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Safety and exploratory functional effects of topical cord blood serum in glaucoma patients

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To evaluate the safety, tolerability, and potential functional signals associated with cord blood serum (CBS) eye drops as adjunctive treatment in patients with open-angle glaucoma (OAG) already under intraocular pressure (IOP)-lowering therapy. In this monocentric prospective pilot study, 20 OAG patients (37 eyes) received topical CBS eye drops 8 times daily for 60 days, in addition to their standard hypotensive therapy. Ophthalmic evaluations were performed at baseline (V1), end of treatment (V4), and 60 days after discontinuation (V5), and included best-corrected visual acuity (BCVA), IOP, visual field (VF), pattern electroretinography (PERG), and retinal nerve fiber layer (RNFL) thickness. Statistical analyses assessed changes in functional and structural parameters. The treatment was well tolerated, with no adverse events and no significant changes in IOP or BCVA. Visual field mean deviation (MD), PERG parameters, and RNFL thickness showed non-significant variations across visits. A statistically significant RNFL thinning was observed in the infero-temporal sector between V1 and V4, although likely due to outlier effects. Linear mixed model analysis showed a significant increase in N95 amplitude at V5 compared to V4 when baseline MD was considered as a covariate. CBS eye drops were safe and well tolerated in this glaucoma population. Although no statistically significant functional or structural improvement was observed, some exploratory signals suggest potential neuroretinal involvement that warrants further investigation in larger, controlled studies.

Keywords Glaucoma, Cord blood serum, Eyedrops, BDNF, NGF, Neuroprotection

Glaucoma refers to a multifactorial group of diseases characterized by optic neuropathy with distinctive visual field defects. Glaucomatous damage to the optic nerve is attributed to the progressive degeneration and apoptosis of retinal ganglion cells (RGCs)[1]. To date, intraocular pressure (IOP) remains the primary targeted risk factor for halting optic damage progression[2,3]. However, despite successful IOP control by medical therapy, some patients exhibit progressive worsening of visual field defects with RGC and optic nerve degeneration and abnormalities in pattern electroretinography (PERG) and visual evoked potentials (VEP) responses[4,5].

To address this issue, various neuroprotective agents have recently gained attention for their ability to prevent RGCs loss, by either stimulating cell survival or inhibiting cell death pathways[6]. Among these, the nerve growth factor (NGF) has emerged as particularly relevant due to his crucial role in the survival, growth and maintenance of specific types of neurons in central and peripheral nervous system, including RGCs[7]. Several preclinical studies have shown that intraocular administration of NGF can inhibit RGC degeneration following mechanical, ischemic or hypertensive injury[8–12]. Moreover, long-term improvements of the visual field, optic nerve function function (measured by PERG and VEP), contrast sensitivity and visual acuity were reported after therapy with NGF in patient with glaucoma, suggesting a role of NGF in enhancing neural conduction along the post retinal visual pathways[11].

Another neurotrophic factor that contributes to RGC survival is brain-derived neurotrophic factor (BDNF), which plays an important role in neural development, synaptogenesis modulation, and neuroprotection[8]. In animal models of ocular hypertension and glaucoma, BDNF has shown a protective effect on RGC dendritic architecture and visual function following injury[13,14]. In addition, preclinical evidence from an in vivo model of retinal neurodegeneration showed that CBS can modulate neuronal stress responses and preserve retinal

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function[15]. Different delivery strategies for neurotrophic factors have been explored in ophthalmology, including topical administration for corneal disorders, intravitreal injections in experimental optic nerve damage, and systemic administration in preclinical models, each demonstrating biological activity in ocular tissues[11,16].

The topical delivery of a pool of neurotrophic factors, including NGF and BDNF, via cord blood serum (CBS), has been studied in ocular surface diseases involving nerve damage, such as neurotrophic keratopathy and severe dry eye. These studies demonstrated its ability to regenerate injured corneal nerve infrastructure[17–21]. However, CBS has not yet been investigated in glaucoma patients to address the progression of visual field damage.

Given its biological properties, CBS may represent a valuable adjunct to conventional IOP-lowering therapies. Thus, the aim of this study is to evaluate the safety of CBS administration in glaucoma patients and to explore whether its addition to IOP-lowering therapy can influence the anatomical and functional damage caused by the disease.

Methods.

