A Life that is born makes Life Grow

Cord Blood: current experiences and future programmes

Biological eye drops

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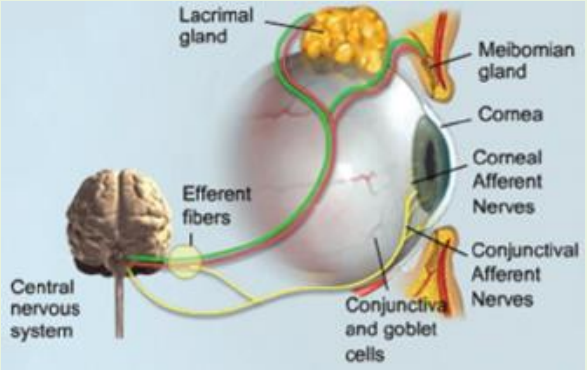
INTRODUCTION



- Failure of the ocular surface epithelium occurs in many clinical conditions with differing pathogeneses; the core of medical management is to stabilize or promote healing of healthy corneal epithelium.
- Dry eye (DE) syndrome is a very common disorder that may also represent a sight threatening disease and a potential cause of blindness as irreparable ulceration, perforation or scarring can also occur in untreated or uncontrolled severe cases.
- First line treatment in DE syndrome, including the application of artificial tears, topical anti-inflammatory agents, therapeutic contact lenses and punctual occlusion, lacks the biologically active components present in normal tears that are critical for a healthy epithelium

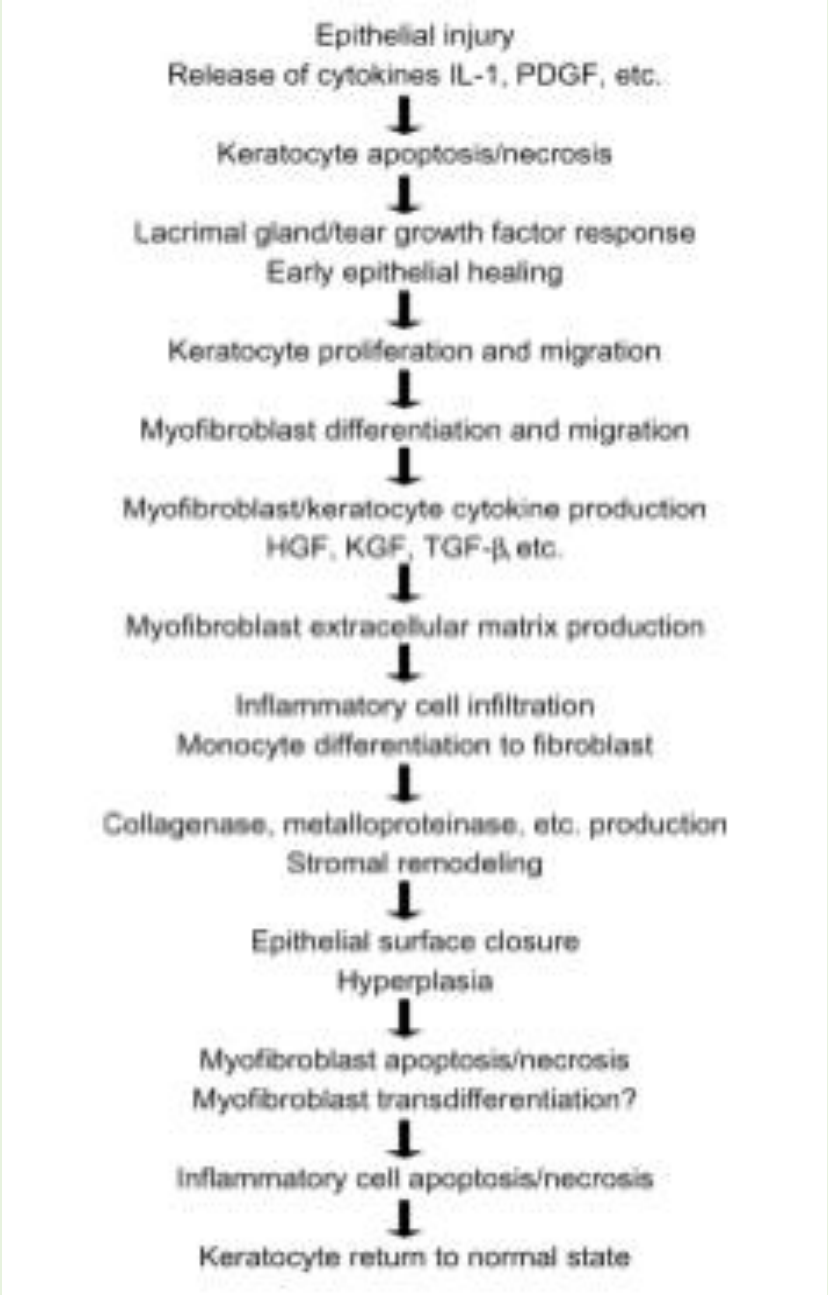
Biological eye drops

- Blood derived Biological eye drops, including autologous serum (AS), platelet rich plasma (PRP), and homologous serum, like cord blood serum (CBS), have been introduced in the treatment of many ocular diseases, because they contain a high concentration of biologically active components (mucin, neuropeptides, fibronectin, vitamin A, alpha 2 macroglobulin) and growth factors (EGF, TGF beta1), that are usually found in tear film and are essential for regulating the proliferation, differentiation and maturation of ocular surface epithelium.
- These natural components may support the healing of injured ocular surface epithelium, in case of diminished tear growth factor content, as may occur in DE disease.

Biological eye drops

- More recently some Authors have suggested what could be the next generation of biological eye drop therapy for severe ocular surface disease: eye drops derived from human mesenchymal stem cell (hMSC)-conditioned medium, that may have antiapoptotic and/or proepithelial wound healing effect via a paracrine mechanism. (Oh JY et Al. Invest Ophtalmol Vis Sci 2014; Roddy GW et Al. Stem Cells 2011).
- The challenge for researchers in this field will be to identify and test the optimum hMSC culture conditions and protocols to produce safe, tolerable and effective hMSC conditioned medium eye drops without the proangiogenic factors also known to be produced by these cells.

Process of corneal damage repair



Klenkler B et al., Exp Eye Res. 2004

	Cord Blood Serum	Autologous Serum	Tear Substitutes	NSAID Steroids	Cyclosporin A
pH/Osmolarity	natural	natural	chemically buffered controlled	chemically buffered controlled	chemically buffered controlled
Preservatives	free	free	present	present	present
Patients compliance	optimal	optimal	good depending upon type	products burn	products burn
Chemical components	absent	absent	present	present	present
Ocular nutrients	present	present	present only in the last generations of products	absent	absent
Growth factors	present	present	absent	absent	absent
Anti-inflammatory properties	present, natural substances (direct effects)	absent (presence of pro-inflammatory cytokines in	absent (only diluting, indirect effects)	present, chemical compounds	present, chemical compounds

Autologous eye drops

- The use of AS in the ophthalmic setting dates back at least two decades, but despite its proven efficacy there is no standard procedure for preparation, quality control, storage and administration.

Int Ophthalmol Clin. 2000 Fall;40(4):113-22.

Serum application for the treatment of ocular surface disorders.

Tsubota K[1], Higuchi A.

Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease

Y Ogawa[1,3], S Okamoto[2], T Mori[2], M Yamada[1], Y Mashima[1], R Watanabe[2], M Kuwana[3], K Tsubota[1,4], Y Ikeda[2] and Y Oguchi[1]

PERSPECTIVE

Autologous serum eye drops for ocular surface disorders

G Geerling, S MacLennan, D Hartwig

Br J Ophthalmol 2004;88:1467–1474. doi: 10.1136/bjo.2004.044347

Cornea:
September 2008 - Volume 27 - Issue - pp S25-S30
doi: 10.1097/ICO.0b013e31817f3a0e
Symposium 1

Autologous Serum Eye Drops for the Treatment of Dry Eye Diseases

Kojima, Takashi MD*; Higuchi, Akihiro PhD†; Goto, Eiki MD‡; Matsumoto, Yukihiro MD†; Dogru, Murat MD†; Tsubota, Kazuo MD†

Autologous eye drops

- In more recent years, the platelet lysate obtained from autologous platelet-rich plasma has attracted increasing interest because it is also a source of a variety of growth factors with an important role in the wound-healing process in many tissues

Curr Pharm Biotechnol. 2012 Jun;13(7):1257-65.

