Supplementary material Br J Ophthalmol

SUPPLEMENTARY FILE 1

Adult donors were requested to sign an informed consent after having been explained the scientific rationale of the study. Blood samples were collected from 105 donors by venipuncture at the time of donation. All 107 Cord Blood (CB) units were collected at the time of delivery after compilation of a donor selection questionnaire and signing informed consent; all steps from the recruitment to the processing and registration of CB were performed according to standard operating procedures and guidelines issued by the Foundation for the Accreditation of Cellular Therapy (FACT). Cord blood was collected from spontaneous term births free of complications (≥37th week of pregnancy) and Caesarean births decided by trained and qualified health personnel. CB collection for transplantation purposes was performed when the placenta was still in utero, and for ophthalmic purpose, samples from ex utero placenta vessels were transferred in 9 ml Vacumtube (Biomed Device, Italy) without any anticoagulant. The medium volume of collected samples was 7±1,5ml. Adults' PB was collected in 9 ml Vacumtube (Biomed Device, Italy) without any anticoagulant during normal donation. For further processing, the unit and the related samples were sent to the Processing Facility (PF) laboratory of the CB Bank opened 24/24hrs, 7/7 days, where it went through a series of checks and tests to establish the blood characteristics and its suitability for preservation and therapeutic use. Maternal infectious disease markers (HIV, HCV, HBV Treponema pallidum, CMV, Toxoplasmosis and HTLVI/II) evaluations were performed. Suitable samples were centrifuged at 2.800 g for 10 min and serum samples were transferred in sterile tubes under laminar flow hood and stored at -80°C. Both cord and adult serum samples had an average volume of 4 ± 0.5ml. Serum from both sources were tested for the presence of selected growth factors: IL-1β, IL-4, IL-6, IL-10, IL-13, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), epidermal growth factor (EGF), transforming growth factor (TGF)-α, TGF-β1/β2/β3, insulin growth factor (IGF)-1 and IGF-2. The differential analysis of the levels has been the subject of a separate study, quote in the manuscript with ref [21] Sera with a value of EGF <800 pg / ml were excluded...

To prepare CBS and adult eye drops, sera of same blood group were thawed and pooled to obtain the needed amount of serum to treat all patients, diluted to 20% with refrigerated sterile phosphate buffered saline with aseptic technique, and filtered (Millex HV 0.4 mm). The eye drops solution was then aliquoted using the COL-20 medical device (Biomed, Modena, Italy) to prepare unidose vials, packed, frozen, and stored at – 80°C. Patients were instructed to administer one drop per eye for 8 times a day, after having thawed 1 vial the evening before the day of use.