THERAPIES BASED ON SUBSTANCES OF HUMAN ORIGIN (SoHO)

Brief report

Characterization of growth factors in platelet-poor plasma for eye drop applications from umbilical cord blood and from allogeneic adult donor serum

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INTRODUCTION

Dry Eye Disease (DED) is a multifactorial ocular surface disorder characterized by tear film instability, inflammation, and epithelial damage, with a major impact on quality of life, especially in women and the elderly. Severe immune-mediated forms[1] often remain refractory to conventional topical treatments[2]. In this setting, blood-derived eye drops (ED) have become an established non-transfusion application of human blood components in ophthalmology, due to their content of growth factors (GF) and bioactive mediators that support corneal epithelial repair. Platelet-derived factors, including epidermal growth factor (EGF); vascular endothelial growth factor (VEGF); fibroblast growth factor (bFGF); platelet derived growth factor (PDGF), play a central role in ocular surface regeneration, and allogeneic adult blood serum ED (AB-S) currently represent the most standardized and regulated biological product used for the treatment of DED[2].

Over the past three decades, human umbilical cord blood (CB) from healthy newborns has been widely used as a source of stem cells for hematopoietic transplantation, alongside other sources of hematopoietic progenitors. However, the clinical efficacy of CB transplantation is closely correlated with the cellular content, particularly the number of total nucleated cells (TNC), a critical parameter for hematopoietic engraftment and immune reconstitution in the recipient[3]. It has been observed that an adequate TNC dose is present in less than 10% of collected CB units, resulting in a high discard rate of biological material and a significant limitation in its effective clinical utility. This limitation stimulated growing scientific interest in alternative uses of CB as a source of biological materials suitable for novel therapeutic applications[4].

In this framework, platelet-poor plasma derived from CB (CB-PPP) is an attractive substance of human origin (SoHO) for the treatment of inflammatory ocular surface diseases, due to its unique content of immunomodulators[5]. Compared to AB-S, CB-PPP is expected to differ biologically because of its neonatal origin. In fact, CB is characterized by an immunologically immature and tolerogenic profile, with a different balance of cytokines, growth factors, and anti-inflammatory mediators compared to adult peripheral blood[6-8]. In addition, CB-PPP is obtained from anticoagulated blood without clot formation, unlike AB-S, which is derived after coagulation and platelet degranulation. This difference in processing may further influence the composition and concentration of bioactive molecules, particularly platelet-derived growth factors.

The aim of this descriptive, non-comparative pre-clinical study was to characterize the content of EGF, VEGF, bFGF, PDGF-AA, -BB, -AB in CB-PPP and in AB-S in order to provide

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a biological basis for the evaluation of CB-PPP in ocular surface applications (NCT07089511). The selected growth factors were chosen based on their well-established role in corneal epithelial repair, angiogenesis, and stromal regeneration, as well as their documented presence in blood-derived ocular therapies. In addition, their selection was supported by previous literature on cord blood-derived products and by the availability of validated multiplex assays enabling their simultaneous and reliable quantification[2,7].

MATERIALS AND METHODS

The study was carried out during January 2024 - June 2025. EGF, VEGF, bFGF and PDGF-AA, -BB, -AB, were determined in 20 CB-PPP and 6 AB-S units using a customized Luminex assay plate (Luminex xMAP platform, Luminex Corporation, Austin, TX, USA).

CB was collected with informed maternal consent from term, healthy newborns in commercial JMS (Singapore, code 811-1009) and Macopharma (Tourcoing, France; code: MSC1210PU) collection bags. Cord blood units were obtained from term deliveries, units were stored and transported at 4°C and, if deemed unsuitable for clinical transplantation, processed for research use. Inclusion criteria were: nucleated cell count below threshold for hemopoietic transplantation, collection within 48 hours, absence of clots, and net CB volume ≥50mL. All units underwent standard infectious disease screening. CB-PPP was prepared by differential centrifugation following a standard "multicomponent" fractionation procedure using the BioNest ABC commercial system (White Nest Pharma, Milan, Italy), aimed at obtaining leukoreduced red blood cells (CB-RBC), platelet rich plasma (CB-PRP) and CB-PPP, as described in detail elsewhere[9].