The role of "eye platelet rich plasma" (E-PRP) for wound healing in ophthalmology.

Alio JL[1], Arnalich-Montiel F, Rodriguez AE.

Jpn J Ophthalmol. 2012 Nov;56(6):544-50. doi: 10.1007/s10384-012-0175-y. Epub 2012 Sep 13.

Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis.

Kim KM[1], Shin YT, Kim HK.

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Author Manuscript
Cochrane Database Syst Rev. Author manuscript; available in PMC 2014 August 27.

Published in final edited form as:
Cochrane Database Syst Rev. ; 8: CD009327. doi:10.1002/14651858.CD009327.pub2.

Autologous serum eye drops for dry eye

Qing Pan[1,2], **Adla Angelina**[3], **Andrea Zambrano**[4], **Michael Marrone**[5], **Walter J Stark**[4], **Thomas Heflin**[4], **Li Tang**[6], and **Esen K Akpek**[4]

- The authors identified four eligible randomized controlled trials (RCTs) among 30 studies reviewed, in which AS was compared with artificial tear treatment or saline in individuals with DE of various etiologies.
- The meta analysis showed inconsistent results for AS efficacy.
- Well-planned, large scale, high quality randomized controlled trials are needed, stratified by age and severity of DE, comparing AS to artificial tears or other treatments, as well as evaluating various concentrations of AS.

Homologous eye drops

- Homologous blood sources (from adult blood donors and from cord blood donors) have recently been proposed , with the advantage that they can be routinely obtained as quality/microbiologically-controlled products from blood banks, where a supply may be kept and stored in advance to be readily dispensed.

Homologous eye drops

- Use of homologous products may also enable us to treat patients with serious immunologically jeopardised diseases associated with DE.
- These would be damaged by autologous products which contain non homogeneous levels of GFs and high levels of pro-inflammatory cytokines.

CBS eye drops

- Several authors have demonstrated the safety and efficacy of CBS eye drops in the treatment of severe DE with or without Sjogren's syndrome (SS-I), ocular Graft versus host disease (GVHD), persistent corneal epithelial defects, recurrent corneal erosions, chemical burns and neurotrophic keratitis.
- CBS eye drops seem to be more effective than AS in decreasing symptoms and keratoepitheliopathy in patients with severe DE syndrome, and increasing goblet cell density in SS patients.

Am J Ophthalmol. 2007 Jul;144(1):86-92. Epub 2007 May 9.

Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome.

Yoon KC[1], Heo H, Im SK, You IC, Kim YH, Park YG.

CONCLUSIONS: Umbilical cord serum eye drops were more effective in decreasing symptoms and keratoepitheliopathy in severe dry eye syndrome and increasing goble density in Sjögren syndrome compared with autologous serum eye drops.

Comparative role of 20% cord blood serum and 20% autologous serum in dry eye associated with Hansen's disease: a tear proteomic study

Somnath Mukhopadhyay,[1] Swarnali Sen,[2] Himadri Datta[2]

Br J Ophthalmol 2015;**99**:108–112. doi:10.1136/bjophthalmol-2013-304801

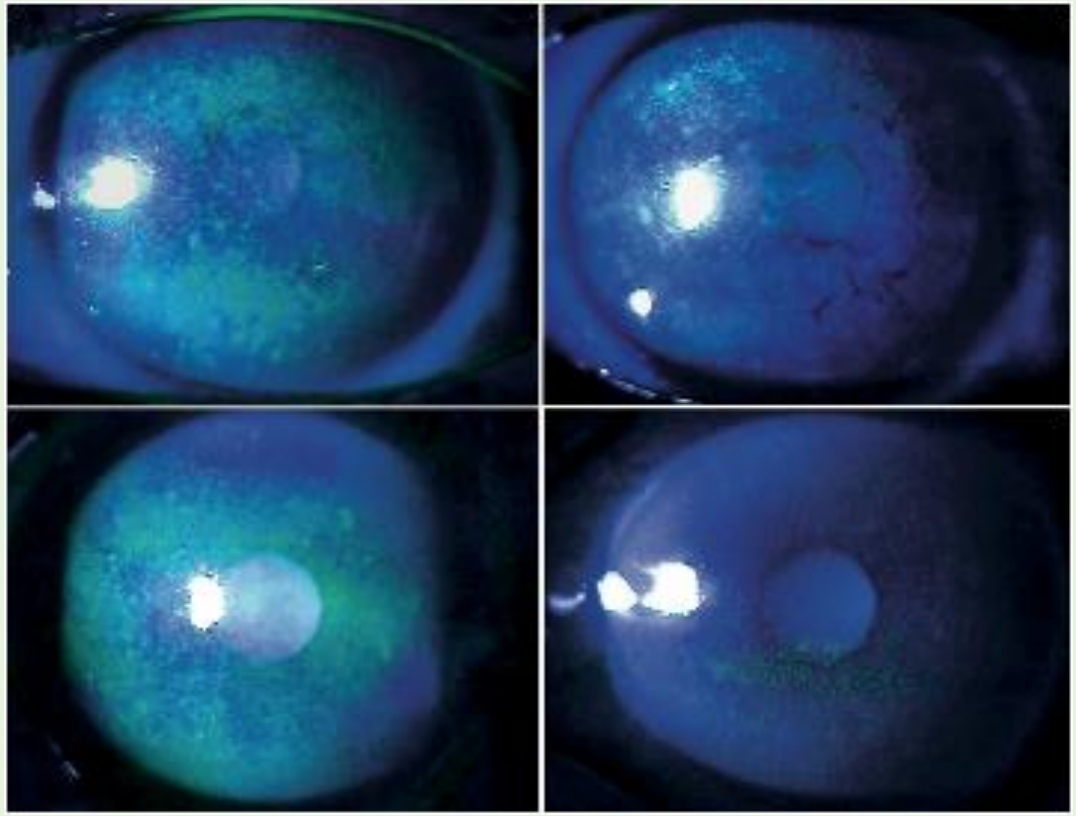
Comparison of Autologous Serum and Umbilical Cord Serum Eye Drops for Dry Eye Syndrome

KYUNG-CHUL YOON, HWAN HEO, SEONG-KYU IM, IN-CHEON YOU, YOON-HA KIM, AND YEOUNG-GEOL PARK

AMERICAN JOURNAL OF OPHTHALMOLOGY JULY 2007

T0
AS
(day 0)

T0
CBS
(day0)



T1
AS
(day 60)

T1
CBS
(day 60)

CBS eye drops

- Some other studies evaluating the effects of CBS eye drops in treating corneal epithelial defects, have given encouraging results.
- The studies confirm that CBS contains essential substances for the proliferation, differentiation and maturation of the normal ocular surface epithelium, like Epidermal growth factors (EGF), vitamin A and Transforming growth factor -β.1 (TGF-β.1),
- Epidermal growth factor in particular plays a central role in corneal wound healing

Application of Umbilical Cord Serum Eyedrops for the Treatment of Dry Eye Syndrome

Kyung-Chul Yoon, MD, PhD, Seong-Kyu Im, MD,* Yeoung-Geol Park, MD, PhD,* Young-Do Jung, MD, PhD,† Seong-Yeul Yang, MD, PhD,† and Jin Choi, MD, PhD‡*

Cornea • Volume 25, Number 3, April 2006 *Umbilical Cord Serum Eyedrops for Dry Eye*

TABLE 2. The Concentrations of EGF, Vitamin A, and TGF-β1 in Umbilical Cord Serum and Normal Peripheral Blood Serum

	Concentration (ng/mL)		
Components	Umbilical Cord Serum (mean ± SD)	Peripheral Blood Serum (mean ± SD)	*P*
EGF	0.48 ± 0.09	0.14 ± 0.03	<0.01
TGF-β	57.14 ± 18.98	31.30 ± 12.86	<0.01
Vitamin A	230.85 ± 13.39	372.34 ± 22.32	0.03

EGF, epidermal growth factor; TGF-β, transforming growth factor β.

use of umbilical cord serum were observed. The mean concentrations of EGF, TGF-β, and vitamin A in umbilical cord serum and peripheral blood serum are shown in Table 2. EGF and TGF-β concentrations of umbilical cord serum were significantly higher than those of peripheral blood serum. Vitamin A concentration of umbilical cord serum was significantly lower than that of peripheral blood serum.

