conditions, metabolic defects, immunodeficiencies and hemoglobinopathies (3).

The clinical efficacy of placental blood transplantation is similar to that obtained using the other classic sources of hematopoietic stem cells (bone marrow, peripheral blood mobilized with growth factors) and to the promising results of more recent, innovative HLA-haplo-identical transplantation procedures (4.5). This type of transplant offers the advantage of easy and prompt access to a highly motivated, family donor who shares with the patient half (haplo) genetic heritage, typically a parent or child of the patient. The haplo-identical transplantation, mainly applied to patients lacking an HLA identical donor and any valid alternative therapeutic options, was in the past burdened by a high incidence of graft versus host disease (GVHD, a severe and not infrequently fatal immunologic transplant complication) if the infused cells were not subjected to extensive lymphocyte removal, or insufficient immunological reconstitution after transplantation if, in order to prevent GVHD, they

were deprived of most lymphocyte subpopulations. The current haplo-identical transplantation protocols have significantly reduced the risks of GVHD using cytotoxic drugs capable of electively removing the most reactive lymphocyte components against the recipient's tissues and organs, thus preserving the ability of the transplanted cells to reconstitute the patient's immune system by the cellular populations without such aggressiveness.

These important therapeutic advances have progressively reduced the number of cord blood transplants in recent years and stimulated the structural, organizational and administrative review of the networks of public placental blood banks, which often fail to cover their high operating costs with the reimbursement fee generated from the units distributed to national and international transplant centers (about $ 36,000 per unit distributed by US public banks).

Furthermore, the data collected in the first 20 years of clinical use of cord blood for the purpose of hematopoietic transplantation showed that less than 10% of the donations collected contained a number of total nucleated cells (TNC), a parameter related to the number of hematopoietic stem cells) sufficient to perform the transplantation in safe conditions. As a result, most public banks, in order to avoid costly characterization and cryopreservation procedures of cord blood units with low probability of transplantation use, currently discard more than 90% of the units collected. This high waste fraction, in addition to generating a significant and unproductive economic burden, represents a potential disincentive to the solidarity donation of cord blood.

The current economic difficulties of numerous public banks (6), the recent decline in the transplantation of cord blood compared to the haplo-identical transplant and the high percentage of waste of the collected units have led some administrators to propose the revision of some programs of collection and drastic reduction in the number of currently operating public banks.

Should we close the public cord blood banks?

The therapeutic success of placental blood hematopoietic transplantation has helped to stimulate the creation of a large number of public banks in the last decade of the last century. The activation of numerous banks equipped with expensive cryopreservation infrastructures has gradually proven to be operationally unnecessary and economically unsustainable in many countries (7.8). In more recent years, many programs have been consolidated according to a "hub and spoke" model, by significantly reducing the cryopreservation sites while maintaining peripheral operations for the selection of cord blood donors and the collection of cord blood.

A careful analysis of the relationship between the costs and the social and health benefits of the national network of 19 public cord blood banks currently operating in the USA, has been published by the RAND Corporation (9). The authors of this analysis, commissioned by the U.S. Department of Health and Human Services, list the main advantages and disadvantages of the different stem cell sources used for haematopoietic transplantation, documented by numerous published studies: ready availability of frozen units, better compatibility and less immunological aggressiveness, but lower cellular dose, slower transplant take and higher hospitalization costs for stem cells obtained from cord blood; high cellular dose, faster engraftment for the classic cellular sources represented by the bone marrow and the mobilized peripheral blood, but higher risk of severe GVHD and longer times to select non-family donors registered in the international registers of hematopoietic stem cell donors.

An interesting element of the analysis published by the RAND Corporation is represented by the

social value ("corporate value") of the system of public banks in the US. The authors of this analysis compared the annual costs of the current system (60-70 million US dollars) and the social value generated by the accessibility to transplantation and the survival of transplanted patients, estimated using a value of 100,000 dollars for each year of life earned thanks to cord blood transplantation. Based on these data and this analysis model, the RAND Corporation estimated an annual social value of the US public cord blood banking network between 883 and 1700 million US dollars, about 20 times higher than the costs.