This was a mono centric prospective pilot study enrolling adult open angle glaucoma (OAG) patients at the Ophthalmology Unit, I.R.C.C.S. Azienda Ospedaliero-Universitaria di Bologna between March 2018 and May 2023 (with a stop between March 2020 and March 2022, due to COVID-19 pandemia). Patients were recruited from the cohort of those undergoing regular follow ups in our glaucoma service for at least 5 years. The study was performed in respect to the principles in the Declaration of Helsinki, it was approved by the Comitato Etico Indipendente dell'Azienda Ospedaliero-Universitaria di Bologna – Policlinico S. Orsola-Malpighi (128/2017/U/Sper, approved November 7th, 2017) and registered in an international registry (NCT03609125, clinicaltrials.gov, 23/07/2018).

Patients

To be eligible for the study, patients had to meet all the inclusion criteria both at the screening and the baseline visit. Inclusion criteria were as follows: physical and mental ability to undergo the required examinations, patients were required not to show signs of disease progression at enrolment but to present a stable and measurable visual field (VF) defect consistent with glaucoma, mean deviation (MD) between − 3 and − 20dB, average retinal nerve fiber layer (RNFL) thickness ranging from 60 μm to 90 μm in at least one eye. Best-corrected visual acuity (BCVA) of better than 20/200 in both eyes, IOP less than 21mmHg controlled by IOP lowering therapy.

Exclusion criteria included primary or secondary closed angle glaucoma, unilateral blindness, optic nerve atrophy, use of systemic steroids or other immunosuppressive drugs, undergoing chemotherapy, history of ocular herpes simplex or zoster, diagnosis of uveitis or other ocular inflammatory diseases, evidence of corneal keratopathy, opacification or any lack of optical clarity, diabetic macular edema and/or diabetic retinopathy, a history of malignancy, pregnancy, lactation, or a history of using drugs with known retinal toxicity.

Study design

This was a longitudinal, single-group, non-randomized clinical trial conducted over five study visits. No formal sample size calculation was planned, as appropriate for a pilot safety study, the sample size was arbitrarily set at twenty patients. Enrolment was conducted in the Ophthalmology Unit at the IRCCS AOU di Bologna, patients underwent a complete ophthalmic examination, including BCVA, biomicroscopy, IOP measurement with the Goldmann applanation tonometer, fundus evaluation, and gonioscopy. Additionally, the most recent VF test and RNFL thickness profile obtained with optical coherence tomography (OCT) were reviewed. If either of these exams was older than 6 months, it was repeated during the same visit. The evaluation of patient eligibility according to the inclusion criteria was performed by an experienced glaucoma specialist (EL). Patients who agreed to participate in the trial provided written informed consent and were then scheduled for visit 1. All visits were performed late in the morning (range 12.00 am-2.00 pm), to reduce circadian variability.

Visit 1 (V1): At the initial visit, patients underwent a VF test in the study eye, followed by a PERG test. A comprehensive ophthalmic evaluation was then performed, including BCVA, biomicroscopy, IOP measure with Goldmann applanation tonometry, and a fundus evaluation. Optical coherence tomography (OCT) scans were obtained to assess the RNFL thickness. Patient eligibility was re-evaluated by the same specialist.

Visit 2 (V2): Patients who entered the study received CBS eye drops for the initial 30 days of treatment. They were instructed on the treatment regimen, including the proper storage temperature and place for the eye drops.

Visit 3 (V3): This visit was scheduled 30 days after V2. Patients were queried about any local or systemic side effects and any changes in their therapy regimen. All complaints and considerations were recorded. Patients who did not experience side effects or require withdrawal from the study were provided with another 30-day supply of CBS eye drops.

Visit 4 (V4): This visit took place 60 days after the start of CBS therapy (V2). Patients were asked about treatment side effects, and all complaints and considerations were recorded. The same evaluations and examinations as those conducted at the baseline visit were repeated. Patients were then instructed to discontinue CBS therapy.

Visit 5 (V5): This final visit was scheduled 60 days after V4. A complete examination was conducted using the same tests and evaluations as in the previous visits.

A 60-day treatment period was selected in line with prior CBS studies showing good tolerability, and to ensure a conservative exposure appropriate for this pilot safety design.