Cornea. 2011 Jul;30(7):744-8. doi: 10.1097/ICO.0b013e31820d850f.

Application of umbilical cord serum eyedrops for recurrent corneal erosions.

Yoon KC[1], Choi W, You IC, Choi J.

CONCLUSIONS: In the treatment of recurrent corneal erosions, umbilical cord serum eyedrops may be effective in reducing the number of recurrences.

Chonnam Med J 2014;50:82-85

Use of Umbilical Cord Serum in Ophthalmology

Kyung Chul Yoon*

Owing to the high concentrations of essential tear components, growth factors, and neurotrophic factors in UCS, UCS eye drops can be safely and effectively applied in intractable ocular conditions such as severe dry eye disease with or without Sjögren's syndrome, ocular GVHD, persistent epithelial defects, neurotrophic keratopathy, recurrent corneal erosions, ocular chemical burn, and surface problems after corneal refractive surgery.

Int J Ophthalmol, Vol. 7, No. 5, Oct.18, 2014 www. IJO. cn
Tel:8629-82245172 8629-82210956 Email:ijopress@163.com

·Clinical Research·

Umbilical cord blood serum therapy for the management of persistent corneal epithelial defects

Elif Erdem [1], Meltem Yagmur [1], Inan Harbiyeli [2], Hande Taylan-Sekeroglu [3], Reha Ersoz [1]

Here we have our own 2012 study

CLINICAL SCIENCE

Efficacy of Standardized and Quality-Controlled Cord Blood Serum Eye Drop Therapy in the Healing of Severe Corneal Epithelial Damage in Dry Eye

Piera Versura, BSD, Vincenzo Profazio, MD,* Marina Buzzi, BSD,† Alessandra Stancari, PharmD,‡ Mario Arpinati, MD,§ Nazzarena Malavolta, MD,¶ and Emilio C. Campos, MD**

Cornea, 2012

Purpose: We standardized quality-controlled cord blood serum (CBS)–based eye drops and evaluated the efficacy of 1-month CBS treatment in the healing of diseased corneal epithelium in severe dry

Conclusions: Heterologous CBS-based eye drops represent a promising therapeutic approach in the healing of severely injured corneal epithelium and in subjective symptom relief. These drops can be obtained as readily available and quality-controlled blood derivative from cord blood banks on a routine basis.

First European trial on standardized preparation and clinical evaluation of CBS eye drops

Oftalmology Unit, Bologna University

Emilia-Romagna cord blood bank (ERCB) Transfusion Service

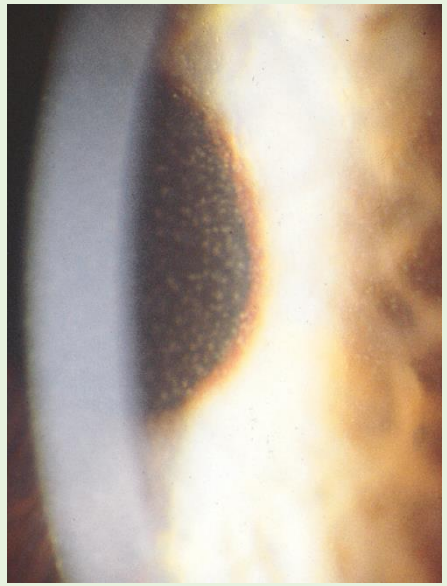
Pharmacy Service

EudraCT: 2008-005757-38

Clin Trial Gov Id NCT01234623

Seventeen GVHD and thirteen SS-I patients were included in the study. They had experienced AS therapy in their previous clinical history, exhibiting severe corneal involvement, at the time of enrolment, graded according to the Oxford grading level (16). Informed consent was obtained from each enrolled subject.

Inclusion and exclusion criteria

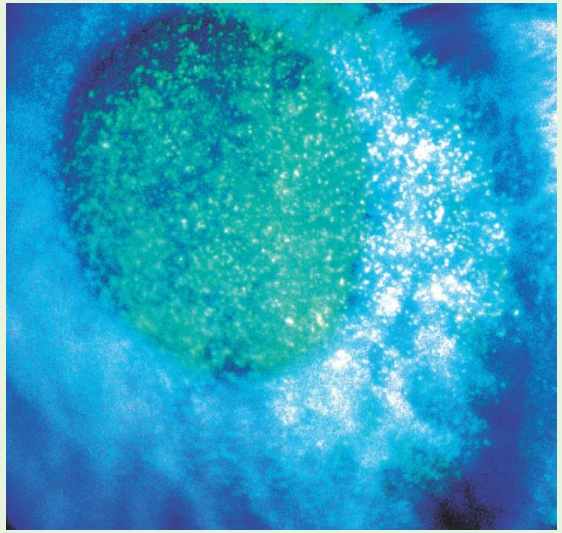


Inclusion

- ✓ GVHD and SS type I with severe corneal epithelial defects (Oxford grade 5)
- ✓ resistant to or dependent on topical steroids
- ✓ resistant or unable to receive autologous serum

Exclusion

- ✓ previous (one year) ocular surgery
- ✓ contact lenses
- ✓ punctal plugs or cauterization

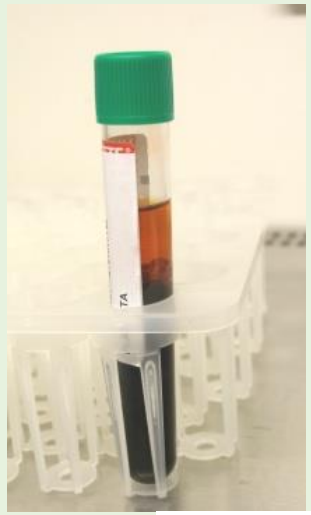


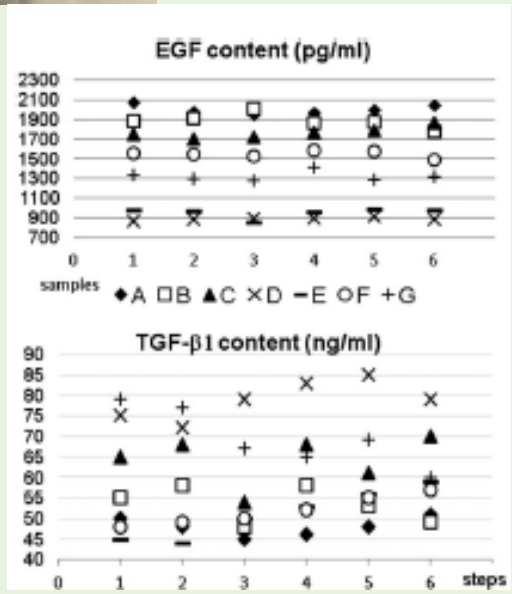
50% of the patients had been treated with topical Cyclosporine A

The entire cohort had to stop all concomitant treatments, except for tear substitutes, 4 days before starting CBS therapy (washout period).

Standardization of eye drop preparation

- A mean volume of 80 ml of CB was collected from the umbilical vein and clotted for two hours at room temperature. After centrifugation at 3,800 g for 10 minutes, the serum has isolated under a laminar flow hood and frozen at -80°C. In the other case blood samples were collected from ex utero placental vessels with a sterile syringe and transferred into Vacutest tubes without any anticoagulant.
- Sterility tests demonstrated that all batches remained sterile after handling and storage. The CB serum levels of EGF , TGF-β1 were maintained throughout the process.