The analysis by Kapinos et al. (9) finally reports some recommendations aimed at improving the system and ensuring its economic sustainability, which can also be useful in other operational contexts: 1) increase the genetic diversity of donations collected and the cellular threshold for banking, in order to increase the probability of transplant use of cryopreserved units; 2) plan accurate financing programs for banks; 3) develop programs ("contingency plans") to ensure the conservation of the units even in the event of interruption of the activities of some banks; 4) change the reimbursement mechanisms by separating the costs of acquiring cord blood units from the costs of transplantation; 5) promote a greater exchange of knowledge between banks and clinicians; 6) offer banks the option of selling the smaller volume units, currently wasted, for programs aimed at developing new transplant procedures and alternative uses.

Also in Italy, similar to the previous reorganization of the network of French public banks (8), the National Blood Center has promoted the start of a process of revision of the organization of the Italian network of public banks (ITCBN) by adopting in February 2016 a high cellular threshold for units frozen for hematopoietic transplantation (TNC> 120 x 10^7 and CD34 + cells> 2 x 10^6 or TNC> 160 x 10^7 independently of the CD34 + cell count) (10). As expected, the high cellular threshold adopted nationally determined a very high discard rate of the units collected in 2017 (93.1%). Similar percentages of waste are currently reported in other countries. It is likely to be expected that the high banking threshold deliberated in Italy in 2016 will be maintained - if not increased - in the future, considering that only 26.9% of the 41,164 units that made up the ITCBN inventory at the end of 2017 contained a TNC number> 150 x 10^7, a value below which the probability of transplant use of the units is significantly reduced.

More recently, in the session of December 13, 2018, the Italian Permanent Conference for Relations between the State, the Regions and the Autonomous Provinces of Trento and Bolzano sanctioned the agreement on the document entitled 'Scheme type of agreement for the sale of blood and its products for laboratory use and for the production of in vitro diagnostic medical devices', which regulates the transfer to the industry of biological materials, some of which are obtained from cord blood, described in table 1. The document also specifies that the sale of biological materials can be aimed at a) laboratory activities (eg: matrices, additive solutions, etc.); b) production of in vitro diagnostic medical devices.

Table 1. Biological materials that can be transferred to industry in accordance with the agreement of 13 December 2018 of the Italian Permanent Conference for Relations between the State, the Regions and the Autonomous Provinces of Trento and Bolzano.

a) whole blood
b) concentrated red blood cells
c) fresh frozen plasma
d) apheresis plasma
e) apheresis platelets
f) buffy-coat
g) platelets from buffy-coat pools
h) platelet concentrates from cord blood
i) platelet-rich plasma from cord blood
j) platelet-poor plasma of cord blood
k) other: (describe)…

Novel uses of placental blood

The high availability of units collected after accurate medical history of donor mothers and not suitable for hematopoietic transplantation due to insufficient cellular content, but also compliant with the safety requirements related to transmissible diseases, has stimulated the development of programs aimed at identifying alternative uses of cord blood. Alongside numerous and specific scientific reasons, the intention to fully exploit the generous solidarity decision expressed by the many families that donate placental blood to the community represents a significant ethical motivation to support these programs (11).

The first programs, developed in the early 2000s and consolidated in the following years, involved some ophthalmic applications of serum obtained after coagulation of cord blood (12-19). At the same time, the platelet fraction - very rich in tissue regeneration factors (table 2) - obtained from cord blood with simple differential centrifugation procedures, was used to produce gels for the treatment of skin ulcers and mucous lesions (20-27), eye drops for the treatment of corneal lesions (unpublished data) and additives for cell culture media (28-30).

Table 2. A partial list of tissue regeneration factors present in platelets.