Study treatment

CBS eyedrops were prepared as previously reported by our group[21]. Briefly, the CBS was obtained from mothers with vaginal or caesarean section delivery after informed consent. Blood samples, collected from the umbilical vein, were clotted for 2 h at room temperature. After centrifugation at 3000 rpm for 15 min, the serum was carefully isolated, and frozen for the quarantine period. To normalize biological variability of the donors and

standardize the preparations, ten pools from ten CBS matched AB0 groups were prepared, tested for BDNF levels to minimize inter-donor variability and ensure consistency of a known neurotrophic component (ELISA, kits from Bio-Techne-R&D systems), and finally diluted by 20% in sterile saline solution, filtered, aliquoted into COL-20 medical device (Biomed, Modena, Italy) in single dose one-day vials containing 1 mL of product. Vials were packed, frozen, and stored at − 80 °C[20,22] until dispensing. Therapy consisted in the administration of the CBS eyedrops in the study eye at a dose of 1 drop 8 times per day during the waking period, 30 min after the use of any other IOP-lowering topical medication. This treatment regimen was followed for a duration of 60 days. Patients were instructed to store the vials at a temperature of − 20 °C in the home fridge throughout the entire treatment duration, to thaw each vial for the daily administration and maintain it at 4 °C, to avoid contamination, then discard any eventual residue.

Patients did not discontinue their IOP lowering therapy which consisted of an association of various molecules: Timolol 1%, Latanoprost 0.005%, Brinzolamide 1%, Bimatoprost 0.03%, Dorzolamide 2%. For statistical purposes, treatments were categorized into number of molecules utilized/day (Table 1). A washout was performed only for baseline visit planning in terms of time separation from prior CBS use (in case of compassionate previous exposure), not for hypotensive therapy.

Withdrawal criteria included unexpectedly severe progression of glaucoma as measured by VF testing, BCVA, or by examination of the optic nerve; intolerance or allergy to the CBS preparation; local or systemic toxicities; or changes in IOP monitored throughout the study that differed from those expected during glaucoma (considered timing, consistency across visits, and response to therapy adjustments) and that were identified to be potentially related to the CBS treatment.

Functional and morphological outcomes

The functional outcomes studied to assess efficacy on glaucoma progression included mean, median, and distribution of changes in VF, PERG and RNFL thickness across V1, V4, and V5. Specifically, VF was performed with a 30–2 pattern and SITA standard strategy with the Humphrey Visual Field Analyser (software version 1.5.2.431, HFA 3.0, Zeiss, Germany). MD and pattern standard deviation (PSD) indices were collected for each examination.

The PERG was recorded simultaneously for both eyes using the RetimaxPlus system (CSO, Florence, Italy) and according to the ISCEV guidelines (International Society for Clinical Electrophysiology of Vision). After topical anaesthesia with Oxibuprocaine 0.4%, sterile HK loop electrodes were positioned on each lower conjunctival fornix (active electrode), standard skin surface electrodes were taped on the temples (reference electrode), and on the ear lobe (ground electrode), the inter electrode resistance was less than 5 kΩ. The patient sat on a chair at a distance of 57 cm from the television screen (resolution 1,024 × 768; size 34 inches) and fixed binocularly on a red cross at the center of the screen, which subtended a visual angle of 48.89 degrees. Exams were performed with an appropriate refractive correction for the working distance, and patients were allowed to blink freely. The PERG stimulus was first presented as a full-screen black-and-white squares (contrast: 20%; spatial frequency: 0.3 cycles/degree/cpd; temporal frequency: 15 Hz). The number of samples acquired, mediated, and processed with discrete Fourier transform was 300 and the acquisition time was 133 milliseconds.

	Eyes *n* = 37
Gender (males/females)	17 males (31 eyes) 3 females (6 eyes)
Age (years ± SD)	70.24 ± 12.46
Glaucoma diagnosis	
POAG	15 (40.5%)
Pseudoesfoliative	2 (5.7%)
Post-AAC	3 (8.1%)
Therapy (drops)	
1	4 (10.8%)
2	10 (27%)
3	6 (16.2%)
IOP (mean ± SD)	13.73 ± 2.59 mmHg
BCVA (LogMar ± SD)	0.34 ± 0.31
Global RNFL thickness (median; 25–75% percentile)	58 (49.50; 63.50) µm
VF parameters (median; 25–75% percentile)	
MD	− 11.65 (− 14.78; − 6.99) dB
PSD	8.87 (4.34; 11.69) dB

Table 1. Patient's demographic and baseline characteristics. SD, standard deviation; POAG, primary open angle glaucoma; AAC, acute angle closure; IOP, intraocular pressure; CI, confidence interval; BCVA, best corrected visual acuity; RNFL, retinal nerve fiber layer; VF visual field; MD, mean deviation; PSD, pattern standard deviation.