Adult blood was collected (mean volume: 420 mL) after informed consent in transfer blood collection bags without anticoagulant and stored at 4°C overnight to promote clot formation, platelet derived GF release and serum separation in compliance with the EDQM guide[10]. The units were centrifuged at 4,070 *g* (Cryofuge 6000i, Kendro Laboratory Products GmbH, Hanau, Germany) for 10 min, serum was manually expressed into a transfer bag, and a second centrifugation (2,900 *g*, 5 min) was performed to remove residual RBC.

AB-S samples were obtained from individual allogeneic donations intended for the routine production of serum eye drops for clinical use, without pooling. Donor demographic characteristics (e.g., age and sex) were not available, as samples were anonymized and the study was focused on the characterization of the final biological product rather than on donor-related variables.

After preparation, CB-PPP and AB-S samples were aliquoted and stored at −80°C until analysis. All samples were thawed only once and analysed. Thawing was performed at room temperature and samples were gently mixed prior to assay to ensure homogeneity.

White blood cells (WBC), RBC, and platelets counts were determined in CB-PPP and AB-S with a Sysmex XN-1000 system (Sysmex Corporation, Kobe, Japan). Data were collected and analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and are reported as median and range. Statistical analysis was limited to descriptive statistics only.

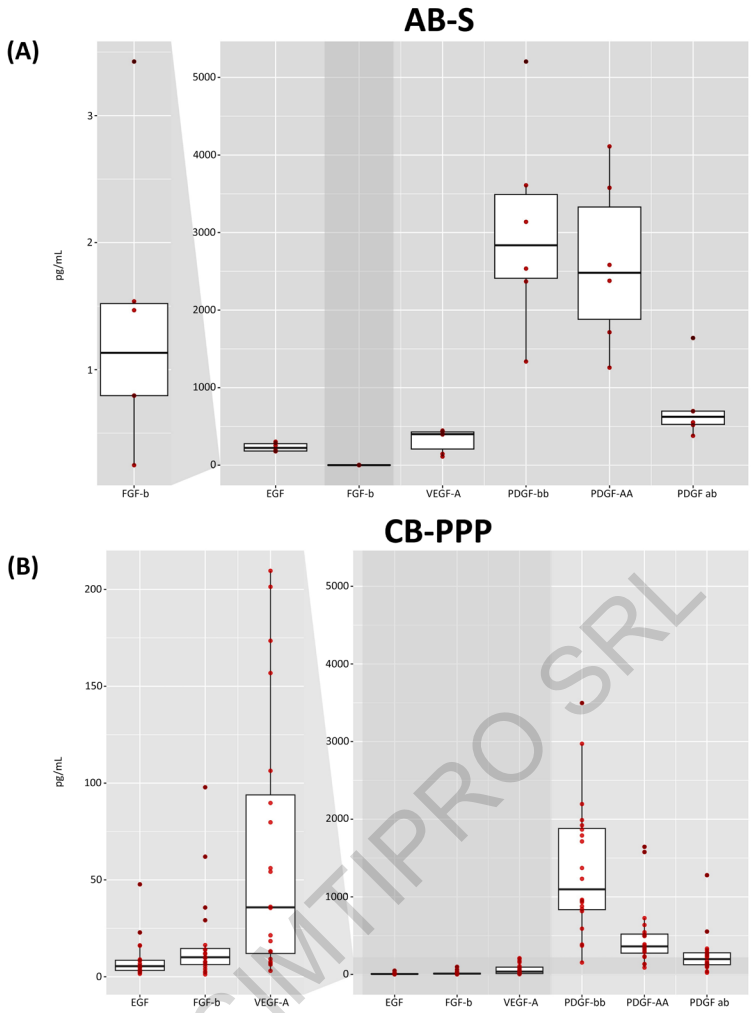
Given the descriptive and exploratory nature of the study, the limited sample size, and the imbalance between groups (CB-PPP No.=20; AB-S No.=6), no inferential statistical analysis was performed in order to avoid overinterpretation of the data. Instead, individual variability was considered relevant and results are reported using descriptive statistics.