- Brain derived neurotrophic factor (BDNF)
- Basic fibroblast growth factor (bFGF)
- Bone morphogenetic protein (BMP)
- Connective tissue growth factor (CTGF)
- Epidermal growth factor (EGF)
- Hepatocyte growth factor (HGF)
- Insulin-like growth factor-1 (IGF-1)
- Matrix metalloproteinase (MMP)
- Platelet derived growth factor (PDGF)
- Platelet factor 4 (PF4)
- Stromal cell derived factor-1 (SDF-1)

- Transforming growth factor-beta (TGF-beta)
- Tissue inhibitor of metalloproteinases (TIMP)
- Vascular endothelial growth factor (VEGF)

More recently, neonatal red blood cells have been used in Europe and Africa in experimental protocols of allogeneic transfusion in the pediatric context, particularly in term or premature infants (31.32). Some of these latter programs have been stimulated by previous studies that have suggested a possible causal relationship between the transfusion of red blood cells from adult donors, containing adult hemoglobin characterized by a high release of oxygen to the tissues, and some complications of prematurity, including retinopathy of prematurity (ROP), broncho-pulmonary dysplasia (BPD) and necrotizing entheropathy (NEC). The alternative use of red blood cells from cord blood, which is the object of ongoing studies, is based on the hypothesis that fetal hemoglobin, present in neonatal red blood cells and characterized by a high affinity for oxygen, can prevent or reduce oxidative damage generated in the non-fully developed organs of premature infants by the high oxygen release of adult hemoglobin (33-35).

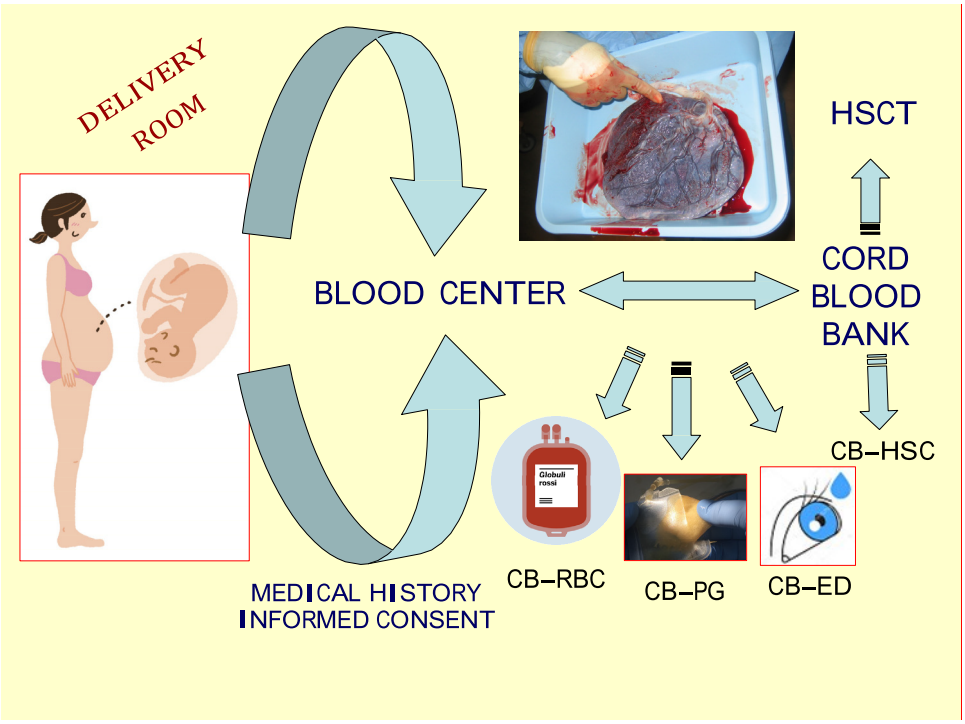
How to obtain 'Baby Cells' in adequate quantities?

In almost all countries where a network of public cord banks is operating, more than 90% of units donated for hematopoietic transplantation cannot be used for this purpose and are therefore potentially available, with the explicit informed consent of the donor parents, for alternative uses.

For example, in Italy in 2017 11,996 cord blood units collected in 285 delivery rooms were donated to the public banks of the ITCBN network, of which only 825 (6.3%) were cryopreserved and included in the inventory available for hematopoietic transplantation (10). The organizational network technically formed to collect, process, store and distribute cord blood for clinical use purposes represents a great cultural and professional value, developed in over 25 years of activity. Several thousands of units donated each year are already available in Italy, which could be used to prepare alternative therapeutic products. The low percentage of collections made in relation to the number of deliveries registered at national level in 2017 (11,996 / 464,000 = 2.6%) indicates a high potential availability of cord blood which is now discarded as hospital waste.