The pattern presentation (approximately 4 min) was preceded by an un-modulated uniform field (approximately 1 min) of the same mean luminance (blank), which was used to evaluate the background noise level. Wave width P50-N95 and wave latency P50 and N95 were collected and compared between the study visits.

RNFL thickness was measured with Spectralis Swept Source OCT (software version 1.10.2.0, Heidelberg Engineering, Germany).

Statistical analysis

Statistical analysis among different times of each measure were first carried out by the Friedman test for repeated measures, the Wilcoxon signed-rank test paired for each eye at the different time points was used for post-hoc analysis. This is necessary to consider the correlation between repeated measures on the same eye. Friedman and Wilcoxon tests were chosen to account for non-normality of the data. For each outcome the test was corrected for multiple testing with the Benjamini-Hochberg method, since each outcome was tested three times, for all possible combination of time steps. P values are given following this correction.

Then a more thorough analysis was carried on the 4 PERG measures (P50 amplitude, P50 latency, N95 amplitude, and N95 latency) fitting Linear-Mixed Effect models for repeated measures, with MD as covariate. The structure of the random effects was tested for each model between the following: random intercept only, random intercept and slope with diagonal covariance matrix and random intercept and slope with full covariance matrix. For all the models the likelihood ratio test gave as result that the best model was the one with random intercept only. The non-independence of measures from eyes of the same patient was tested in the linear mixed models, but not retained in the final model because not significant. The time was introduced into the model as a factor variable, since we only have 3 time steps, of which the reference level was the initial time.

Results

A total 20 patients (37 eyes) were enrolled in the study, their demographic characteristics are shown in Table 1. All patients administered CBS eye drops for the entire study period and were fully compliant with the treatment regimen. During the study, patients continued their clinical standard of care for glaucoma. Results from the BDNF level testing in the CBS preparations showed that the amount of BDNF delivered in each eye, for each day treatment consisted of 1.5 ± 0.2 ng BDNF.

No premature withdrawal, study discontinuation, major protocol deviation, or adverse event was registered. IOP and BCVA did not show statistically significant differences across visits, in both Friedman and Wilcoxon tests (Table 2, all $p > 0.05$). Specifically, IOP remained below 21 mmHg throughout the study period for all patients (Fig. 1), and BCVA remained stable across all three time points.

Mean deviation (MD) values (Fig. 2) did not differ significantly across visits ($p > 0.05$). At V4, a numerical increase in MD was observed in 17 eyes (from 11 patients, 45.9%), with a median difference of 1.69 dB (range: 0.32 to 2.89 dB). Among these, 4 patients showed bilateral increase, while 7 in one eye only. Conversely, twenty eyes showed numerically stable or slightly more negative MD values.; their median change was − 0.81 dB (range − 2.34 to − 0.19 dB). From V4 to V5, eleven of the seventeen eyes that had shown numerically higher MD values at V4 exhibited a partial return toward baseline (median MD variation: +0.63 dB, range − 1.22 to + 1.82 dB). All changes were not statistically significant.

No association was found between MD variation and baseline MD ($p = 0.13$), patient age (coefficient = 0.04; $p = 0.66$), defect location (coefficient = 1.63; $p = 0.48$), or concurrent therapy (coefficient = 1.95; $p = 0.46$). These associations were explored using linear mixed models, testing the best random effect structure by likelihood ratio test. The best fit was the one with only random intercept, showing no significant correlation between the time variation of MD and the baseline individual value.

