

EMOCOMPONENTI  
AD USO NON  
TRASFUSIONALE:  
STATO DELL'ARTE  
E SVILUPPI FUTURI

21 GENNAIO 2020

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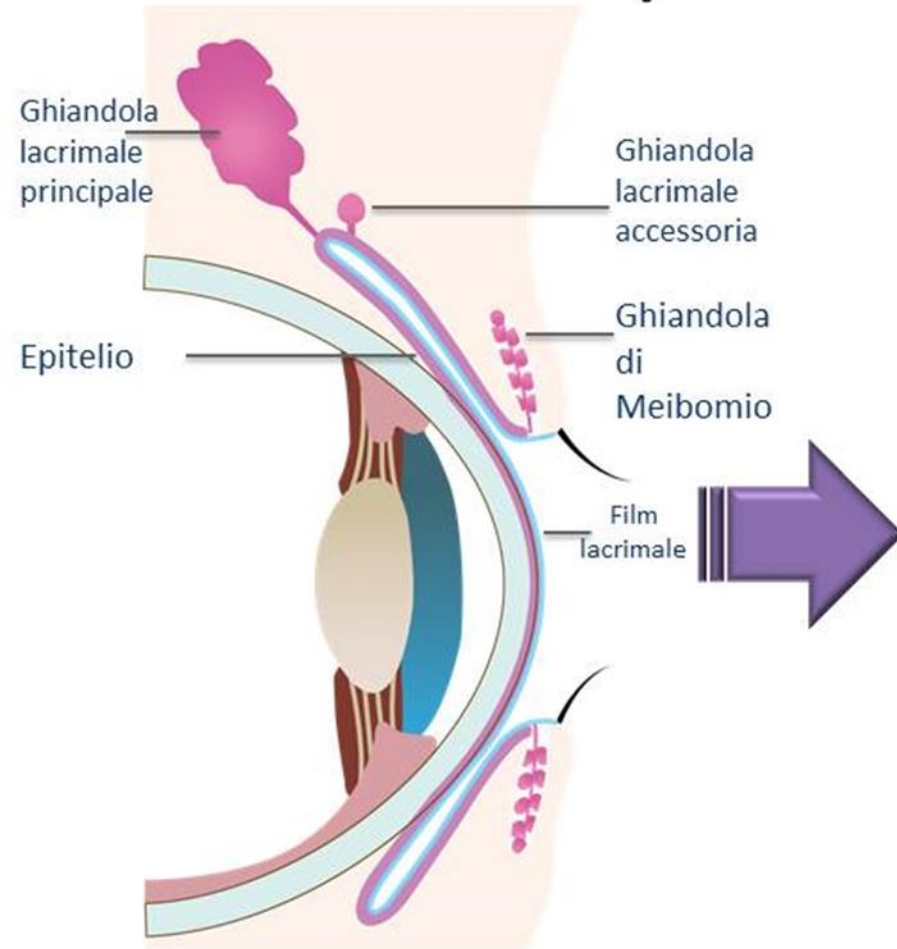
# Emocomponenti ad uso non trasfusionale in oculistica (Il sierocollirio)

Piera Versura

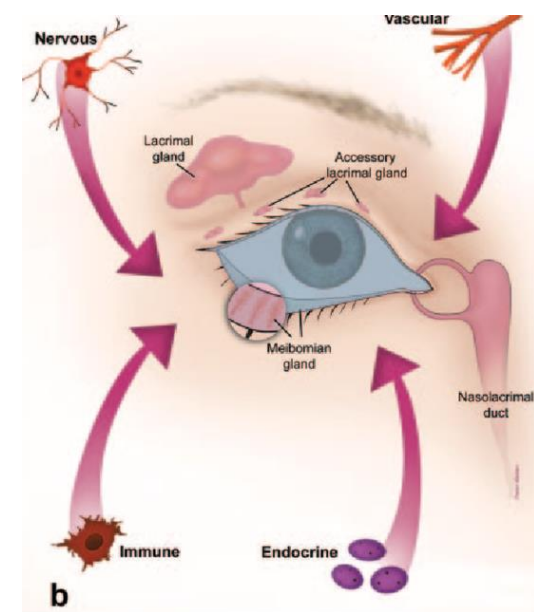
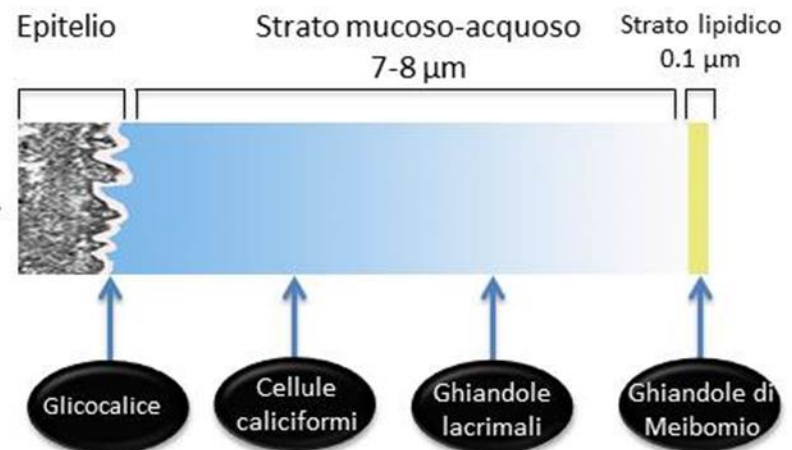
DIMES Alma Mater Studiorum Università di Bologna  
UO Oftalmologia Laboratorio Analisi Superficie Oculare  
e Ricerca Traslazionale - TFOS Board of Directors

