A LIFE THAT IS BORN MAKES LIFE GROW.
CORD BLOOD: CURRENT EXPERIENCES AND FUTURE PROGRAMMES

Milan (Italy), June 5th - 6th, 2015
Auditorium Don Giacomo Alberione
Via Giotto, 36

Organized by
MILANO CORD BLOOD BANK
CENTRO TRASFUSIONALE

FONDAZIONE IRCCS CA’ GRANDA
OSPEDALE MAGGIORE POLICLINICO, MILAN

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FONDAZIONE INTERNAZIONALE MENARINI

ABSTRACT BOOK
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Current aspects and future developments in cord blood transplant

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Umbilical cord blood is an alternative hematopoietic stem cell source for patients with hematologic diseases who can be cured by allogeneic hematopoietic cell transplantation. Initially, umbilical cord blood transplantation was limited to children, given the low cell dose infused. Both related and unrelated cord blood transplants have been performed with high rates of success for a variety of hematologic disorders and metabolic storage diseases in the pediatric setting. The results for adult umbilical cord blood transplantation have improved, with greater emphasis on cord blood units of sufficient cell dose and HLA match, and with the use of double umbilical cord blood units and improved supportive care techniques. Cord blood expansion trials have recently shown improvement in time to engraftment. Umbilical cord blood is being compared to other graft sources in both retrospective and prospective trials. The growth of the field over the last 25 years and the plans for future exploration will be discussed.
The Italian Project on Cord Blood Platelet Gel

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Cord blood (CB) platelet gel (CBPG) is a new blood component originally developed by the Milano Cord Blood Bank for allogeneic topical therapeutic applications mainly focussed on skin ulcer repair (US patent no. 8,501,170 B2, granted to Fondazione Ca’ Granda Ospedale Maggiore Policlinico on August 6, 2013). CBPG consists of a platelet concentrate (PC) which is stored frozen until the time of use, when it is thawed and a gel trapping platelet fragments is formed by the addition of an activator (Ca gluconate, thrombin or batroxobin). Its development was prompted by the high content of growth factors (GF) in CB platelets, the high microbiological safety profile of CB as an allogeneic blood component and the large availability of allogeneic CB units collected for hemopoietic transplant purposes which are otherwise unsuitable for public banking due to small stem cell dose.

In 2009, 14 public CB banks belonging to the Italian Cord Blood Network (ITCBN), the Proteomics Laboratory of the Tuscia University and the Blood Transfusion Service of the Alessandria Hospital were involved in a national research project coordinated by the Italian National Blood Centre (Centro Nazionale Sangue, CNS) aimed at the standardization of CBPG.

CB units collected after maternal informed consent and showing negative maternal and familial history of transmissible diseases, negative infectious markers screening, total nucleated cell count below the threshold for banking for hemopoietic transplant purposes (set at \(1.5 \times 10^9/\text{CB unit}\)), platelet count above \(150 \times 10^9/\text{L}\) and volume greater than 50 ml, underwent soft spin centrifugation within 48 hours of collection to obtain platelet-rich plasma (PRP). PRP was centrifuged at high speed to remove most of the platelet poor plasma (PPP) and to obtain a PC with a target volume of 10 mL and a platelet
concentration of $0.8 - 1.2 \times 10^6$/uL. Before CBPC cryopreservation at temperatures below -40 °C, the PPP was tested for sterility. Quality control data from CB, PRP, PC and PPP were managed with a single database used by all the participating banks. A cost analysis of the CBPG production process was carried out in one bank, which also determined the percentage of collected CB units which could be used for routine production of CBPG based on the selection criteria used in this study.

After a pilot exercise carried out in January – October 2013, during November 1st, 2013 – December 31st, 2014, the participating banks produced 1080 CBPCs with mean and SD volume of 11.4 ± 4.4 mL and platelet concentration of 1003 ± 229 x 10^9/L. Total platelet count per CBPC unit was 11.3 ± 4.9 x 10^9. Platelet recovery from CB was 47.7 ± 17.8%. About 1/3 of CB units donated for hematopoietic transplant could meet the requirements for preparation of CBPG. Cost of preparation of one unit of CBPC was euro 160.92. Costs of CBPG activation varied depending on the activator (batroxobin and Ca gluconate or Ca gluconate alone). On average, 2 hours were needed for one technologist to prepare 3-4 units of CBPC.