It may also be interesting in this regard to compare the worldwide annual number of adult donor whole blood donations - about 100 million - with the number of births (and placentas) - about 73 million. Assuming for adult whole blood donations and for cord blood units an average volume of 450 and 100 mL respectively, it can be estimated that the total blood volume annually eliminated worldwide with the placenta (7.3 million liters) corresponds to about 1/6 (16%) of 45 million liters of blood collected by adult donors. It would obviously be inappropriate to neglect the need for adequate organization and infrastructure to systematically collect and manufacture novel cord blood products: could these activities (anamnesis of the mothers, collection of informed consent, collection, processing, characterization and distribution of the new products) be entrusted entirely to blood transfusion centers (figure 3), considering the consolidated professional skills and infrastructural characteristics of these structures?

Figure 3. A possible hospital organizational model of Multicomponent Cord Blood Banking. CB: cord blood; RBC: red blood cells; PG: platelet gel; ED: eye drops; HSC: hemopoietic stem cells; HSCT: hemopoietic stem cell transplant.



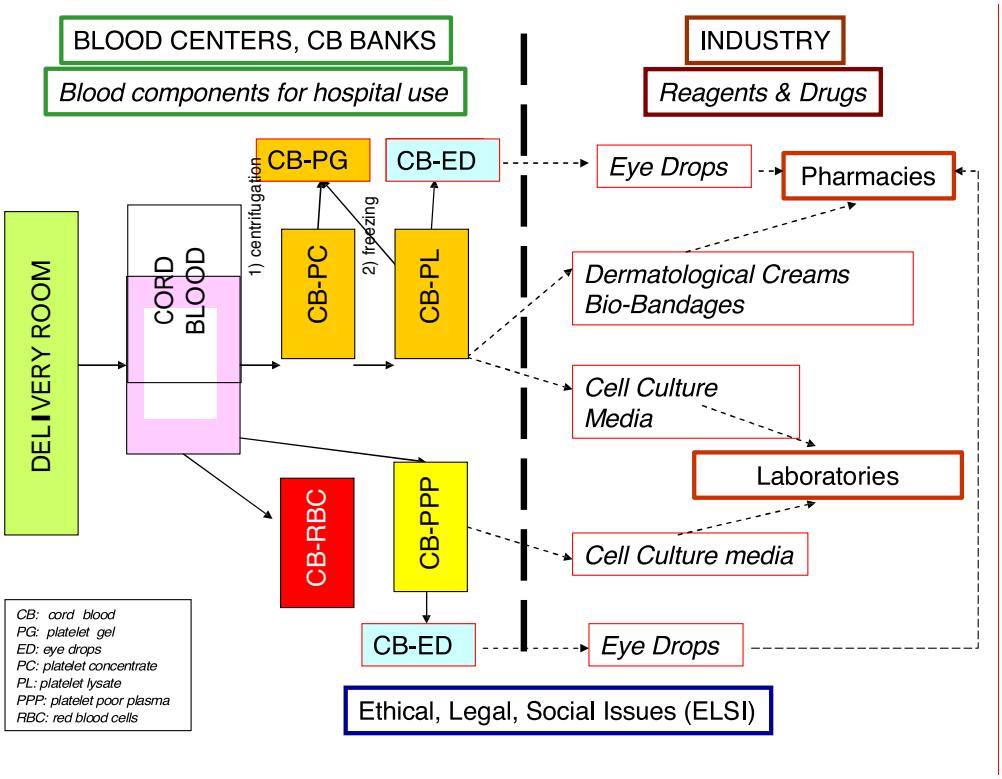
Conclusions.

This article is not aimed at demonstrating the clinical validity or cost-effectiveness of alternative uses of cord blood, nor to propose definitive organizational models for these activities. Many studies in this field are in fact still ongoing and, despite the promising clinical results obtained mainly with dermatological and ophthalmic applications, few randomized studies of adequate statistical power have been published so far that allow for a complete evaluation of the efficacy of the new therapies compared to traditional treatments. Therefore, further studies are needed before promoting the use of these new therapies outside of research projects.