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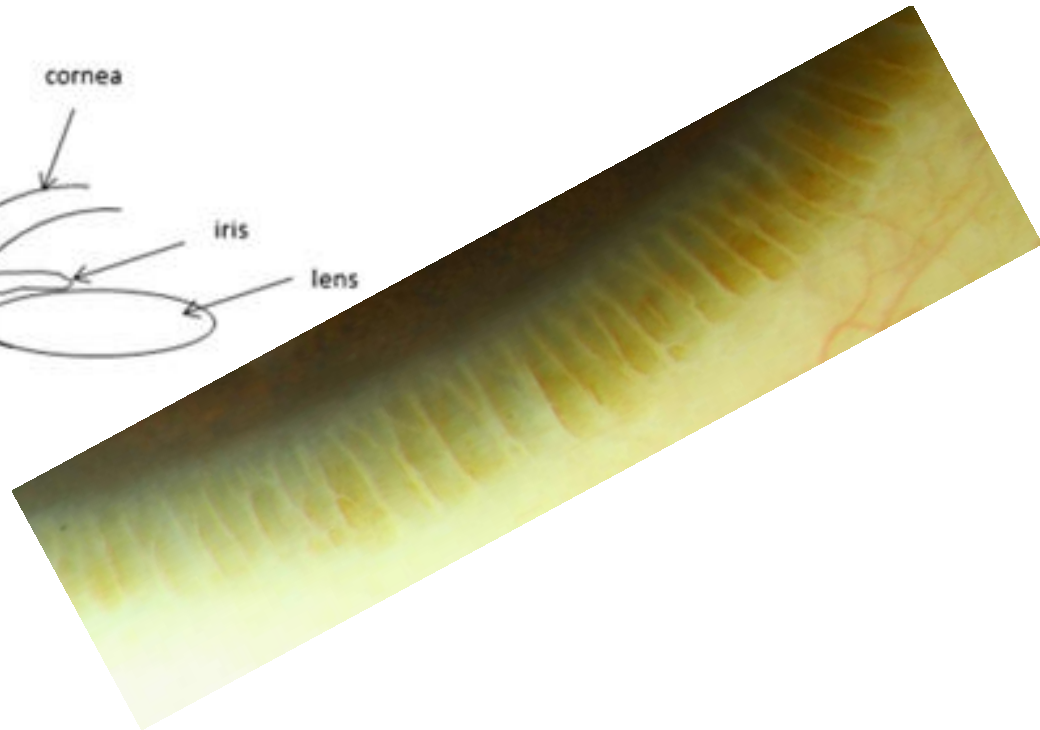
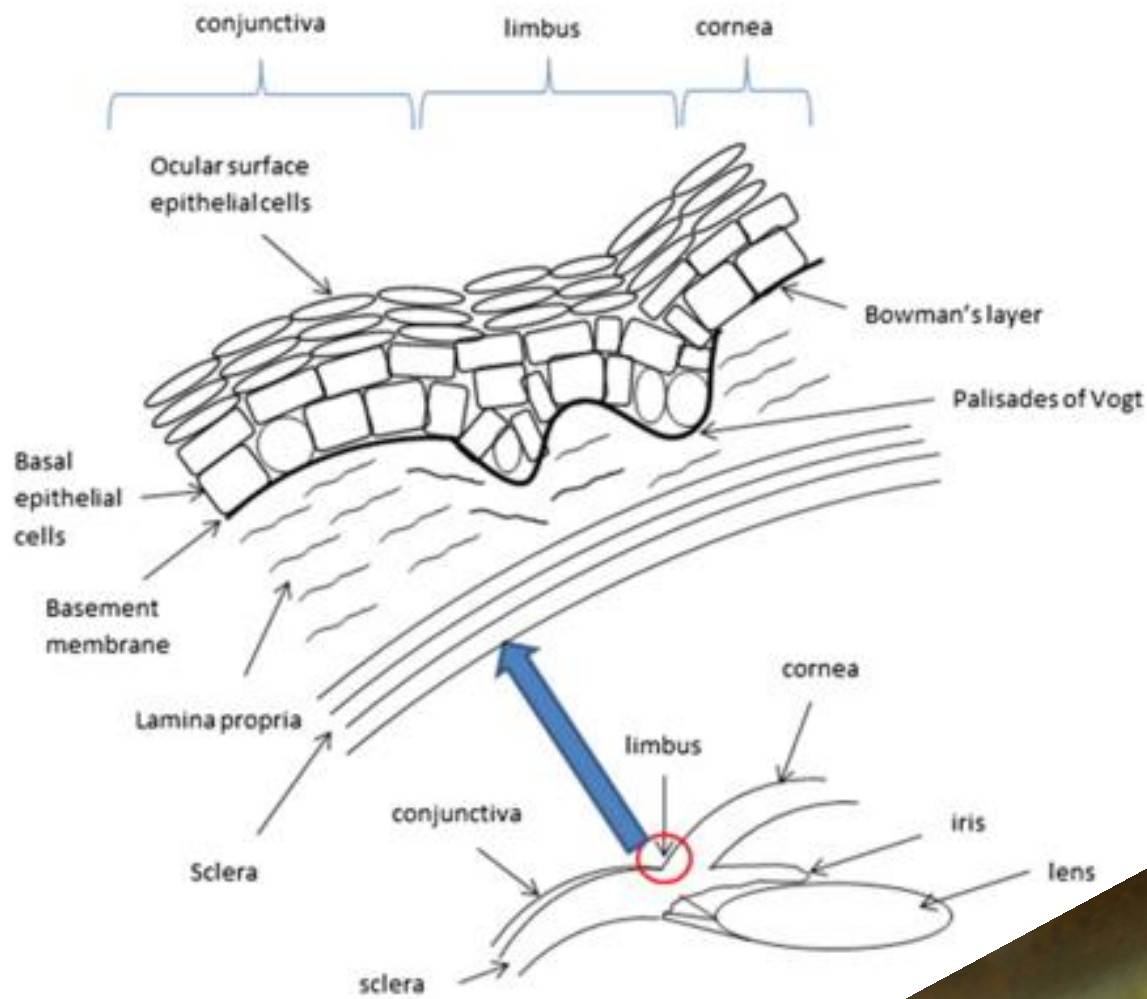
# La superficie oculare



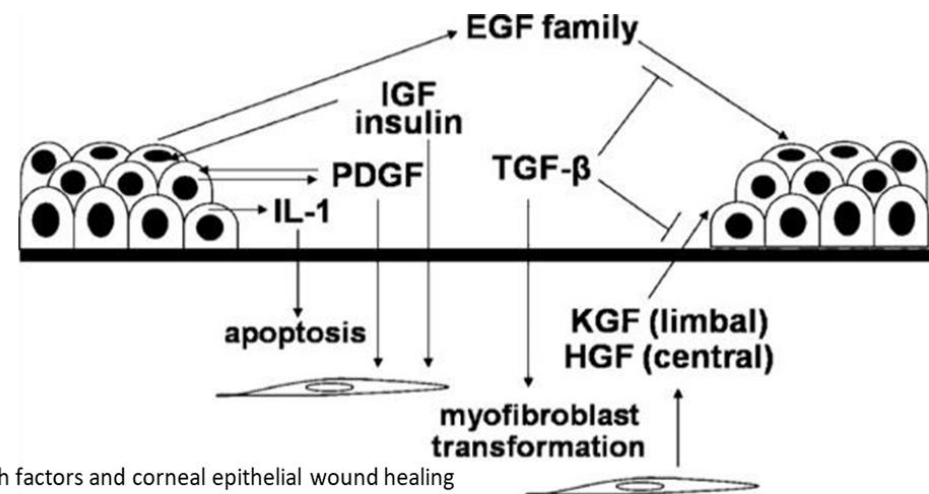
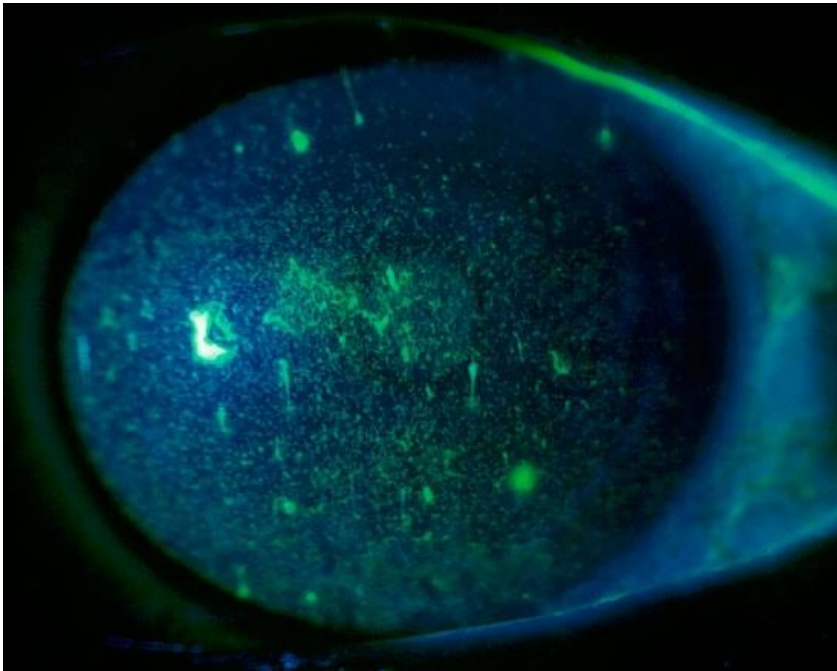
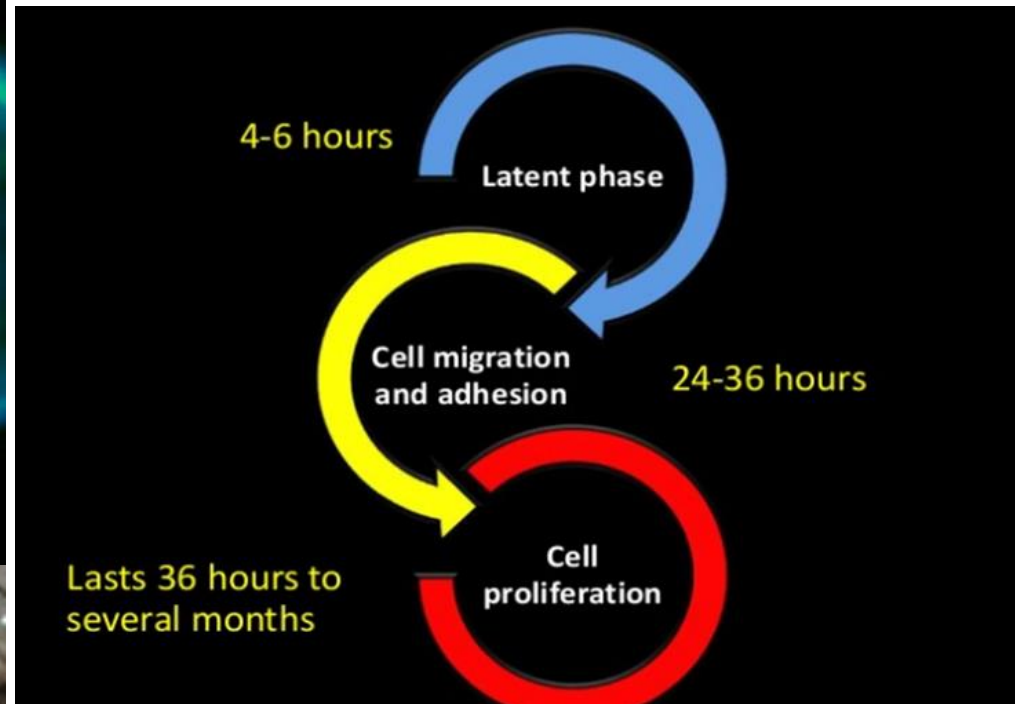
## Film lacrimale pre-oculare