RESULTS

CB-PPP volume showed a median (range) of 40 mL (21-62). Median (range) platelet, WBC and RBC counts were: 11×10^3/µL (5-20), 0.06×10^3/µL (0-0.31) and 0×10^6/µL (0-0.2) respectively. AB-S volume showed a median (range) of 187 (167-192) mL, while platelet, WBC and RBC counts were below the lower detection limits. Individual GF values are shown in **Figure 1**, highlighting marked inter-sample variability in both groups. Outlier analysis was performed using the Tukey method (1.5×IQR). A limited number of outliers were identified in the CB-PPP group, ranging from 0 to 2 values depending on the growth factor analyzed. However, these represented only a very small proportion of the samples and did not appear to drive the overall observed differences.

The results of GF evaluations are reported in **Table I** and show a distinct quantitative distribution between CB-PPP and AB-S. EGF, VEGF and PDGF-AA, -AB and -BB

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Figure 1 - Distribution of growth factor concentrations in platelet-poor plasma units derived from cord blood (CB-PPP) and allogeneic adult eye drops serum units (AB-S)
Individual data points are shown together with box plots indicating median and interquartile range.

Table I - *Growth factor analysis of platelet-poor plasma units derived from cord blood (CB-PPP) and allogeneic adult eye drops serum units (AB-S)*

Growth Factor	CB-PPP (No.=20) Median (range) pg/mL	AB-S (No.=6) Median (range) pg/mL
EGF	5.45 (1.52-47.70)	224.0 (179.29-303.24)
VEGF	35.83 (3.05-209.64)	401.26 (111.31-446.99)
bFGF	10.07 (1.19-97.85)	1.13 (0.25-3.43)
PDGF-AA	362.05 (87.86-1644.86)	2,480.44 (1258-4110.96)
PDGF-BB	1,097.11 (153.14-3496.79)	2,836.29 (1337.20-5204.53)
PDGF-AB	197.06 (22.26-1278.43)	624.14 (379.42-1640.61)

EGF: epidermal growth factor; VEGF: vascular endothelial growth factor; bFGF: fibroblast growth factor; PDGF-AA/AB/BB: platelet derived growth factor. All analytes were measurable in all samples, and no values below the limit of detection were observed.

levels were lower in CB-PPP compared to AB-S, whereas bFGF levels were higher in CB-PPP. When expressed as fold-change, EGF, VEGF and PDGF isoforms were approximately 41-fold, 11-fold, 7-fold, 3-fold and 2.6-fold lower in CB-PPP compared to AB-S, respectively, while bFGF was approximately 9-fold higher.

DISCUSSION

Different formulations of biological ED derived from blood serum and platelet lysate have been used in laboratory studies and successful experimental clinical trials[6,11]. The aim of this study was to improve the knowledge on CB-PPP as an additional potential blood-derived

ophthalmic product for the treatment of DED and other ocular surface diseases.

Through a standardized closed-system process, a cell-depleted plasma fraction was reproducibly obtained from non-transplantable CB units, compliant with quality and safety requirements and containing a biologically relevant concentration of GF. This approach enables the valorisation of otherwise unusable CB units, generating a standardized human-derived product consistent with current SoHO regulatory principles[12].

In this context, the GF profile identified in CB-PPP reflects the major biological pathways involved in corneal repair and ocular surface homeostasis. EGF and PDGF isoforms act as central regulators of epithelial migration, proliferation, chemotaxis, and stromal cell modulation, while bFGF contributes to epithelial and stromal repair by supporting cell survival and barrier integrity, with cytoprotective and anti-inflammatory effects reported in experimental dry eye models. VEGF, the principal pro-angiogenic mediator, exerts a trophic role but, when overexpressed, is implicated in pathological neovascularization in inflammatory ocular surface diseases[8,13]. Together, these mediators define an integrated trophic and immunobiological network that provides a coherent biological rationale for the regenerative and therapeutic potential of CB-PPP and for the clinical efficacy of blood-derived eye drops in DED and ocular surface disorders.