Functional outcomes are summarized in Table 2. None of the PERG parameters showed statistically significant changes between visits (all $p > 0.05$). A non-significant variation toward increased amplitude at V4 was observed in 12 eyes (43%, 4 patients in both eyes, and 8 patients in one eye) for P50 and in 19 eyes (51.4%, 5 patients in both eyes, and 9 in one eye) for wave amplitude in P50 and N95, with median increases of + 0.93 µV (range: 0.16 to 4.21) and + 0.76 µV (range: 0.31 to 3.99), respectively. In contrast, 25 eyes (67.6%) for P50 and 18 eyes (48.6%) did not show improvement, with median changes of − 0.75 µV and − 0.55 µV, respectively. In

	Visit 1	Visit 4	Visit 5	*P* value
IOP mmHg	14 (12; 16)	14 (11.25; 18)	13 (12;16)	0.1
BCVA (Logmar)	0.34 ± 0.31	0.34 ± 0.31	0.34 ± 0.31	1
MD dB	− 11.65 (− 14.78; − 6.99)	− 9.34 (− 15.22; − 5.61)	− 9.26 (− 15.65; − 5.29)	0.62
PSD dB	8.87 (4.34; 11.69)	8.68 (4.34; 11.69)	8.09 (4.19; 11.93)	0.31
PERG microV				
P50 amplitude	2.31 (1.19; 3.36)	2.08 (1.24; 3.52)	1.64 (1.03; 4.01)	0.07
P50 latency	50.29 (46.88; 54.20)	48.83 (46.88; 53.22)	50.78 (46.88; 58.11)	0.43
N95 amplitude	2.99 (1.77; 4.10)	2.94 (1.94; 4.55)	2.33 (1.44; 3.81)	0.22
N95 latency	107.20 (95.75; 122.60)	110.00 (98.14; 118.8)	107.90 (97.78; 118.8)	0.47

Table 2. Functional outcomes. Results are reported as median (25%; 75% Percentile) or mean± standard deviation. IOP, intraocular pressure; MD, mean deviation; PSD, pattern standard deviation; PERG, pattern standard electroretinography. *P* values are by the Friedman test.

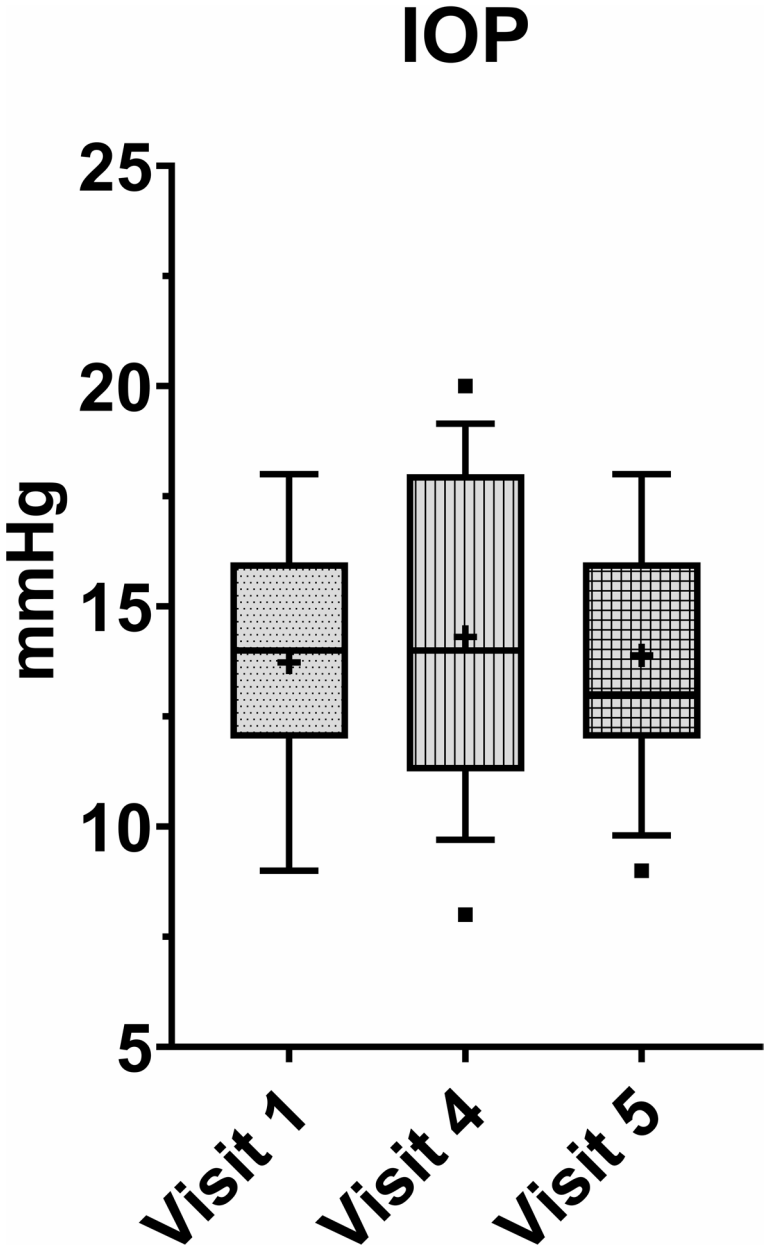

Fig. 1. Boxes and whiskers of IOP measurements in the 3-study visit. IOP: intraocular pressure. Line at median; plus, sign at mean; bar at 5 and 95% percentile.