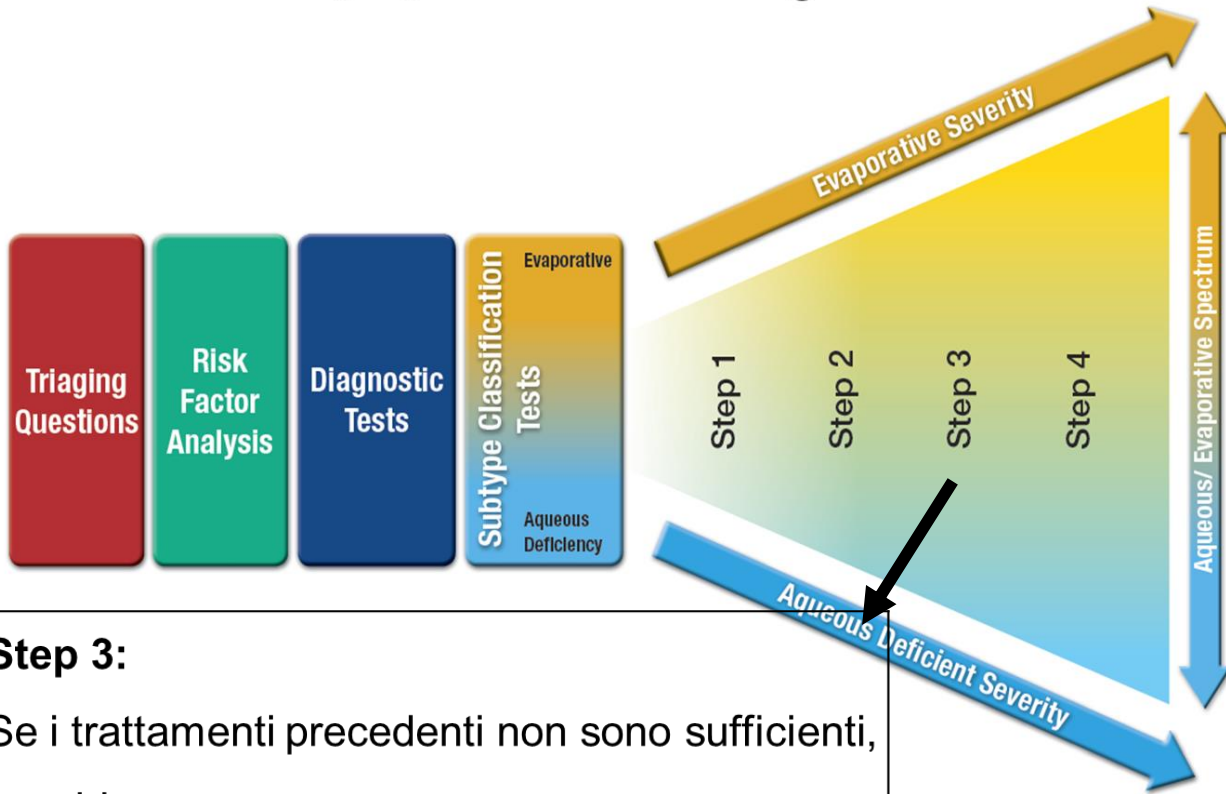
# Corneo conjunctival limbus



## Corneal wound healing steps



# Dry Eye Disease Management



## Step 3:

Se i trattamenti precedenti non sono sufficienti, considerare:

- Secretagoghi per via orale
- Sostituti lacrimali biologici
- Lenti a contatto terapeutiche
  - Morbide
  - Rigide sclerali



## TFOS DEWS II Management and Therapy Report



Lyndon Jones, FCOptom, PhD <sup>a,1,\*</sup>, Laura E. Downie, BOptom, PhD <sup>b</sup>, Donald Korb, OD <sup>c</sup>,  
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## SOSTITUTI LACRIMALI BIOLOGICI

- ✓ Siero autologo
- ✓ Siero da sangue cordonale
- ✓ Preparati piastrinici (plasma ricco di piastrine; plasma ricco in fattori di crescita; lisato piastrinico)

# Rationale for the use of blood-based products

- growth factors EGF, VEGF-A, IGF-1, TGF- $\beta$   
fibronectin, albumin

cell migration  
antiapoptotic effects ,  
stromal repair process  
wound healing