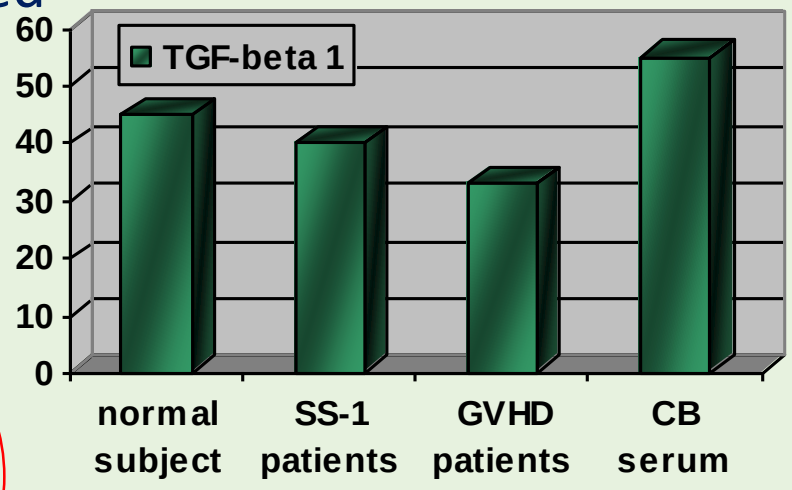


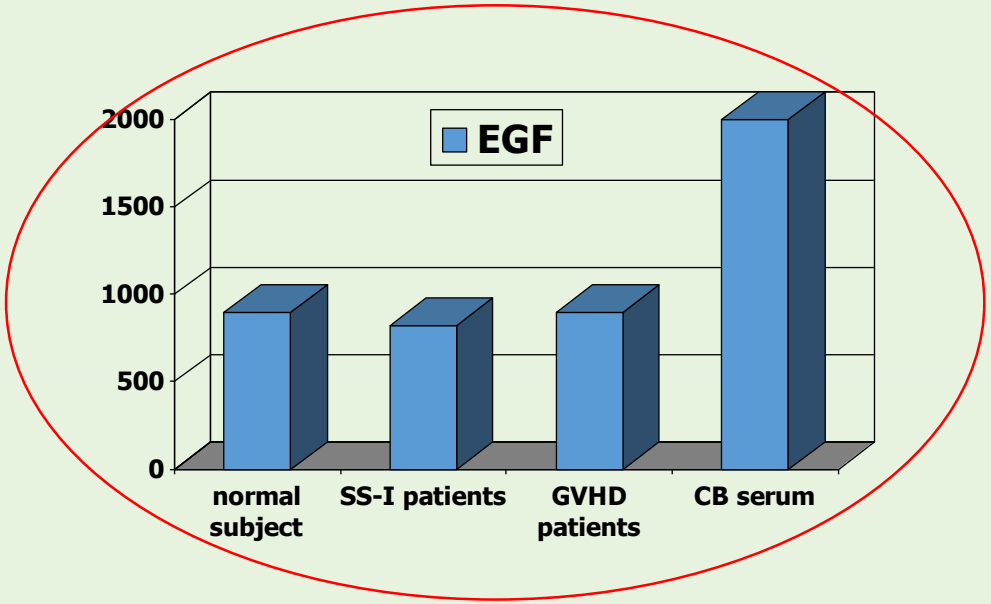


specifically as follows: 1: freshly collected CBS; 2: after the quarantine period; 3: after dilution; 4: after filtration; 5: after 1 month of freezing; 6: after 2 months of freezing. A considerable biological variability was shown in both EGF (700–2100 pg/mL) and TGF-β1 (800–1900 ng/mL) over the samples analyzed, but no significant variation was observed throughout the whole process, suggesting that the procedure does not affect or reduce the content of both growth factors.

EGF and TGF β1 dosage

The concentration of EGF and TGF β1 in CB units was determined using a Quantikine Human EGF Immunoassay Kit according to manufacturer's instructions.





Graphics show the difference in concentration of EGF and TGF- β1 among normal subjects, SS-1 patients, GVHD patients and Cord Blood Serum.
Note that the concentration of EGF and TGF- β1 in SS-1 and GVHD patients is less than normal subjects while in the case of Cord Blood Serum it is higher.

Targeting growth factor supply in keratopathy treatment: comparison between maternal adult peripheral and cord blood sources for preparation of topical eye drops.

Versura P[1], Buzzi M[2] , Giannaccare G[1] , Terzi A[2] , Fresina M[1] , Velati C[2] , Campos EC[1]

Results EGF , TGF-β and VEGF levels were significantly higher in CB-S than in PB-S (median respectively 1254.4 vs 646.0 pg/ml, 51.3 vs 38.4 µg x ml and 686.8 vs 30 pg x ml, always p<0.0001) whereas IGF content was significantly higher in PB-S than in CB-S (respectively 159.9 vs 53.5 pg x ml, p<0.0001). In CB-S, CD34+ cell concentration appeared to be related to EGF, IGF and TGF-β levels whereas WBC appeared in relationship with EGF and TGF-β levels. VEGF levels showed no relation with the hematological parameters considered. PLT

Discussion. Data showed a differential GFs content in the two blood sources, with a higher contribution from CB. Each GF selectively regulates cellular processes involved in corneal healing, so the use of PB or CB should be targeted for specific GF supply on the basis of type and severity of keratopathy.

GFs	source	Median	Min	Max	p
EGF	CB-S	1254.4	516.8	2824.0	<0.0001
	PB-S	646.0	283.8	1754.0	
IGF-1	CB-S	53.5	40.3	81.2	<0.0001
	PB-S	159.9	76.7	352.0	
TGF-	CB-S	51.3	30.2	75.1	<0.0001
	PB-S	38.4	19.8	52.8	
VEG	CB-S	686.8	31.9	1856.0	<0.0001
	PB-S	30	21	35	

Conclusive comment

this study showed that EGF and TGF-β supply may be significantly higher in CB-S as primary source. CB-S is also the selected choice for products to treat corneal nerve damage as VEGF supply from PB-S may be negligible. Conversely, PB-S can be indicated as primary source for products to accelerate keratinocyte metabolism and heal stromal defects, due to its higher IGF content.