The ITCBN cooperative project yielded a large number of standardized CBPC units that will be used in an international controlled randomized clinical trial on the repair of diabetic foot ulcers.
Cord Blood Platelet Lysate as Gmp Culture Media

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Platelet gel derived from peripheral blood is widely applied in many clinical fields of surgery as biomaterial containing growth factors with high proliferative properties. In 2010 we studied and patented a platelet gel derived form cord blood. In this study, due to the crucial role of the factors released by the platelet gel, the aim was to study the effects on cell culture, immunophenotype and function of mesenchymal stem cells exposed to platelet lysates of these two gels as substitute of the animal serum.

Since our findings nicely show that the use of the peripheral versus the cord blood platelet lysate can differently influence the mesenchymal stem cell commitment, we can suggest that cord blood platelet lysate shows excellent proliferative properties as cell culture supplement.

Moreover, we investigated an interesting application of cord blood platelet gel by in vitro and in vivo experiments for the treatment of a common postoperative complication in thoracic surgery, known as prolonged air leak.

We observed that cord blood platelet gel accelerates the repair of pleural damage and stimulates the development of pleural adhesions. Both properties could be particularly useful in the management of prolonged air leak and to reduce inflammation.

In conclusion, cord blood platelet lysate stimulates cell growth and reparative processes so it results to be an excellent candidate to be used as GMP culture media in regenerative medicine.
Proteomics tools for profiling of platelet (PLT) derivatives

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Besides classical transfusion therapy, PLTs can be used in several regenerative treatments (including chronic wound, skin and soft tissue repair), mostly as PLT gel (PG) preparations. PG contains a high concentration of a large number of known and unknown biological factors, which are primarily proteins involved in inflammation and tissue repair. Although activated PG derived from adult peripheral blood has been extensively used for topical therapy of various clinical conditions, few observations on PG from umbilical cord blood (CB) have been reported so far. Interestingly, literature few experimental data show that the levels of growth factors (GFs) contained in CB-derived PG (CB-PG) are higher than those contained in PG produced from adult peripheral blood, suggesting that CBPG may represent a preferable source for tissue engineering applications. A comprehensive characterization of CB-PG is still missing. Therefore, the innovative goal of our research was to use proteomics technologies in order to profile biologically active components in these novel blood components by means of mass spectrometry-based quantitation methods (untargeted label-free and targeted multiple reaction monitoring approaches). Attention was focused on GFs by using “proteotypic peptides” (i.e. peptides with sequences unique to the target protein and thus selected as surrogates for the parent protein). Prior to in-solution tryptic digestion, however, samples were subjected to pre-fractionation techniques to improve the detection of low-abundance proteins. This approach may represent a rapid method for quantification of GFs that bypasses using of expensive antibodies and solves the problem of false positives due to the cross-reactivity of ELISA tests.
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Allogeneic platelet gel and bedsores in elderly patients

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We evaluated growth factor contents and clinical efficacy of allogeneic platelet gel (PG) prepared with standard blood banking procedures from routine buffy coat platelet concentrates (PCs). The PGs were used to treat 11 hypomobile elderly patients unable to undergo autologous blood processing, previously ineffectively treated with advanced medications for 8-275 weeks. PGs were prepared by platelet activation with human thrombin or commercial batroxobin. Median and range growth factor contents (ng/mL) were: platelet derived growth factor (PDGF-AB/-BB) 112 (31-157) and 20 (3.8-34); transforming growth factor (TGF-b1/-b2) 214 (48-289) and 0.087 (0.03-0.28); basic-fibroblast growth factor (b-FGF) 0.03 (0.006-0.214); vascular endothelial growth factor (VEGF) 1.15 (0.18-2.46); epidermal growth factor (EGF) 4.50 (0.87-6.64); insulin-like growth factor (IGF-l) 116 (72-156).