- vitamin A

squamous metaplasia prevention  
cellular tropism

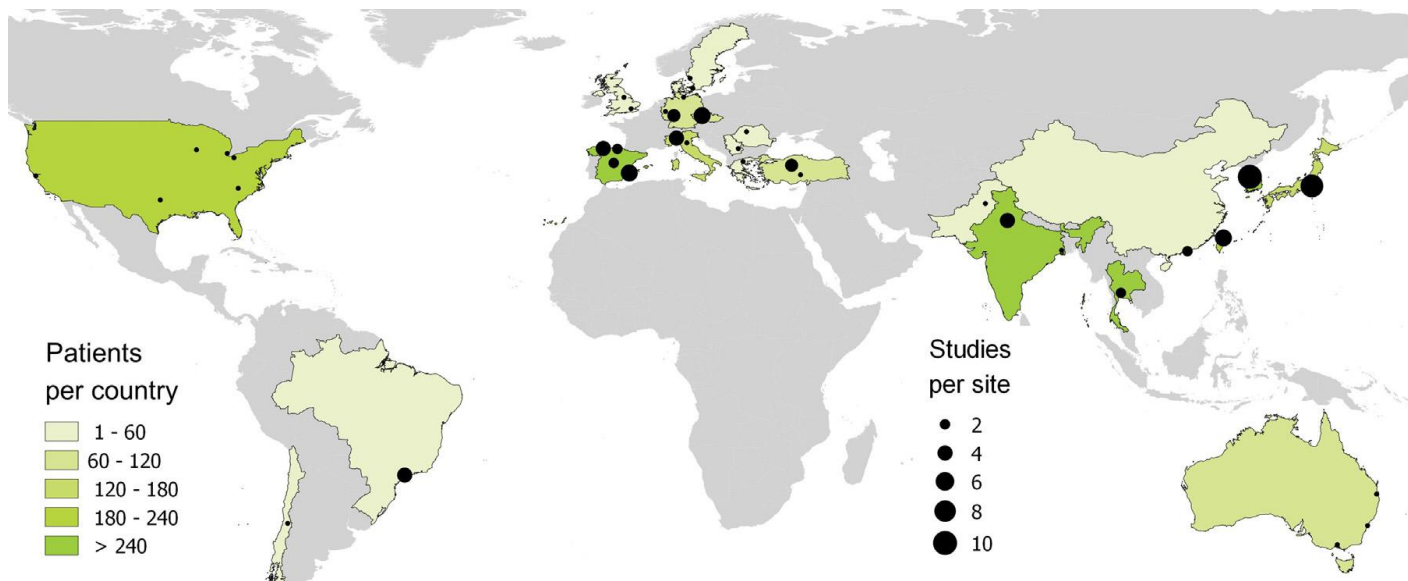
- bacteriostatic components lysozyme

reduce the risk of contamination  
and infection during repair processes

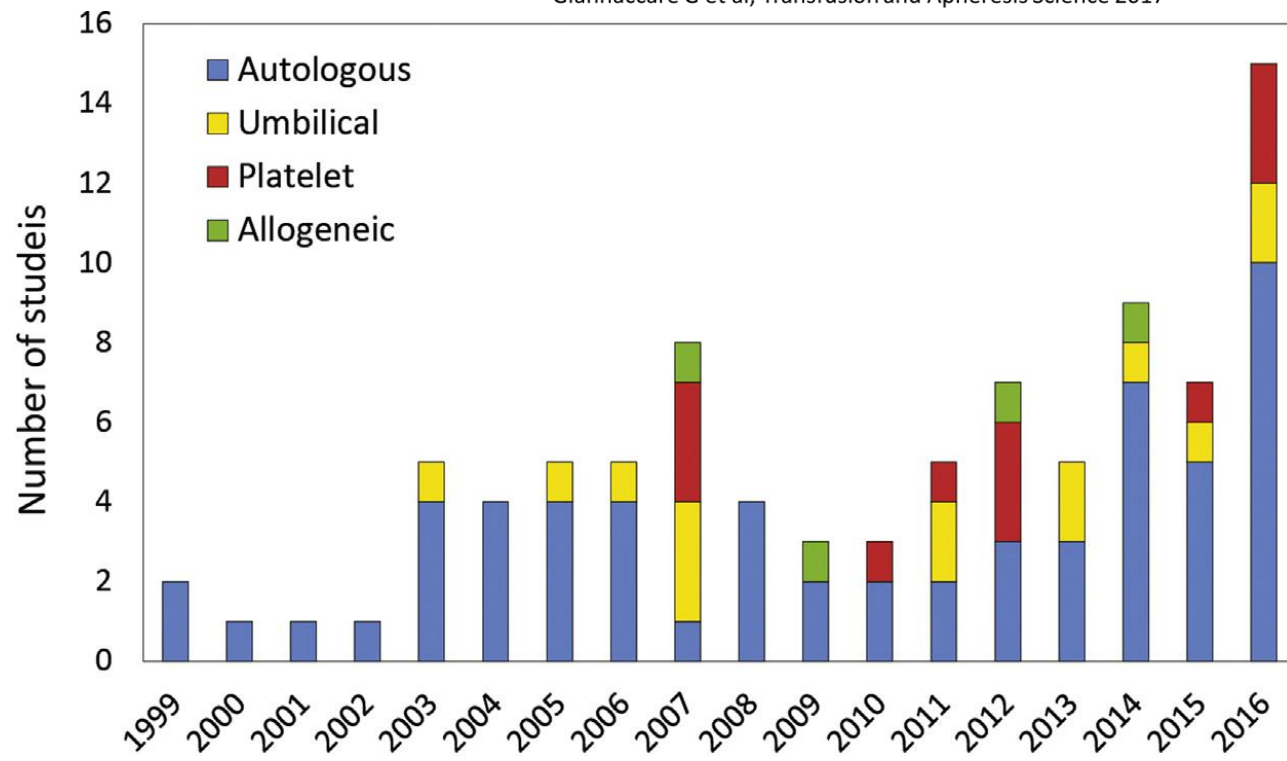
- $\alpha$ -2 macroglobulin

anticollagenase activity

- free of preservatives which potentially induce toxic or allergic reactions
- osmolality and biomechanical properties are similar to those of natural tears



Giannaccare G et al, Transfusion and Apheresis Science 2017



Parameters to be defined in the production of serum eye drops and previously described variations, storage, and application. Geerling G et al, BJO, 2004

Production factor	Published variations
Clotting phase	0–2 days
Centrifugal force	1500 rpm (ca 300 g) to 4000 g (ca 5000 rpm)
Duration of centrifugation	5–20 minutes
Dilution	20%, 33%, 50%, or 100%
Diluent	0.9% NaCl, BSS, 0.5% chloramphenicol eye drops
Container	1–6 ml in insulin syringe or dropper bottle
Storage	–20° to +4° C
Number of daily applications	3 times to hourly

rpm, rounds per minute; g, g force; BSS, balanced salt solution.

# Platelet-Rich Plasma Differs According to Preparation Method and Human Variability

J Bone Joint Surg Am. 2012;94:308-16

Augustus D. Mazzocca, MS, MD, Mary Beth R. McCarthy, BS, David M. Chowanec, BS, Mark P. Cote, DPT, Anthony A. Romeo, MD, James P. Bradley, MD, Robert A. Arciero, MD, and Knut Beitzel, MD