Standardization of eye drop preparation

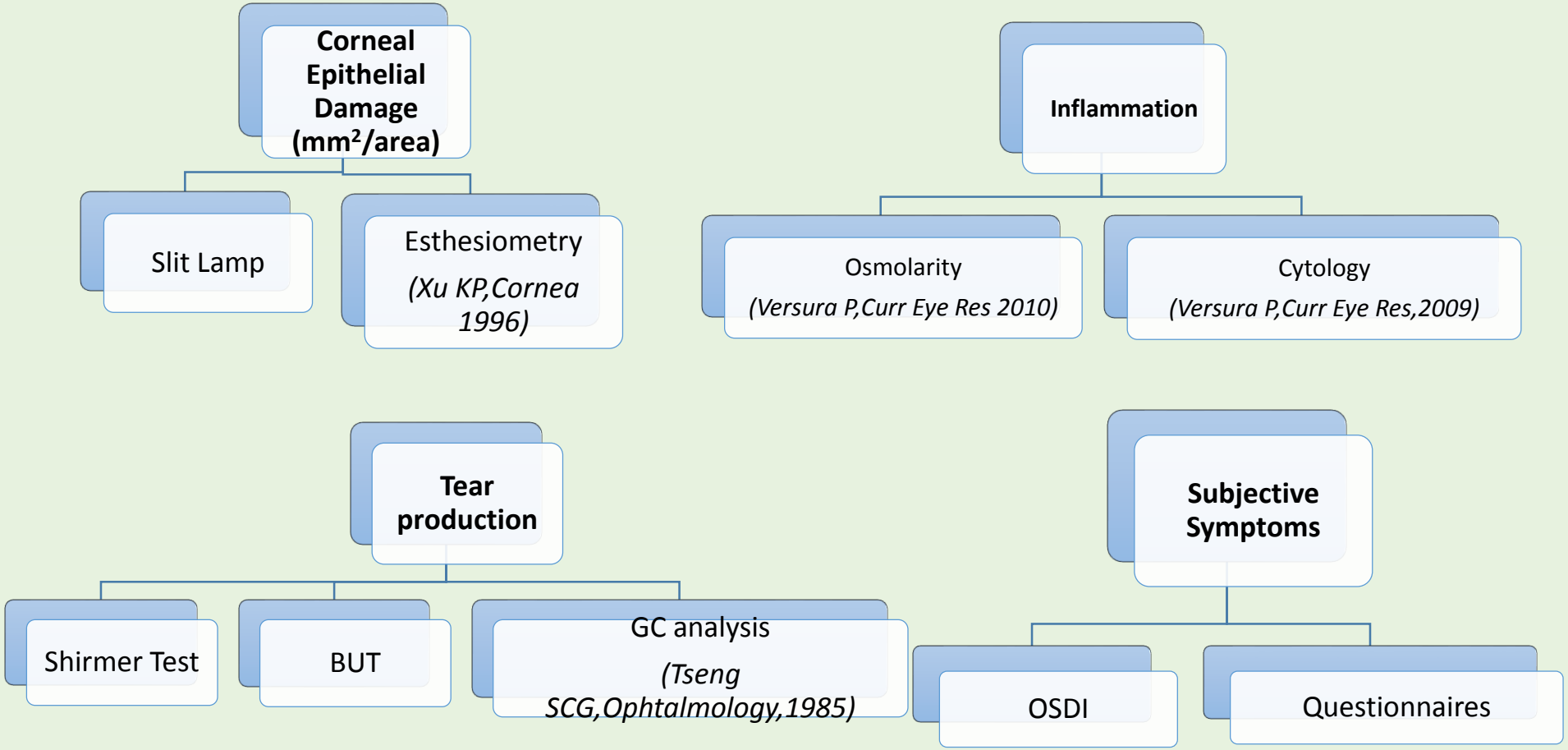
- We decided to standardise EGF content in CBS eye drops.
- A certain variability in EGF levels in CBS may be present and a preliminary dosage of EGF content in CB samples should be performed to control the amount of EGF in the final eye drops.
- CBS with EGF > 1.0 ng/ml was selected as the threshold
- This allows us to avoid varability of composition and improve the homogeneity of clinical results analysis .

Standardization of eye drop preparation

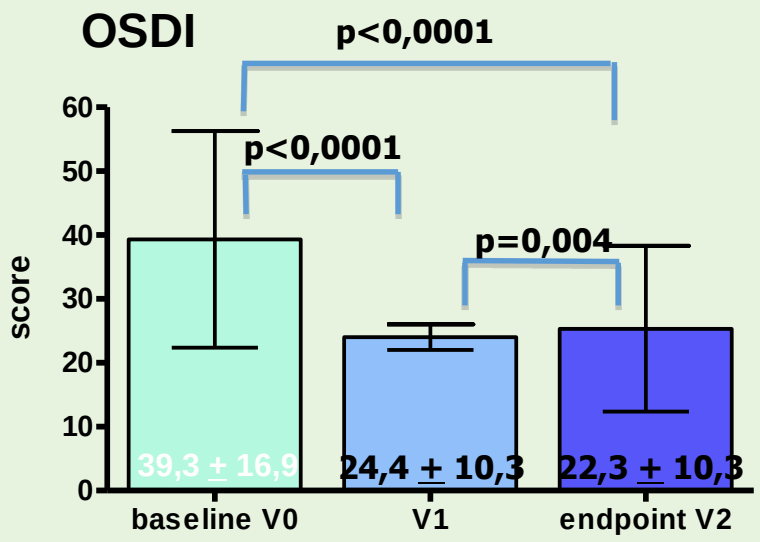
- We demonstrated the healing efficacy of CBS eye drops prepared according to a standardised protocol focusing on obtaining a daily supply of 0.10-0.20 ng/mL EGF, similar to the physiological human tear content.
- The preselected sera were thawed and pooled to obtain the amount of serum needed to treat all patients, diluted to 20% with phosphate buffered saline by an aseptic technique, and filtered (Millex HV 0.4 µm). The preparation was then aliquoted in monodose vials using a COL-20 medical device (Biomed Italy), before being packed frozen and stored.

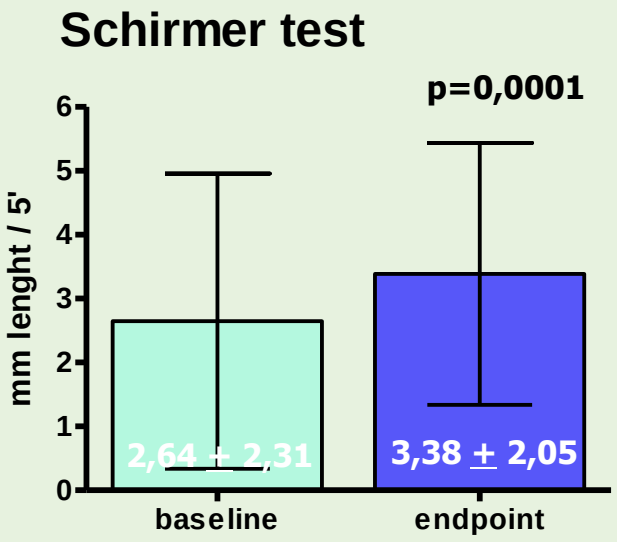
- Patients were instructed to administer one drop per eye for 8 times a day, after having thawed 1 vial the evening before the day of use.

Patients were evaluated for corneal epithelial damage and for discomfort symptoms at baseline (Visit 0-V0), after 15 (V1), after 30 (V2) days of treatment.



Results


OSDI
p<0,0001
p<0,0001
p=0,004
score
60
50
40
30
20
10
0
39,3 ± 16,9
24,4 ± 10,3
22,3 ± 10,3
baseline V0
V1
endpoint V2


Schirmer test
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1
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3,38 ± 2,05
baseline
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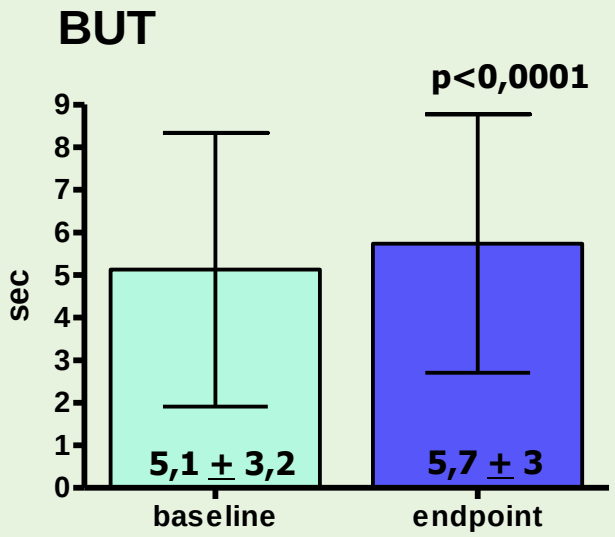
BUT
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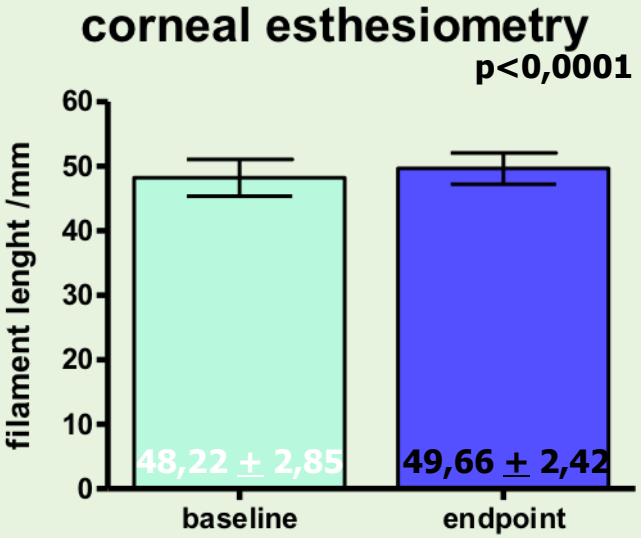
corneal esthesiometry
p<0,0001
filament lenght /mm
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48,22 ± 2,85
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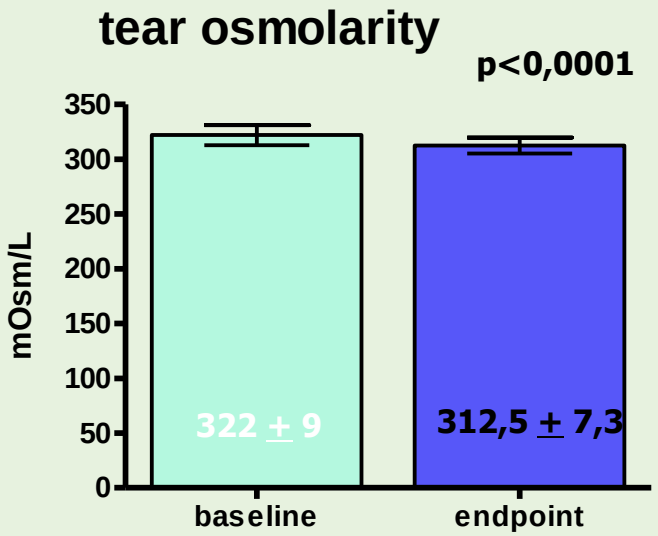
baseline
endpoint