both cases, amplitude values tended to decline again at V5, (median – 1.68 microV, range 0.8, -5.24 for P50; and median – 0.6 microV, range 1.18, – 3.93 for N95). All variations were not statistically significant.

Regarding RNFL thickness (Table 3), a statistically significant decrease was observed in the infero-temporal quadrant between V1 and V5 (Wilcoxon paired $p = 0.03$), while between V4 and V5 or V1 and V4 there was not significant difference (respectively Wilcoxon $p = 0.67$ and 0.08). This change was largely driven by outliers. No statistically significant variations were found in the other quadrants or in global thickness. For transparency, all non-significant variations have been reported uniformly.

When baseline MD was included as a covariate in a linear mixed model, to evaluate the impact of baseline parameters on study outcomes, a significant increase in N95 amplitude at V5 versus V4 was found ($p = 0.03$). No other parameters showed significant associations.

Discussion

Data from this study confirmed the high safety profile of the CBS topically administered eight times per day for two months in patients with glaucoma, with safety sustained for the subsequent two months post-treatment. No adverse events were reported, and intraocular pressure (IOP) and best-corrected visual acuity (BCVA) remained stable throughout the treatment and follow-up period Adverse events such as eye pain and irritation were not

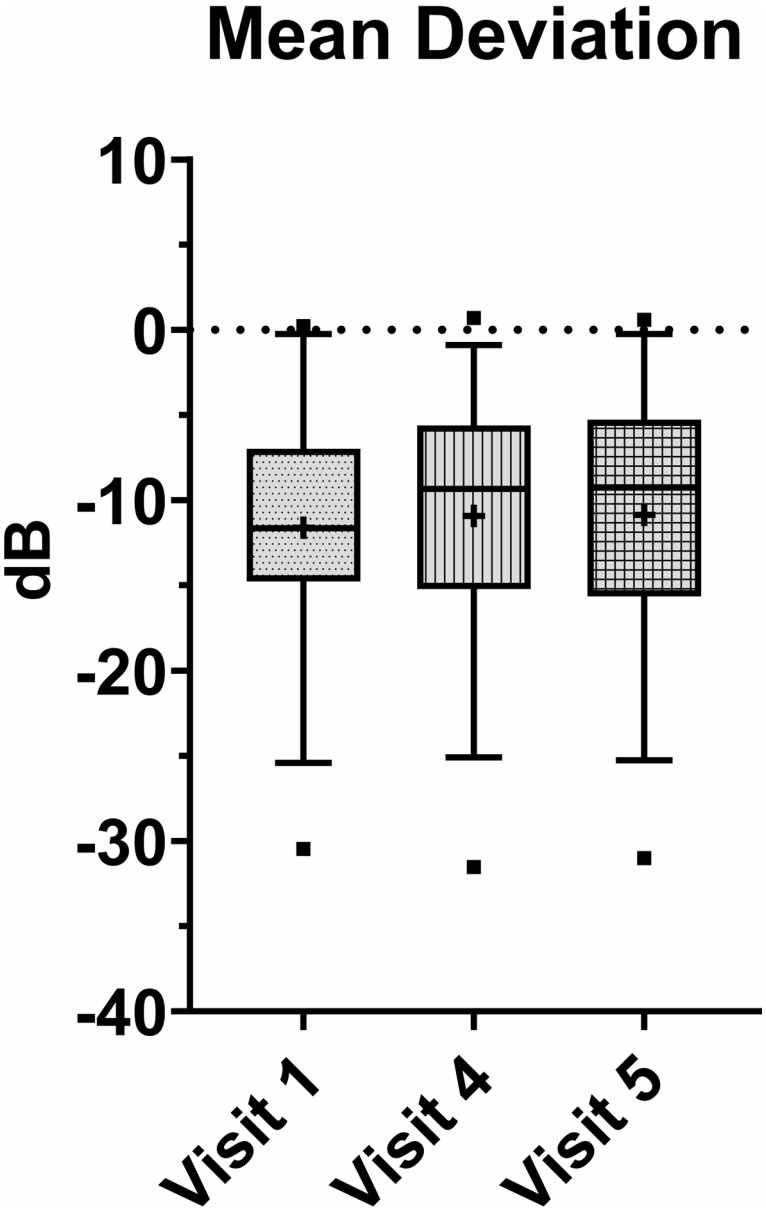

Fig. 2. Boxes and whiskers graph of Mean Deviation values in the 3 study visits.

RNFL thickness sectors	Average thickness (μm)			
	Visit 1	Visit 4	Visit 5	*P* value
Infero-temporal	66 (50.25; 85.25)	55.5 (45; 78.75)	60 (44.5; 78.25)	0.01
Supero-temporal	68 (58; 94)	70 (58; 95.5)	75.5 (60.25; 96)	0.99
Temporal	48 (40; 61)	47 (39.25; 55.75)	46.5 (39.50; 61.75)	0.30
Infero-nasal	56 (44; 78)	56 (46.25; 68.75)	55.5 (49.50; 71)	0.63
Supero-nasal	63 (49; 88)	60 (48; 88.5)	61.5 (47.25; 85.25)	0.80
Nasal	49 (37; 58)	47 (34.75; 54.75)	49 (39.25; 58.75)	0.24
Global	58 (49.5; 63.5)	55.5 (47; 63)	57.5 (47.25; 64)	0.44

Table 3. Structural outcomes: OCT RNFL thickness measured for each sector. Results are reported as median (25%; 75% percentile), *p* values are by the Friedman test.

reported by any patient, which can reasonably be attributed to the nature of CBS, a preparation derived from a substance of human origin (SoHO https://health.ec.europa.eu/blood-tissues-cells-and-organs), free of synthetic components and mimicking tear composition[23].