**TABLE III Growth Factor Concentration Compared Between Separation Methods**

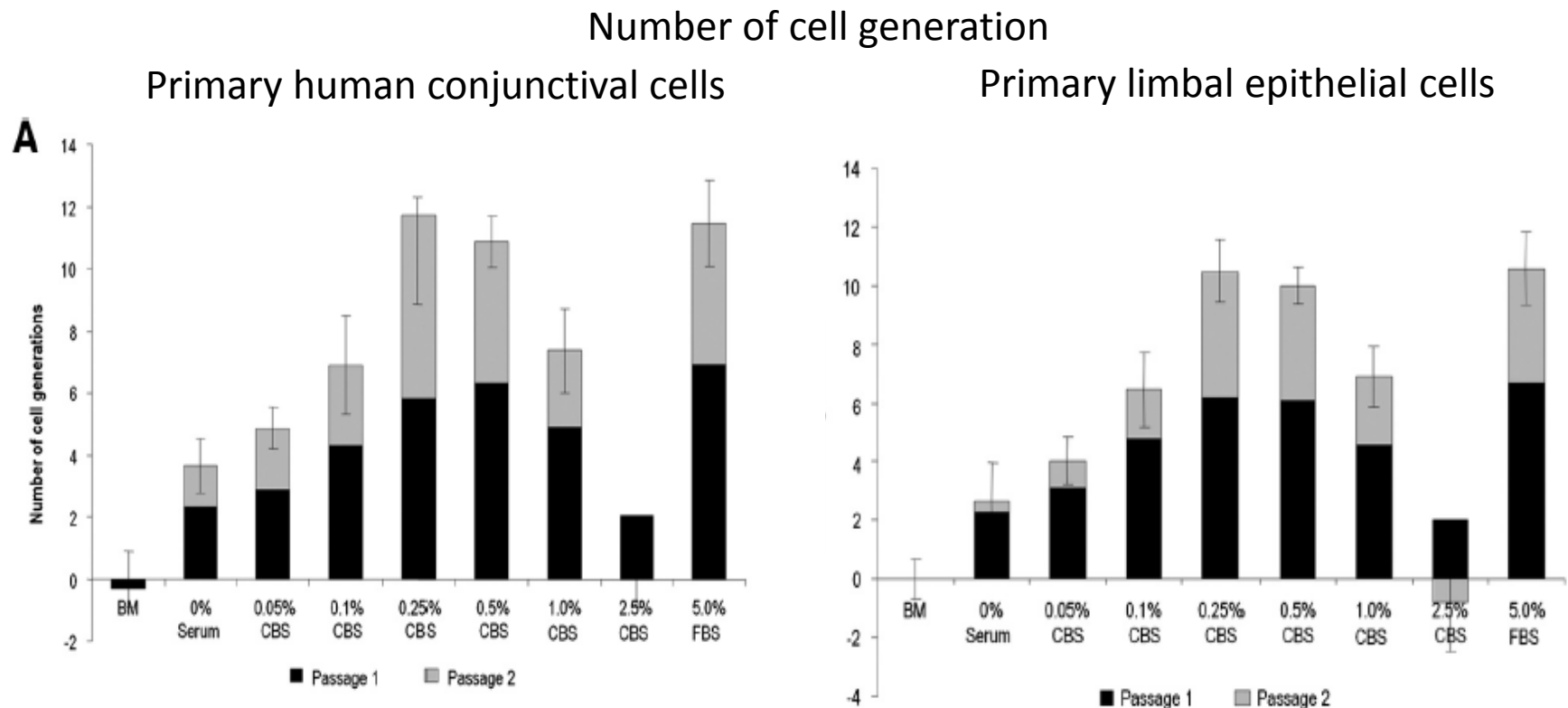
Growth Factor*	PRP <sub>LP</sub> † (pg/mL)	PRP <sub>HP</sub> † (pg/mL)	PRP <sub>DS</sub> † (pg/mL)
EGF	659.8 ± 35.9	2639.5 ± 197.7	670.7 ± 185.1
FGF-2	15.6 ± 2.4	75.2 ± 21.4	15.2 ± 3.4
HGF	645.2 ± 72.1	4277.3 ± 1508.2	581.7 ± 43.2
IGF	64.8 ± 55.4	672.9 ± 378.4	45.1 ± 60.7
PDGF	16,668.1 ± 5512.3	42,273.9 ± 2902.4	12,263.7 ± 3632.7
TGF-β	66,246.2 ± 7620.4	141,286.9 ± 12,576.1	83,011.7 ± 14,129.8
VEGF	138.7 ± 11.2	142.9 ± 12.5	138.7 ± 9.1

\*EGF = epidermal growth factor, FGF-2 = fibroblast growth factor, HGF = hepatocyte growth factor, IGF = insulin-like growth factor, TGF-β = transforming growth factor-beta, and VEGF = vascular endothelial growth factor. †The values are given as the mean and the standard deviation. PRP<sub>LP</sub> = platelet-rich plasma prepared with single-spin method resulting in lower number of white blood cells and platelets, PRP<sub>HP</sub> = alternative method resulting in a high amount of white blood cells and platelets, and PRP<sub>DS</sub> = double-spin method.

# Does too much mean better ?

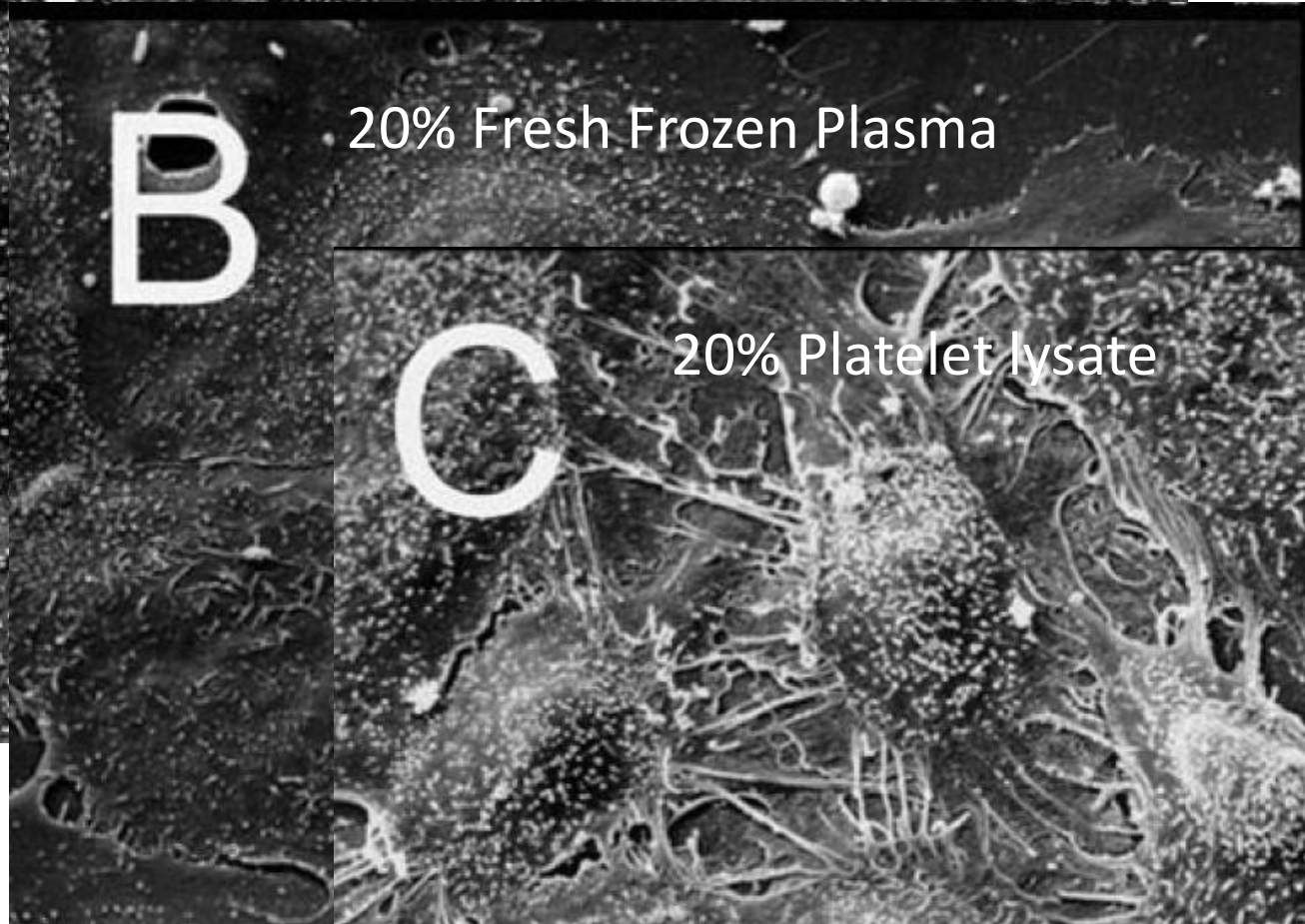
Kruse FE, Tseng SC Growth factors modulate clonal growth and differentiation of cultured rabbit limbal and corneal epithelium. Invest Ophthalmol Vis Sci, 1993