Results


tear osmolarity
p<0,0001
mOsm/L
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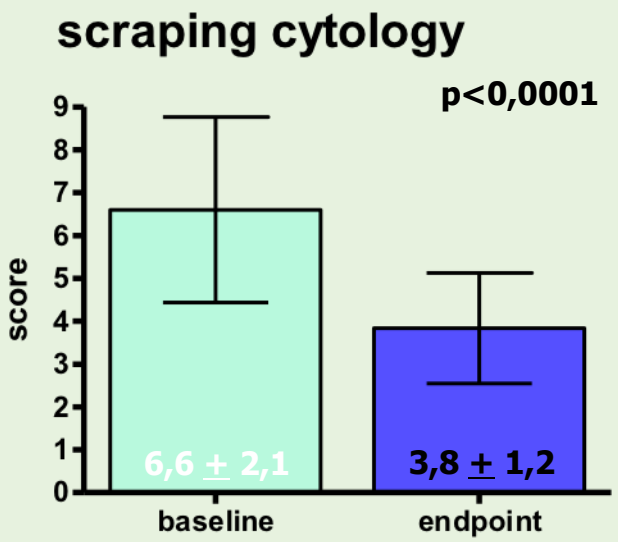
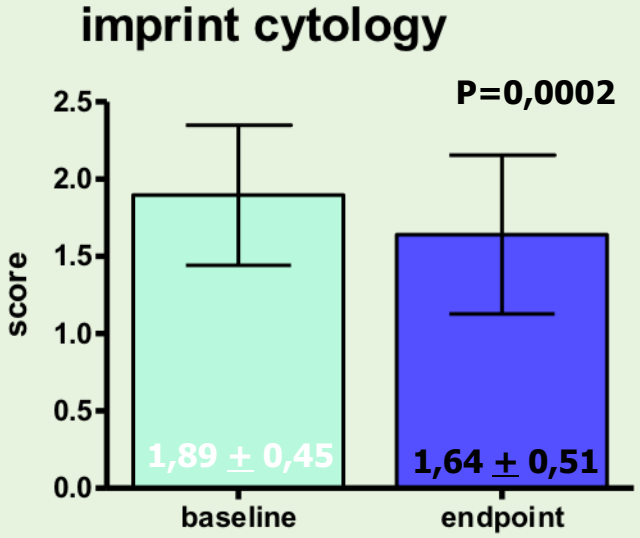
300
250
200
150
100
50
0
322 ± 9
312,5 ± 7,3
baseline
endpoint


scraping cytology
p<0,0001
score
9
8
7
6
5
4
3
2
1
0
6,6 ± 2,1
3,8 ± 1,2
baseline
endpoint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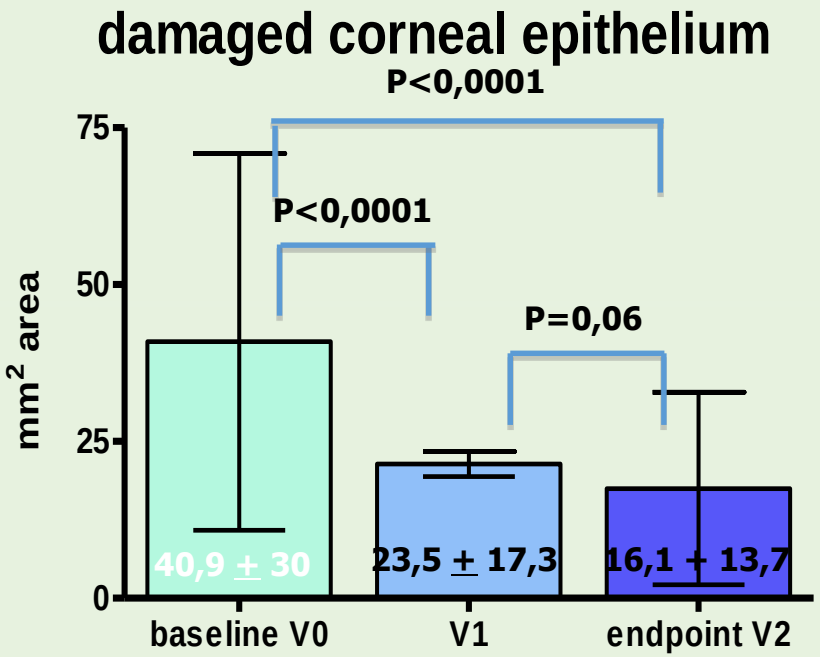
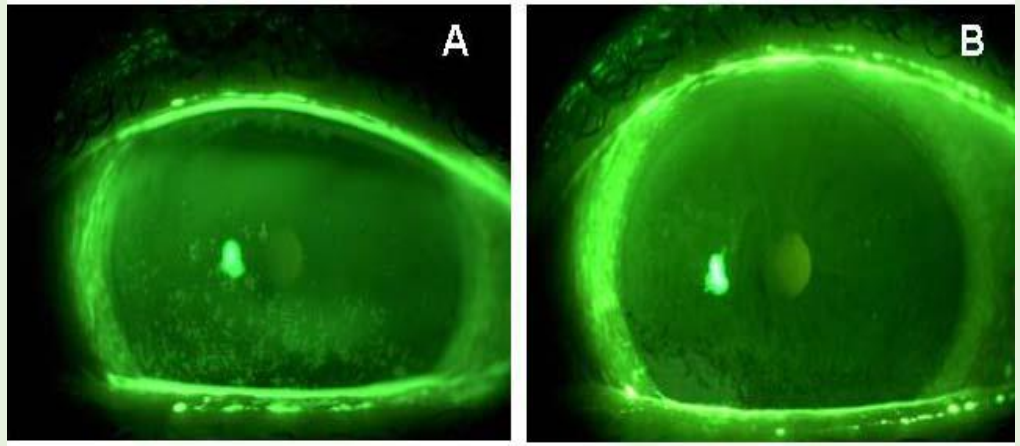
P=0,0002
score
2.5
2.0
1.5
1.0
0.5
0.0
1,89 ± 0,45
1,64 ± 0,51
baseline
endpoint