The rationale for using CBS in glaucoma derives from its content of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which have been associated with modified retinal ganglion cell responses in preclinical models. While these molecules are known to be present in CBS, the pharmacokinetics of their ocular absorption in humans remain largely unknown. It is hypothesized that absorption could occur via transcorneal or transconjunctival routes, potentially reaching the posterior segment through local diffusion or systemic circulation. The administration of neurotrophic factors contained in blood based products has been attempted recently for other retinal diseases through intravitreal delivery[24]. However, no pharmacokinetic analyses were conducted in the present or other studies, and currently, only limited data from animal studies or extrapolated models are available in the literature[13].

The role of CBS has been widely investigated as a treatment for severe ocular surface diseases, showing a successful effect on epithelial healing[20,25–27] and on corneal sub basal nerve layer recovery[22]. The neurotrophin content in CBS, particularly BDNF and NGF[17,19,28], suggests potential ability to influence retinal cellular responses, which warrants further evaluation. BDNF is considered a promising candidate being ubiquitous in the brain and associated with protective and regenerative effects in neural cells[13,29]. It has been successfully investigated in preclinical studies[13,14,15,18,30] demonstrating the ability to modulate retinal responses to various injurious stimuli, including those relevant to glaucoma mechanisms[32]. This effect was observed not only when a solution of BDNF was injected into the vitreous of experimental animals but also when it was applied topically. Levels of BDNF administered with a single drop containing 12 µg/mL have been demonstrated to reach the retina[30] after 6 h, with increased levels in the following 6 h. The treatment used in the present study was prepared and standardized to supply the ocular surface with 1.5 ng/mL daily, a level higher than the physiological content in normal tears[32]. Despite this higher concentration, no pain or discomfort was reported by any patient post-treatment, unlike what has been observed with topical NGF therapies[33,34].

Regarding efficacy, statistical analysis did not demonstrate significant changes in any of the evaluated parameters. The amplitude of the N95 wave was higher in V5 compared to V4 only when baseline MD values were considered in the linear mixed model, suggesting that certain subgroups of glaucoma patients might benefit from the treatment. Given that this was a pilot study focused on tolerance and safety, the short duration of treatment and follow-up, as well as the small sample size and disease stage variability, likely limited the detection of significant changes in functional parameters.