- Increasing concentrations of EGF from 5 ng/ml to 10 and 100 ng/ml resulted in the down-regulation of clonal growth



Ang LPK et al. Ex Vivo Expansion of Conjunctival and Limbal Epithelial Cells Using Cord Blood Serum-Supplemented Culture Medium. Invest Ophthalmol Vis Sci. 2011;52:6138-6147

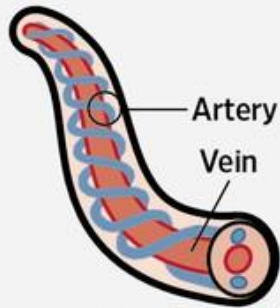
# Do blood derived products work similarly?



# Umbilical cord blood UCB

**1.** Baby is born with umbilical cord and placenta attached.

**2.** After the cord is tied and cut, some blood is left in the blood vessels of the placenta and cord.



Parts of the umbilical cord used in extraction of cord-blood stem cells

**3.** This cord blood is extracted from the umbilical cord using a special collection bag.

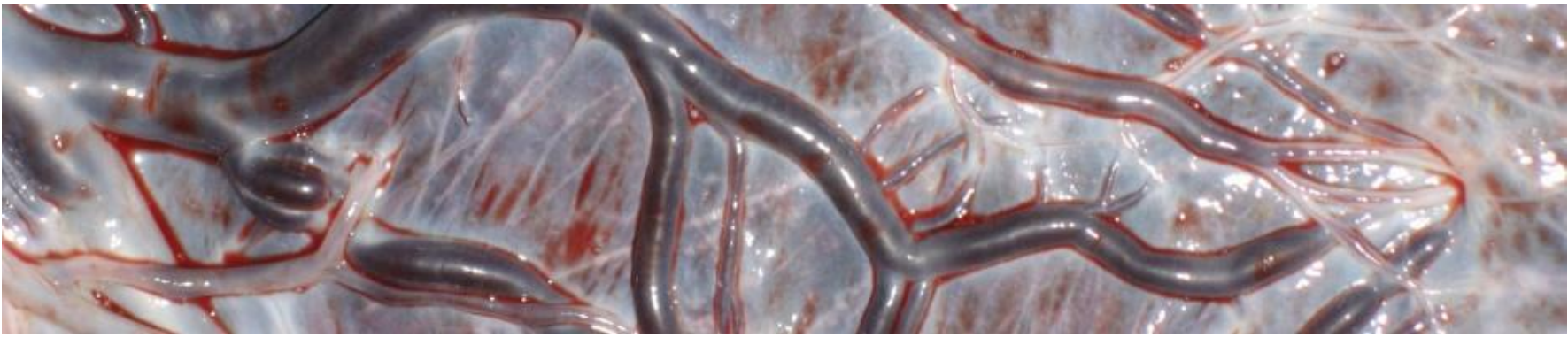


By donating your  
**baby's cord blood,**  
you give  
**patients hope.**

BE  THE MATCH

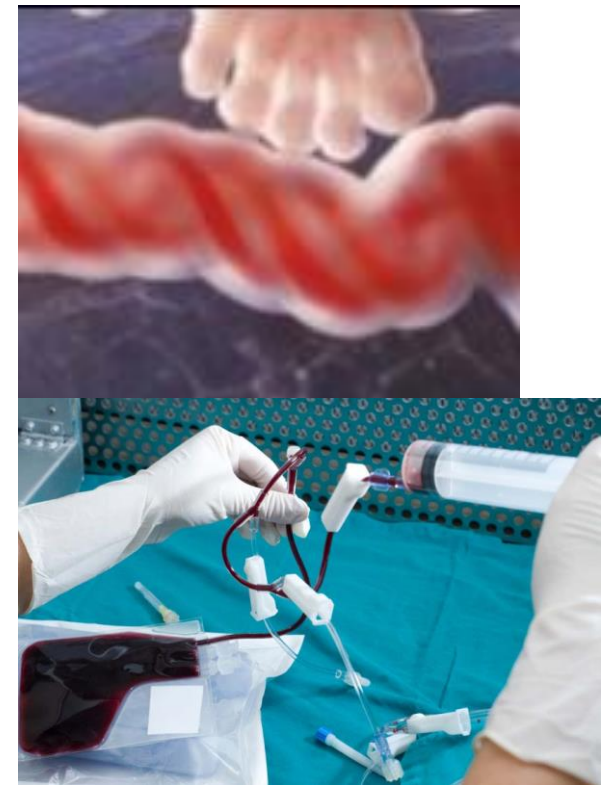
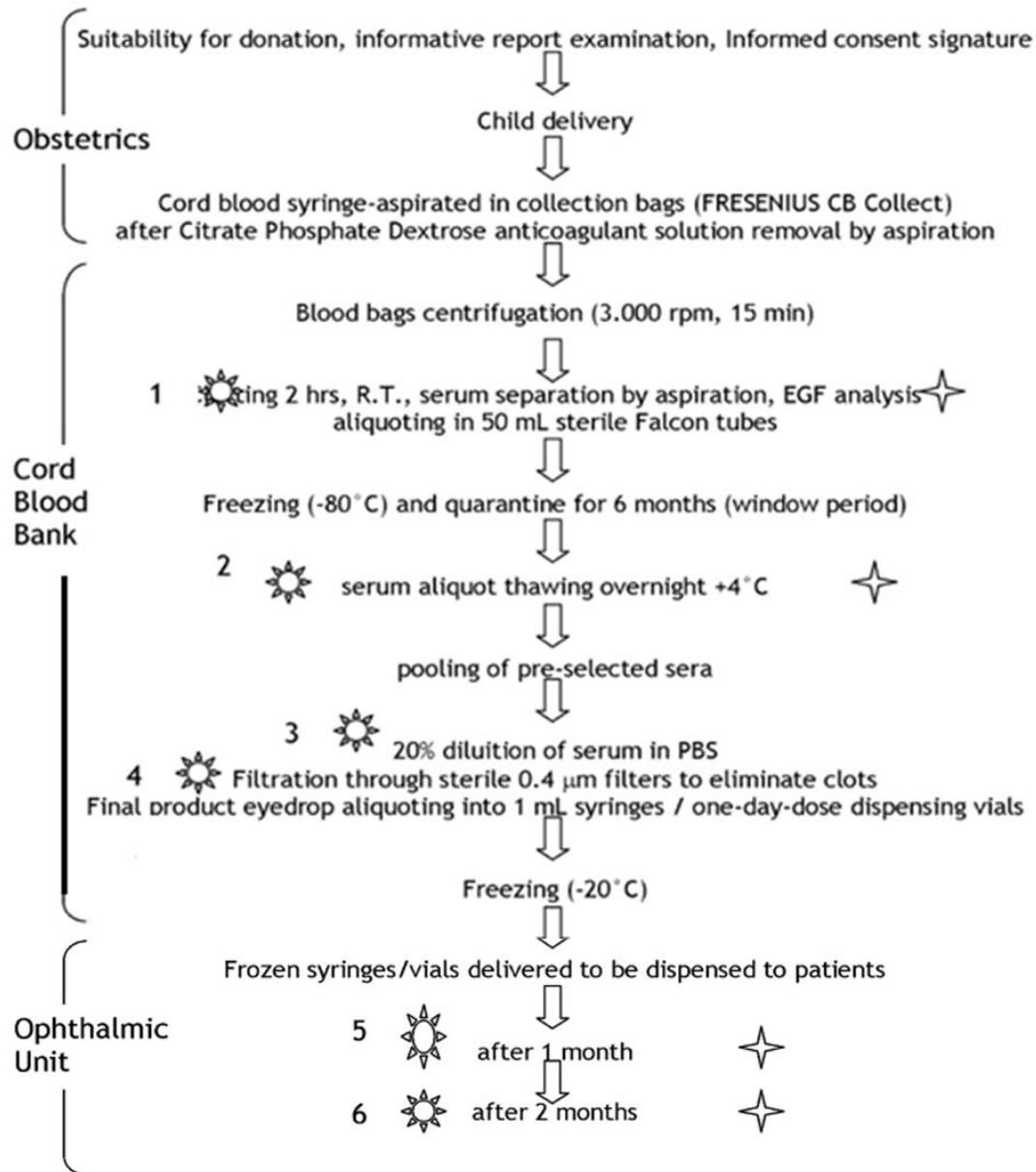