In the clinical study, 222 PGs were used within 2 h of activation to treat 14 chronic skin ulcers in the 11 patients. No improvement was seen in 3 patients with 24, 27 and 30 cm3 ulcers treated respectively for 4, 7 and 8 weeks, due to progressively worsening clinical conditions. The residual 11 ulcers with 3.2 cm3 median size (range 0.2-3.6) in the remaining 8 patients showed 91±14 % reduction after a median of 12 weeks treatment (range 1-20). Cost of PG treatment (19,976 euro) amounted to about 10% of the ineffective advanced medication hospital reimbursement fees (191,236 euro). This study supports efficacy and feasibility of allogeneic PG to treat recalcitrant ulcers in very elderly hypomobile patients for whom autologous blood processing may not be possible.
Complex Wounds: Lower extremities ulcers in blood disorders

Caterina P. Minniti
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The past five decades have seen an improvement in the mortality and morbidity of sickle cell disease (SCD), because of prophylaxis against infectious complications, improved and expanded red cell transfusion therapy, hydroxyurea, and improved supportive care. Since the vast majority of patients in the western hemisphere reaches adulthood and middle age, end organ diseases are more frequent, including vasculopathic complications such as leg ulcers. Their incidence in SCD is unknown, as the only large prospective data comes from the Cooperative Study of Sickle Cell Disease, > 20 years old and skewed toward a young population. Interpretation of studies is complicated by lack of clear separation between prior history of leg ulcers and active ulcer. A recent survey of North American hematologists estimated a 1% SCD incidence of active ulcers, while in a prospective cohort of more than 500 patients: 22% of HbSS and 9% of HbSC self-reported a “history of ulcer.” Regardless, leg ulcers occur ten times more frequently in SCD than the general population and begin at a much younger age.

There is wide geographic variation, ranging from 29.5% of HBSS in Jamaica, to none in Saudi Arabia. Skin ulcerations occur in other blood disorders, such as thalassemia intermedia, hereditary spherocytosis, pyruvate kinase deficiency, congenital dyserythropoietic anemia and HbCC, suggesting that sickling is not a conditio sine qua non for ulcer formation, while chronic anemia and hemolysis are. Recent insights into their pathophysiology may have practical implications, as indicated in a trial of topical sodium nitrite. In the management of leg ulcers, a team of health care professionals needs to address both the immediate consequences of pain, infection and disability, and long term effects on quality of life, employment and stigma.
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Round Table

“The clinical and economical burden of skin ulcers in different countries and conditions: can we spend less and be more clinically effective? What is the role of patients?”
Leg ulcers in Brazil

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Leg ulcers have been a public health problem, generating social and economical damages. In Brazil the GTFe, a specialized technical group in wounds, has created a scale to evaluate the severity of wounds based on the PUSH 3-4 Scale. There are three kinds of treatment: 1\textsuperscript{st} Basic care treatment, 2\textsuperscript{nd} Secondary attention care, 3\textsuperscript{rd} Home care program. Over 10\% of the population above 40 years in Brazil have diabetes. Foot ulcers vary in 2-3\% a year with 4 – 10\% the prevalence. 80\% of these ulcers undergo amputations. In the State of Bahia, SCD with 15 million residents, of which 12 million are of African descent, 1 out of every 658 are born with SCD. In the clinic hospital at the University Federal da Bahia (UFBA), in our SCD ambulatory, our goal for patients with leg ulcers is healing within a reasonable time where the patient could return to normal activities as soon as possible. After the use of VAC and cellular therapy, we perform autologous skin graft or allogenic decellularized skin graft. Autologous transplant of bone marrow is based on the prevalent hypothesis that lineage cells fibroblast derived from a cell trunk of the medular stroma accelerates the healing, increases the number of fibroblast, and establishes rapid closure of ulcers. So far, I have operated on 30 patients with 40 ulcers. Out of the 40 ulcers, 20 have closed without recurrence. Patients with large ulcers need a quantity of stem cells that is difficult to retrieve and administer.
Diabetic foot treatment in India