We attempted to analyse patients by subgroups and observed that those showing a non-significant numerical trend toward increased PERG amplitudes during CBS administration displayed a return toward lower values after discontinuation. Since a decline at V5 was also present in the overall sample, this pattern cannot be uniquely attributed to this subgroup. Rather than implying a specific inducing effect of CBS, it represents only one possible interpretation and should be viewed cautiously. Further studies focused on efficacy are needed to confirm these results.

To our knowledge, the study by Beykyn et al. is the only other trial published in the literature examining the effect of three months of topical delivery of a neurotrophic agent (NGF) in glaucoma patients. However, functional outcomes in that study did not show statistically significant improvement compared to controls[34].

Regarding functional and structural outcomes, our findings did not show statistically significant variations in mean deviation (MD), pattern electroretinography (PERG) parameters, or retinal nerve fiber layer (RNFL) thickness. Several factors could account for these observations. First, the relatively small sample size may have limited statistical power to detect subtle changes. Second, non-significant variations may reflect known sources of fluctuation in glaucomatous measurements, such as regression to the mean, intersession variability, and device-related measurement noise. In visual field testing specifically, a learning effect may have contributed to improvements in some patients, especially due to the higher frequency of testing compared to routine clinical practice.

The increase observed in N95 amplitude at V5 after discontinuation of therapy at V4 was significant in the linear mixed model, but it should be interpreted cautiously. A possible explanation may lie in short-term electrophysiological fluctuations or adaptive responses; however, the finding remained borderline when outliers were excluded ($p = 0.06$), and therefore cannot support strong conclusions.

The infero-temporal RNFL thinning observed between V1 and V5 reached statistical significance ($p = 0.03$), but post hoc inspection indicated that this result was largely driven by a few patients with extensive peripapillary atrophy. After excluding these outliers, the result no longer reached significance. This suggests the variation was not treatment-related, but due to structural artifacts or segmentation variability.

The study has several limitations. These include the small number of participants, absence of a control group, lack of pharmacokinetic data, and the relatively short follow-up.

Finally, an important consideration concerns treatment feasibility. Although all enrolled patients were fully compliant with the protocol, the need for eight daily administrations may represent a significant burden in real-world clinical settings, particularly for glaucoma patients already struggling with complex regimens. This intensive dosing schedule could limit the long-term applicability of the treatment. A future perspective could involve optimizing the formulation by increasing the concentration of neurotrophic factors in each drop, thus reducing the number of daily instillations required. However, such a strategy would require thorough pharmacokinetic evaluation to ensure efficacy and safety, and to date, no such data are available.

In conclusion, CBS eye drops were well tolerated and safe in this glaucoma cohort. Although no statistically significant improvements in functional or structural parameters were observed, some exploratory signals indicate variability in neuroretinal parameters that should be investigated further in larger, controlled studies.

Data availability

The datasets generated during and analysed during the current study are not publicly available due to privacy rules, but are available from the corresponding author on reasonable request.

Received: 2 January 2025; Accepted: 25 January 2026
Published online: 03 February 2026

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Acknowledgements

The Authors would like to express their sincere gratitude to Dr Mauro Cellini, formerly at the Ophthalmology Unit University of Bologna, for his invaluable assistance, expertise and fruitful discussion during the execution of the entire study. The authors wish to acknowledge Prof. Emilio Campos, whose scientific vision laid the foun-

dation for the work.

Author contributions

S.B., L.F., and P.V. conceived and planned the design of the study, but all authors provided critical feedback and helped shape the research, analysis and manuscript; E.L., S.O., and L.F. carried out the clinical study; MB and EB prepared and standardized the product; SP performed the statistical analysis of data; E.L., S.P., S.O., S.B., L.F., and P.V. contributed to the interpretation of the results. P.V., E.L., and L.F. took the lead in writing the manuscript.

Funding

The study was supported by the Fondazione Cassa di Risparmio di Bologna Italy (CARISBO) no. 2019.0547, assigned to PV.

Declarations

Competing interests

A patent, "Blood serum for use in the treatment of neurodegenerative ophthalmologic pathologies", covering the topic of this manuscript, was filed on 15/06/2017 and is owned by University of Bologna, IRCCS AOU BO and University of L'Aquila. P.V., M.B., and S.B. are among the inventors of the patent and participated to this research without any commercial involvement. The other Authors of the manuscript have no competing interest in the issue of the present manuscript.

Additional information

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