In our Center UCB is sampled from the placental vein after SCs extraction from the umbilical vein



# UCB-based eyedrops      rationale

- ✓ Large quantities of the product may be collected at every delivery  
Yoon KC, et al., Ophthalmology. 2005
- ✓ Debilitated patients  
Yoon KC, et al., Ophthalmology. 2007  
Yoon KC et al., Bone Marrow Transpl. 2007
- ✓ Haematological discrasia  
Yoon KC et al., Am J Ophthalmol. 2007  
Yoon KC et al., Cornea. 2011
- ✓ Infectious diseases  
Sharma N, et al., IOVS. 2011  
Ang LP, et al., IOVS. 2011
- ✓ Absence of inflammatory cytokines (GVHD, SS)
- ✓ Higher concentrations of GFs as compared to other blood components for topical use

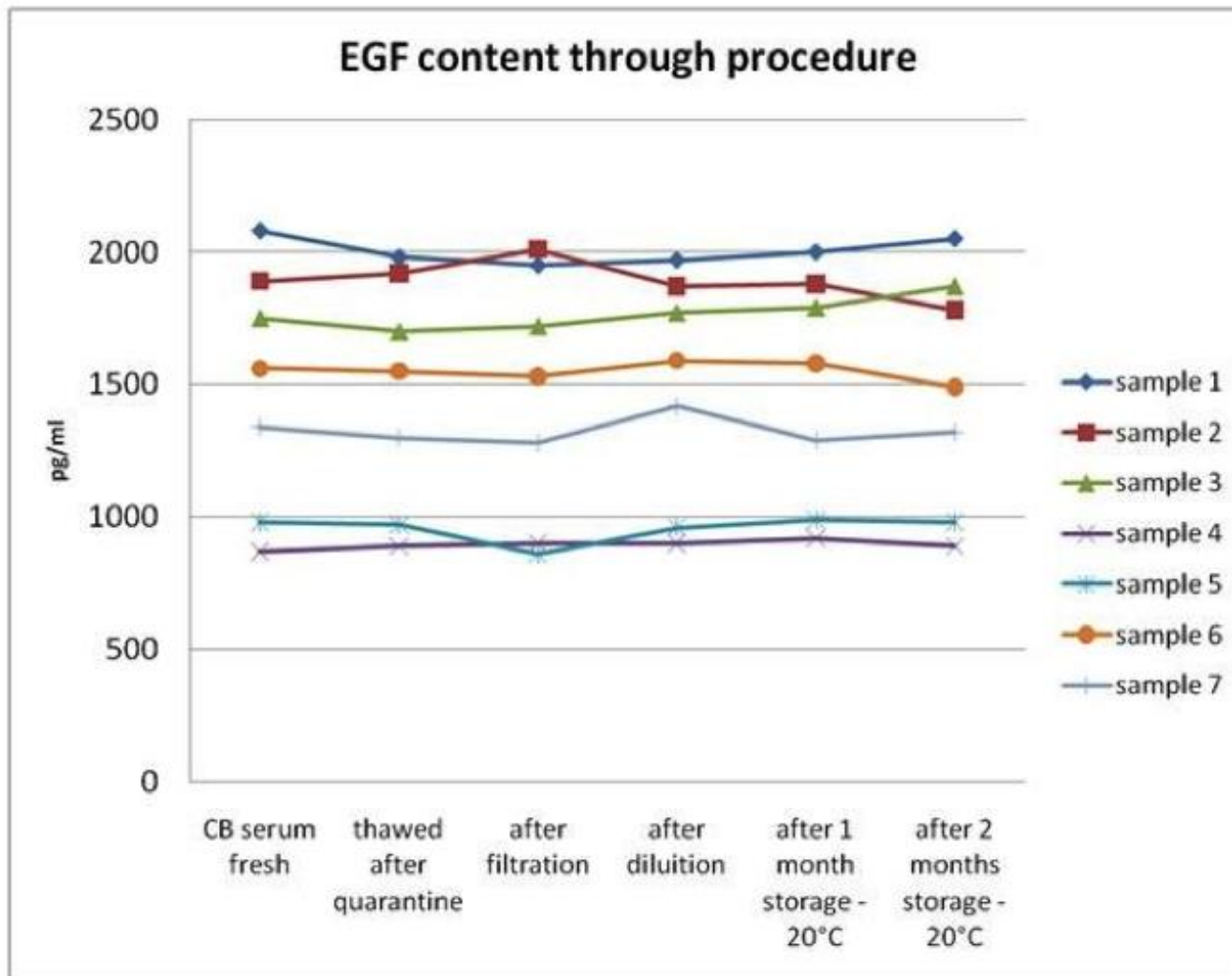


### **Maternal blood (delivery and after 6 m)**

HBV, HCV, HIV1 e 2,  
HCV-NAT, HBV-NAT, HIV -NAT, Siphylis,  
HTLV 1 e 2, CMV, TOXO, WNV)