Furthermore, alternative uses of cord blood require review and harmonization of national and international regulatory systems relating to these activities. This is particularly relevant also due to possible future interest of the industry in the acquisition and pharmaceutical transformation of the donated biological materials (figure 4). The Italian research project 'New reagents, medical devices, blood products and drugs obtained from placental blood' (NUPLA), coordinated by the National

Blood Center and involving some banks of the ITCBN network and the Banc de Sang i Teixits in Barcelona, Spain, has the purpose of promoting scientific studies on biological materials from cord blood and developing the necessary regulatory review at European level of these activities.

Figure 4. A possible operational model aimed at the therapeutic hospital use of the new blood components obtained from placental blood and their transformation by the pharmaceutical industry.



In conclusion, exciting studies on cord blood indicate numerous therapeutic potentialities for different components of this biological material, which do not end with the established use in hemopoietic stem cell transplant. The full development of these activities requires cultural sharing of the available evidences by professionals in transfusion medicine, regenerative medicine and clinical specialties, implementation of laboratory studies and large controlled clinical trials, and regulatory harmonization for the collection, processing and distribution of new reagents, blood components, blood products and drugs obtained from cord blood.

References

1. Broxmeyer HE, Douglas GW, Hangoc G, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci U S A. 1989;86:3828-32.
2. Gluckman E. Milestones in umbilical cord blood transplantation. Blood Rev. 2011;25:255- 9.
3. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood 2013;122:491-8.
4. Fuchs EJ. Related haploidentical donors are a better choice than matched unrelated donors: Point. Blood Advances 2017;1:397-400.
5. Shaw BE. Related haploidentical donors are a better choice than matched unrelated donors: Counterpoint. Blood Advances 2017;1:401-6.
6. Magalon J, Maiers M, Kurtzberg J, et al. Banking or Bankrupting: Strategies for Sustaining the Economic Future of Public Cord Blood Banks. PLoS One 2015;10:e0143440.
7. Pupella S, Bianchi M, Ceccarelli A, et al. A cost analysis of public cord blood banks belonging to the Italian Cord Blood Network. Blood Transfus 2018;16:313-20.
8. Katz G, Mills A. Cord blood banking in France: reorganising the national network. Transfus Apher Sci 2010;42:307-16.
9. Kapinos KA, Briscombe B, Gracner T, et al. Challenges to the Sustainability of the U.S. Public Cord Blood System, RR-1898-DHHS, 2017 (available at www.rand.org/t/RR1898).
10. Centro Nazionale Sangue. Banche di Sangue di Cordone Ombelicale. Report 2017. https://www.centronazionalesangue.it.
11. Petrini C. Umbilical cord blood banking: From personal donation to international public registries to global bioeconomy. J Blood Med 2014;5:87–97.
12. Sharma N, Goel M, Velpandian T, Titiyal JS, Tandon R, Vajpayee RB. Evaluation of umbilical cord serum therapy in acute ocular chemical burns. Invest Ophthalmol Vis Sci 2011;52:1087-92.
13. Versura P, Profazio V, Buzzi M, et al. Efficacy of standardized and quality-controlled cord blood serum eye drop therapy in the healing of severe corneal epithelial damage in dry eye. Cornea 2013;32:412-8.
14. Yoon KC, Oh HJ, Park JW, Choi J. Application of umbilical cord serum eyedrops after laser epithelial keratomileusis. Acta Ophthalmol 2013;91:e22-8.
15. Erdem E, Yagmur M, Harbiyeli I, Taylan-Sekeroglu H, Ersoz R. Umbilical cord blood serum therapy for the management of persistent corneal epithelial defects. Int J Ophthalmol 2014;7:807-10.
16. Versura P, Buzzi M, Giannaccare G, et al. Cord blood serum-based eye drops: the impact of donor haematological and obstetric factors on the variability of epidermal growth factor levels. Blood Transfus 2014;12 Suppl 1:s44-50.
17. Versura P, Buzzi M, Giannaccare G, et al. Targeting growth factor supply in keratopathy treatment: comparison between maternal peripheral blood and cord blood as sources for the preparation of topical eye drops. Blood Transfus 2016;14:145-51.
18. Mukhopadhyay S, Sen S, Datta H. Comparative role of 20% cord blood serum and 20% autologous serum in dry eye associated with Hansen's disease: a tear proteomic study. Br J Ophthalmol 2015;99:108-12.
19. van der Meer PF, Seghatchian J, Marks DC. Quality standards, safety and efficacy of blood- derived serum eye drops: A review. Transfus Apher Sci 2016;54:164-7.
20. Parazzi V, Lazzari L, Rebulla P. Platelet gel from cord blood: a novel tool for

tissue engineering. Platelets 2010;21:549-54.