Results

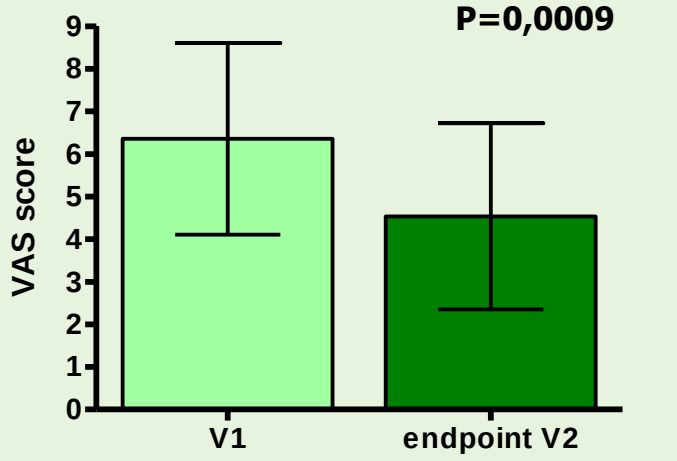


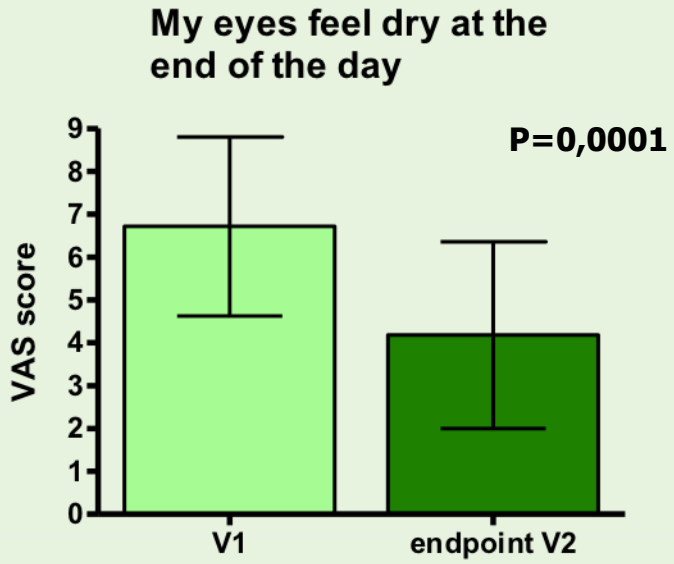


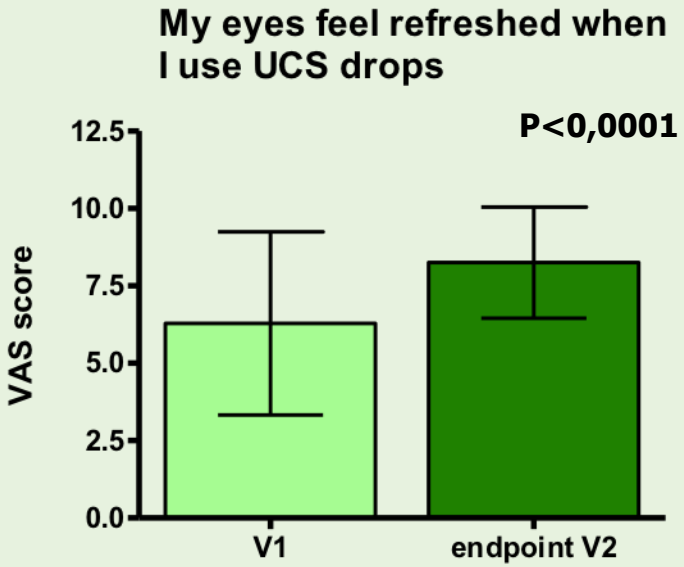
V0 V2

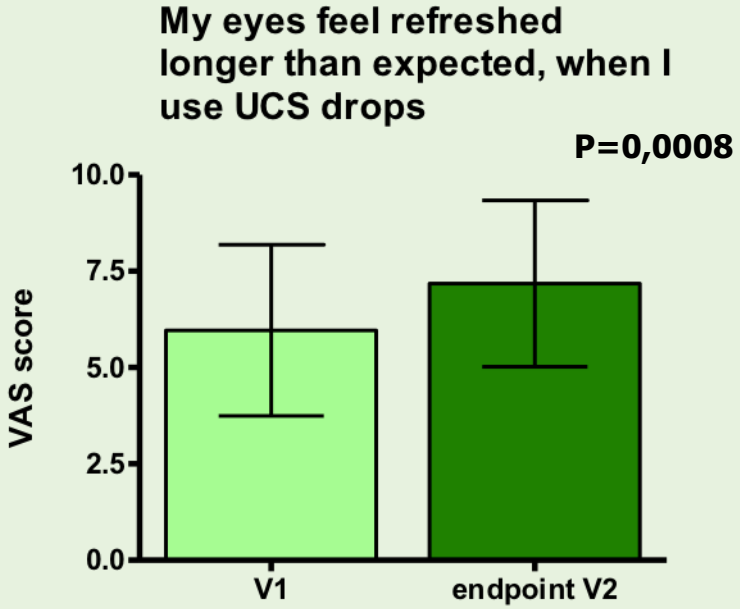
F, 30aa, R.B. SS-I

Satisfaction questions estimated by a VAS score



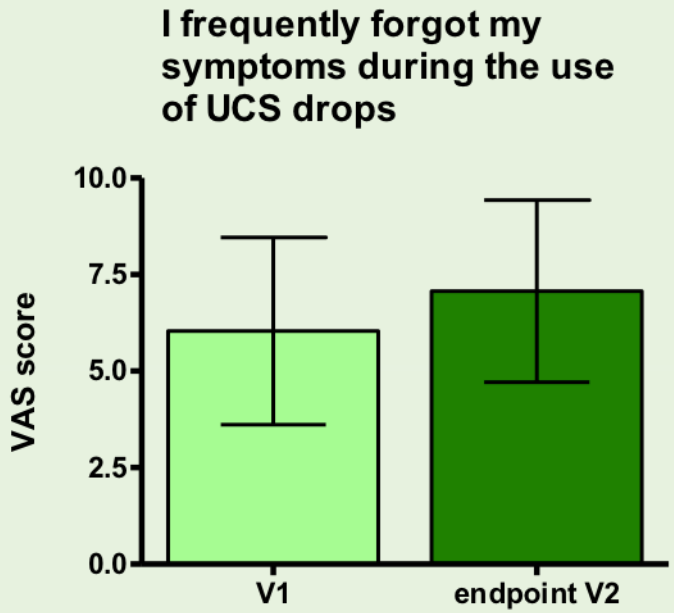


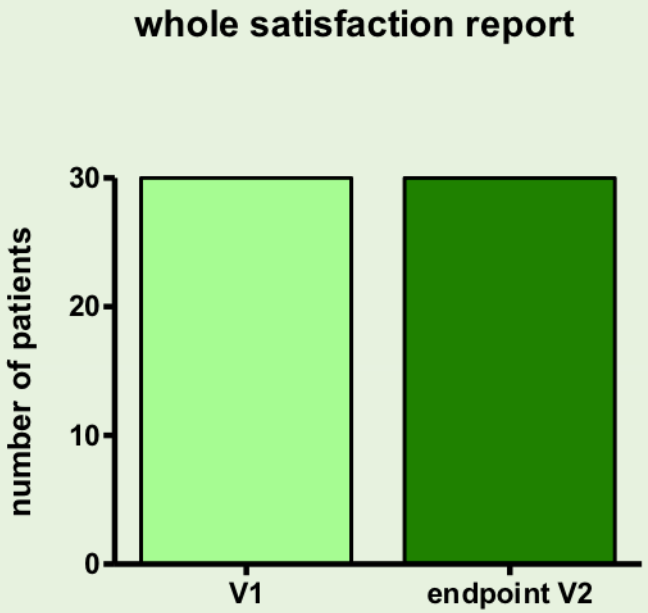




Satisfaction questions estimated by a VAS score

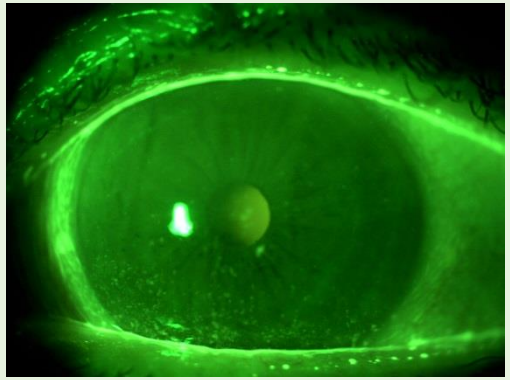
P=0,0002



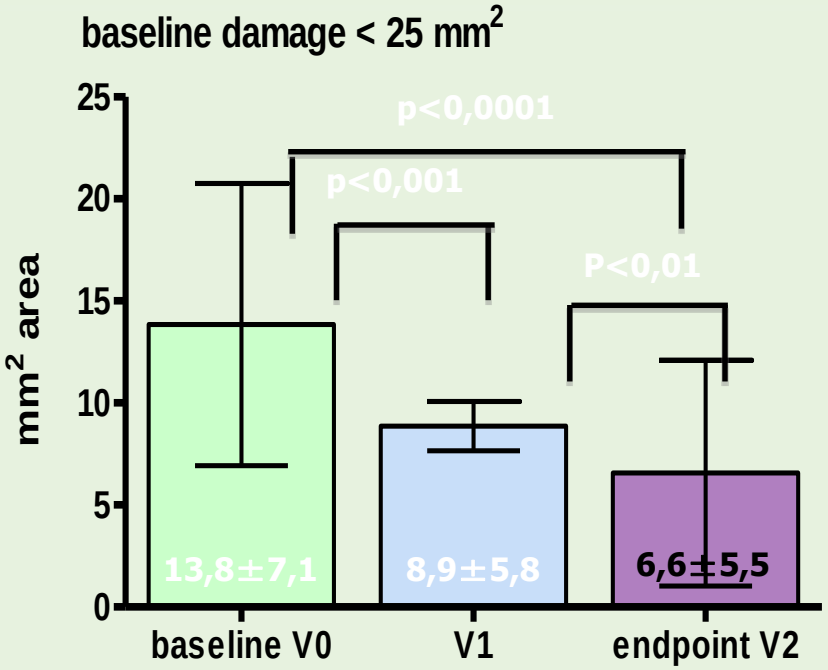


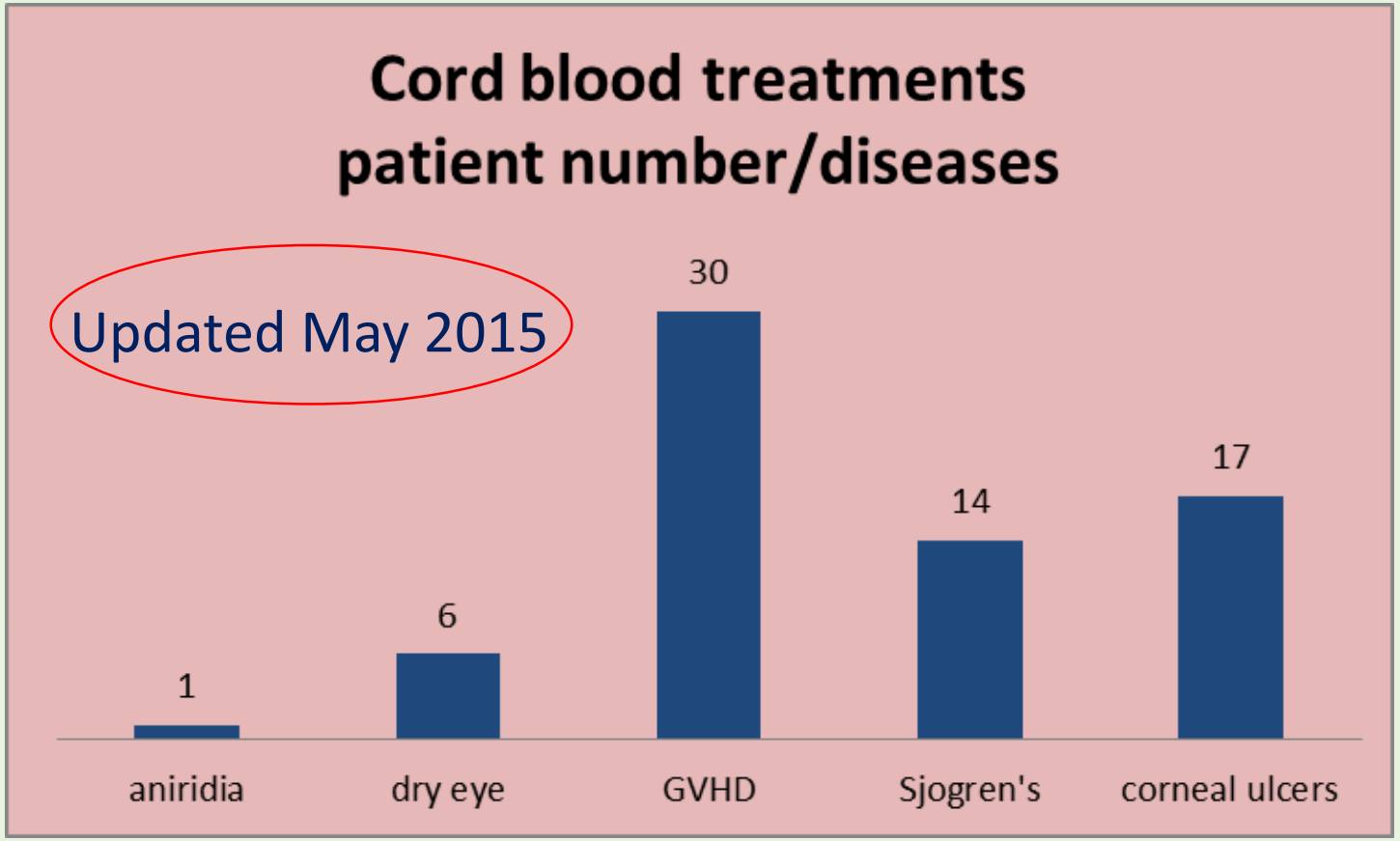
FACTORS PREDICTING RESPONSE

Limited extent of initial corneal damage is the SOLE factor predicting response



Duration of disease, Systemic GVHD, IS treatment and TBI DO NOT correlate with response to CBS therapy.





Pain symptom reduction by 50% in the first week

Number of treatments/patients	30-90 vials
Complete healing	35 out of 69 patients
Reduction over 50% defect	30 out of 69 patients
Reduction less 50 % defect	4 out of 69 patients

CONCLUSIONS

Standardized pooled CB Serum Eye Drops:

- ❖Are safe
- ❖Reduce corneal damage
- ❖Reduce inflammation
- ❖Improve tear production
- ❖Reduce discomfort symptoms

- ❖Have limited duration

….one more take home message

Clinical science

Comparison of umbilical cord serum and amniotic membrane transplantation in acute ocular chemical burns