Consistent with this biological framework, CB-PPP exhibits a quantitatively and qualitatively distinct GF profile from AB-S. Although AB-S shows higher absolute concentrations of several platelet-derived mediators, it is routinely diluted in clinical practice[14], resulting in reduced GF exposure. In contrast, CB-PPP can potentially be used undiluted or with lower dilution, thereby preserving the integrity of its biological content. Notably, CB-PPP displays about 9-fold higher levels of bFGF, a factor implicated in epithelial repair and cellular stress protection, and an intrinsically lower VEGF content, which may reduce the risk of angiogenic stimulation in chronic inflammatory settings[15].

Importantly, the lower concentration of several growth factors in CB-PPP should not be interpreted as reduced biological activity per se, but rather as indicative of a distinct biological profile. In this perspective, CB-PPP may provide a more balanced trophic and immunobiological environment, potentially better suited for conditions characterized by chronic inflammation and dysregulated healing, such as DED.

Alongside its trophic profile, potential immunobiological implications also emerge.

Beyond growth factors, CB-PPP may contain a complex network of bioactive molecules, including cytokines, chemokines, complement-related factors, soluble immune checkpoints, and other immune-regulatory mediators that were not assessed in the present study. These components are known to play a key role in ocular surface homeostasis and inflammation, and their potential contribution to the biological activity of CB-PPP warrants further investigation.

While the present study provides a descriptive characterization of growth factor content, the potential immunomodulatory properties of CB-PPP remain hypothetical and are inferred from the known biological features of cord blood. These aspects should be specifically addressed in future studies designed to investigate immune-related pathways.

Overall, CB-PPP does not represent a quantitatively superior formulation, but rather a biologically distinct product characterized by a balance between regenerative stimulation, controlled angiogenic signalling, and potential immune modulation.

Limitations of this preliminary study include a small sample size, which could be expanded in future, larger determinations. Furthermore, we acknowledge that additional known and unknown GF and immunomodulatory molecules that were not investigated in this study could play a role in the molecular pathways involved in corneal repair.

CONCLUSIONS

In conclusion, this descriptive pre-clinical study provides a characterization of CB-PPP, highlighting a distinct biological profile compared to AB-S. These findings support its potential relevance for ocular surface applications. The use of CB-PPP may also contribute to the valorisation of otherwise discarded CB units. Further preclinical investigations and well-designed randomized clinical trials will be required to define its safety, standardization, and therapeutic efficacy.

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Ethical consideration

The research was conducted ethically; all human samples were collected with written informed consent for research purposes and all experimental procedures were conducted according to the Italian law about the Regulation (EU) 2016/679 (General Data Protection Regulation) articles 5 and 11. All data were anonymous and for these reasons it was not necessary to receive the opinion of the ethics committee (Comitato Etico Territoriale Lombardia 3). The study was approved by the BST ethics committee: PR-BST-409-2019.

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Authors' contributions

TM, SV and PR designed the study. LM and DS performed the experiments and acquired the data; performed the data analysis and wrote the manuscript. PR, DP, MB and JFS reviewed the results and the manuscript. All Authors revised and approved the final manuscript.

Conflicts of interest

PR is co-inventor of patents on novel cord blood products, founder, equity holder and scientific director of Episkey S.r.l., and equity holder and scientific director of White Nest Pharma S.r.l.; DP serves as Section Editor in BT and has received travel and research grants, speaking fees, and teaching fees from Diasorin, Diamed, Diatech Pharmacogenetics, Grifols, Immucor, Macopharma, Ortho Clinical Diagnostics, Terumo, Cerus. All other Authors have no conflicts of interest to disclose.

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