21. Rebulla P, Pupella S, Santodirocco M, et al. Multicentre standardisation of a clinical grade procedure for the preparation of allogeneic platelet concentrates from umbilical cord blood. Blood Transfus 2016;14:73-9.
22. Rosso L, Parazzi V, Damarco F, et al. Pleural tissue repair with cord blood platelet gel. Blood Transfus 2014;12 Suppl 1:s235-42.
23. Tadini G, Pezzani L, Ghirardello S, Rebulla P, Esposito S, Mosca F. Cord blood platelet gel treatment of dystrophic recessive epidermolysis bullosa. BMJ Case Rep. 2015 Jan 8;2015.
24. Tadini G, Guez S, Pezzani L, et al. Preliminary evaluation of cord blood platelet gel for the treatment of skin lesions in children with dystrophic epidermolysis bullosa. Blood Transfus 2015;13:153-8.
25. Gelmetti A, Greppi N, Guez S, Grassi F, Rebulla P, Tadini G. Cord blood platelet gel for the treatment of inherited epidermolysis bullosa. Transfus Apher Sci 2018;57:370-3.
26. Sindici E, Giuliano B, Astesano S, et al. Cord blood platelet gel alone or in combination with photobiomodulation therapy for the treatment of oral ulcerations in patients with epidermolysis bullosa: A pilot clinical comparative study. Photodermatol Photoimmunol Photomed 2017 Nov 15. doi: 10.1111/phpp.12366.
27. Piccin A, Rebulla P, Pupella S, et al. Impressive tissue regeneration of severe oral mucositis post stem cell transplantation using cord blood platelet gel. Transfusion 2017;57:2220-4.
28. Parazzi V, Lavazza C, Boldrin V, et al. Extensive Characterization of Platelet Gel Releasate From Cord Blood in Regenerative Medicine. Cell Transplant 2015;24:2573-84.
29. Ferri AL, Ceserani V, Greppi N, et al. Osteogenic differentiation of adipose tissue-derived mesenchymal stem cells cultured on a scaffold made of silk fibroin and cord blood platelet gel. Blood Transfus 2016;14:206-11.
30. Longo V, Rebulla P, Pupella S, Zolla L, Rinalducci S. Proteomic characterization of platelet gel releasate from adult peripheral and cord blood. Proteomics Clin Appl 2016;10:870-82.
31. Bianchi M, Giannantonio C, Spartano S, et al. Allogeneic umbilical cord blood red cell concentrates: an innovative blood product for transfusion therapy of preterm infants. Neonatology 2015;107:81-6.
32. Hassall OW, Thitiri J, Fegan G, et al. Safety and efficacy of allogeneic umbilical cord red blood cell transfusion for children with severe anaemia in a Kenyan hospital: an open-label single-arm trial. Lancet Haematol 2015;2:e101-7.
33. Del Vecchio A, Henry E, D'Amato G, et al. Instituting a program to reduce the erythrocyte transfusion rate was accompanied by reductions in the incidence of bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis. J Matern Fetal Neonatal Med 2013;26 Suppl 2:77-9.
34. Stutchfield CJ, Jain A, Odd D, Williams C, Markham R. Foetal haemoglobin, blood transfusion, and retinopathy of prematurity in very preterm infants: a pilot prospective cohort study. Eye (Lond) 2017;31:1451-5.
35. Teofili L, Bianchi M, Baldascino A, Papacci P, Vento G. Foetal haemoglobin, blood transfusion, and retinopathy of prematurity. Eye (Lond) 2018;32:1155-6.