Maneesh Singhal

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The spectrum of Diabetic foot ranges from incipient sensory neuropathy to fulminant necrotizing fasciitis. Unfortunately, patients are frequently unaware of the debilitating sequels and disastrous complications that can potentially change the course of their lives. Treatment of this heterogeneous entity is labor intensive, complex, confers significant economic burden and not much evolved. The problem is compounded by lack of education and medical access in developing world. The most effective preventive strategy so far has been patient training and education. There is strong evidence to suggest that consistent preventive care can reduce amputations among patients with diabetes. Loss of protective sensation (LOPS) renders these patients vulnerable to trivial trauma, which can evolve significantly before getting noticed. Since up to 50% of patients with significant neuropathic sensory loss may be completely asymptomatic, searching for early signs of infection, skin breakdown, ulcer formation and skin temperature changes can raise alarm for the alert patient and hence rescue the limb preemptively. Patient should be trained to look and report for discolorations, calluses, wounds, fissures, macerations, nail dystrophy or paronychia on a day to day basis. Feet should be kept dry and moisture avoided. Interdigital spaces can hide a significant story unless routinely examined and taken care of. Skin discoloration or loss of hair growth may be the first signs of vascular insufficiency, while calluses and hypertrophic skin often are precursors to ulcers. Patient training not only minimizes dispensable medical costs, but also reinforces doctor-patient relationship besides gives a positive feedback to the treating doctor in terms of increased compliance, lesser foot complications and eventually, lesser number of limbs lost per year.

Till the time comes when promising but still experimental treatments for diabetic foot become available to the common man, one must bank on the age old wisdom: Prevention is better than cure.
Diabetic foot treatment in India
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References:


Cord Blood: Current experiences and Future program in Iran

Amir Ali Hamidieh¹, Mahin Nikougoftar Zarif²

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Cord blood stem cell transplantation is a lifesaving strategy for patients with hematologic malignancies and some non-malignant disorders. Multiple culture strategies and molecular treatments are used to overcome Cord blood (CB) cell dose limitation. In Iran, Double CB and intra bone injection of hematopoietic stem cells (HSCs) significantly increased the rate of engraftment. Recently, CB and its biological tissues, has been under intense investigation for cell therapy. Mainly, CB derived Mesenchymal stem cells (MSCs) are widely used in a broad spectrum of cell therapy. Immune regulatory properties of MSCs lead to use it for preventing some immunologic disorders. A Clinical trial in HSCs transplantation together with MSCs in Iran, revealed decrease in prevalence of GVHD after HSC transplantation. Also, autologous injection of MSCs to Iranian patients with multiple sclerosis showed immune regulatory effects and decrease the patients symptoms.

Platelet lysate extracted from CB contains lots of growth factors and use to treat wound healing and skin injuries. Treatment of chronic foot ulcers in Iranian diabetic patients with Platelet lysate caused shortening of wound repair period.

CB differentiates into mature cell types and could simply replace damaged tissues and reduce inflammation in the site of injury. CB is also a valuable source for neuronal regenerative medicine. Several trials were done on paraplegia patients based on cell therapy in Iran that showed some signs of repairing spinal cord.

Altogether, the results of clinical trials and cell therapy using CB and relating biological products makes progressive promising future in treatment of wide range of diseases.
Innovative wound healing with stem cells, growth factor and artificial dermis in Japan