Namrata Sharma,[1] Shiv Shankar Lathi,[1] Sri Vatsa Sehra,[1] Tushar Agarwal,[1] Rajesh Sinha,[1] Jeewan S Titiyal,[1] Thirumurthy Velpandian,[1] Radhika Tandon,[1] Rasik B Vajpayee[2,3]

Conclusions Our study suggests that the UCS therapy may be a better alternative to AMT in acute moderate to severe (grades III, IV and V) ocular chemical burns, as it avoids surgical manoeuvre in already inflamed eyes.

Sharma N, *et al. Br J Ophthalmol* 2015;**99**:669–673.

CBS eye drops has significant advantages over amniotic membrane:

- **can be used in patients with poor general health and those unfit for surgery**
- **can be used in children in whom can also be avoided unnecessary general anesthesia**
- **can be administered for prolonged periods whereas, amniotic membrane is retained on the ocular surface for only a limited time period and this may limit the total exposure and availability of the growth factors to the ocular surface**

Where do we could go in the future ……..?

- MAINTENANCE THERAPY WITH REPEATED CYCLES OF CBS EYE DROP TREATMENT…

- EVALUATE THE EFFICACY OF TWO OMOLOGOUS BLOOD SOURCE (ADULT PERIPHERAL BLOOD AND CORD BLOOD) AS GROWTH FACTOR SUPPLY IN THE HEALING OF SEVERE CORNEAL EPITHELIAL DEFECTS WITH A PROSPECTIVE , RANDOMIZED DOUBLE BLIND STUDY

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