Sadanori Akita
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In treatment wounds, in the fresh, acute wounds and most of debrided chronic wounds responds well to external growth factors. In Japan, commercially available and of 10-year-history, namely human recombinant basic fibroblast growth factor (bFGF) can effectively improve the quality of wounds in spectrum of pliability, softness, color-match and visco-elasticity as well as accelerate wound healing. Artificial dermis, which composes two layers and can incorporate the cells and factors to develop the wound bed matrix for preparation. Intractable and prolonged wounds after irradiation, Buerger disease, ulcerative colitis and Crohn’s disease, some new breakthrough concept should be applied with minimal morbidity, easy, safe and effective method. There are clinical evidences that the local somatic stem cells are expressed in the adjacent to of the injured wounds. Among such somatic stem cells, adipose-derived regenerative cells (ADRCs) are highly expected cell source, because the donor-site morbidity is minimal and quickness and easiness of cell processing in a closed circuit. As our consecutive 11 radiation injury clinical series, 2 Crohn’s disease, 1 ulcerative colitis and 1 Burger’s disease, aged 18 to 89 years (55.3 ± 18.1 years) among radiation injury with more than 30 years of duration after sequential 50 Gy of the radiation-induced wound treatment with ADRCs redeemed the regenerative wound healing and local tissue regeneration including tendon, bone, fat and muscle as well as skin re-surfacing with artificial dermis over the exposed bone and tendons and growth factor as external stimuli. Therefore, the strategy of using growth factor, artificial dermis as scaffold and cells from the patients’ own will benefit for treatment of most intractable and difficult wounds, which were in the past so hard-to-heal completely.
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Leg Ulceration in Sickle Cell Anaemia

Olu Akinyanju
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With over 40 million of her population being healthy carriers of the sickle cell trait (HbAS) and over 150,000 babies born annually with sickle cell anaemia (HbSS), Nigeria has the largest burden of sickle cell disorder (SCD) in the world. For unclear reasons, leg ulceration occurs in sickle cell anaemia as in all other chronic hereditary haemolytic anaemias (e.g hereditary spherocytosis and thalassaemia major). Happily, but yet unexplained, the prevalence of leg ulceration in patients with SCD in Nigeria is < 8% which is far less than its reported prevalence of > 70% in the USA and in Jamaica. Nevertheless, the single or bilateral ulcers, which occur more commonly in males than in females, are very slow to heal and have a high rate of recurrence after healing even if by autologous skin grafting. Although bed rest and daily dressings promote healing, these measures are often impracticable because of the patient’s need to attend school or place of work and the expense of hospital admission. Over the years, the ingestion or direct application of various recommended substances have not been shown to accelerate healing or prevent recurrence.

Finally, sickle cell leg ulceration, though not life-threatening, are unsightly, painful and a source of great concern, frustration and depression.

The time is certainly ripe for us to try the effect of cord blood platelet gel on their healing.

References:


Current treatment of corneal lesions

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The external eye is the most crucial part of the body exposed to the outside world. Epithelium of healthy cornea adheres tightly to its basement membrane. Regulation of cellular growth and metabolism is critical to the maintenance of an intact ocular surface and a transparent cornea. For its nutrition, the cornea depends on glucose diffusing from the aqueous humor and oxygen diffusing through the tear film. In addition, the peripheral cornea is supplied with oxygen from the limbal circulation.

Corneal lesions can be the result of many causes: immune related disorders, traumatic injuries, infectious diseases, dry eye syndrome, corneal dystrophies many others.

The pattern of corneal inflammation, or *keratitis*, can be characterized by epithelial and/or stromal and/or endothelial involvement.

The treatment of corneal lesions varies widely according to clinical condition: first removing the triggering event and secondary helping the tissue to restore integrity and transparency.

Treatment of corneal lesions can range from lubrication, protection (starting from contact lenses to amniotic membrane), topical antibiotics antiviral or antifungal eye drops, to immunesoppressive treatment (steroid or cyclosporine).

A completely new developing branch of corneal treatment is now being explored: the biological eye drops.

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Biological eye drops

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Blood derived Biological eye drops, including autologous serum (AS), platelets rich plasma (PRP), and umbilical cord serum (UCS) have been introduced in the treatment of many ocular diseases, because they contain a high concentration of biological active components (mucin, aqueous and lipid layers, vitamin A) and growth factors, that usually found in tears film and are essential for regulating the proliferation, differentiation and maturation of the ocular surface epithelium. Ocular surface disorders including dry eye disease or keratoconjunctivitis sicca are characterized by a decrease in quality and quantity of the tears film and squamous metaplasia of the conjunctival epithelium. Conventional treatment for ocular surface disorders including the application of artificial tears, topical anti-inflammatory agents, therapeutic contact lenses and punctual occlusion are less effective than biological eye drops.

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