### **UCB**

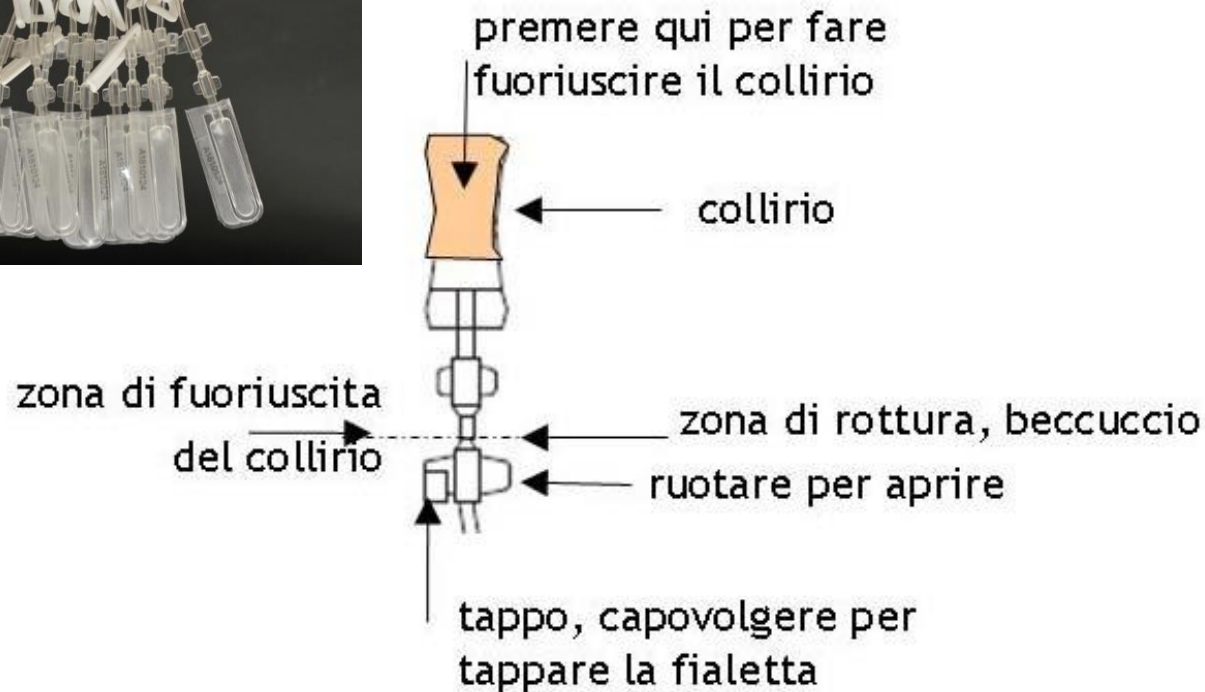
Blood group ABO / Rh,  
sterility  
Mononuclear total cells,  
CD34+ hemopoietic stem cells





Content 0.8 ml /vial

Posology : 8 times/day , 1 drop/eye  
daily supply of 0.10-0.20 ng/mL EGF,  
similar to the physiological human tear  
content.



## 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\**

EudraCT: 2008-005757-38

Cornea, 2013

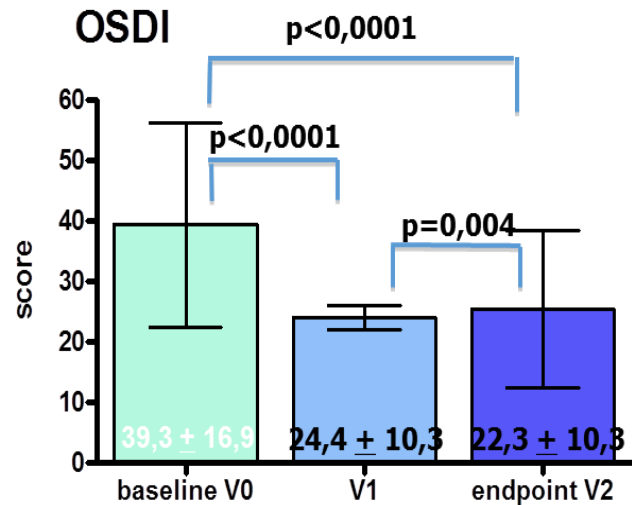
Clin Trial Gov Id NCT01234623

Sterile CBS eye drops were prepared to supply 0,15 ng/eye/day Epidermal Growth Factor and administered for one month in a one-day-dose dispensing.

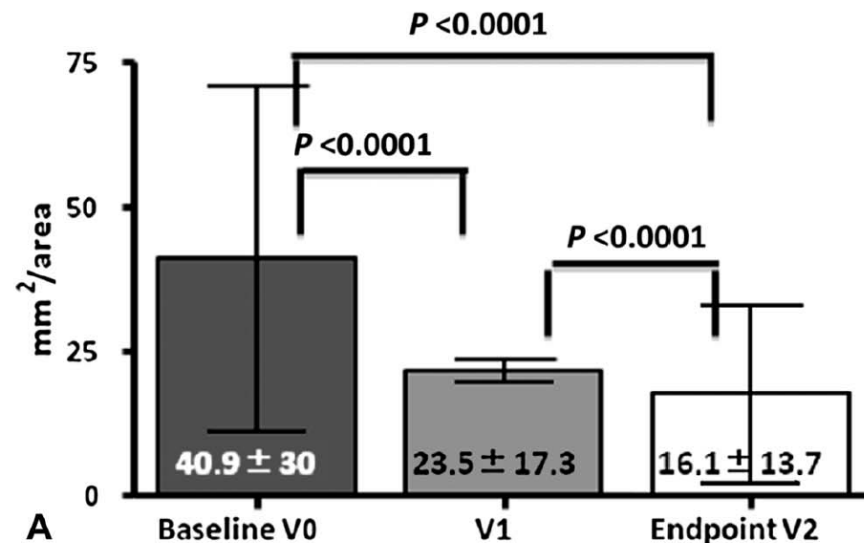
# Methods

V0	V1 (15day)	V2 (30day)	tests
✓	✓	✓	Subjective symptoms (OSDI-VAS score)
✓	✓	✓	Corneal damage (mm <sup>2</sup> ) image
✓		✓	Test Schirmer test
✓	✓	✓	Break up time (BUT)
✓		✓	Tear osmolarity
✓	✓	✓	Corneal esthesiometry -Cochet Bonnet
✓		✓	Imprint Conj cytology
✓		✓	Scraping Conj cytology
✓	✓	✓	Tolerability and satisfaction to treatment

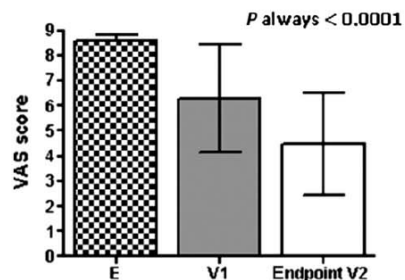
## OSDI



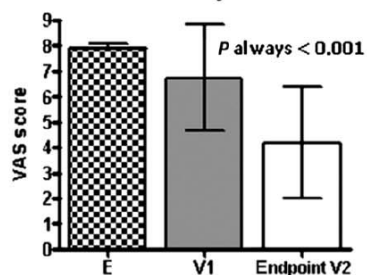
## Damaged corneal epithelium



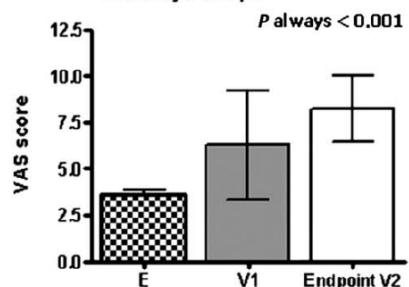
## My eyes feel dry in the morning



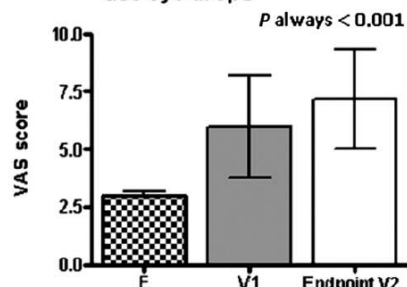
## My eyes feel dry at the end of the day



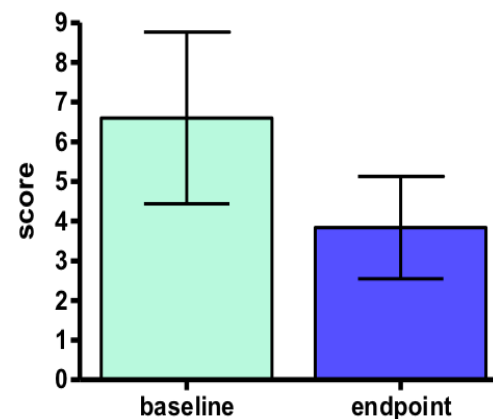
## My eyes feel refreshed when I use eye drops



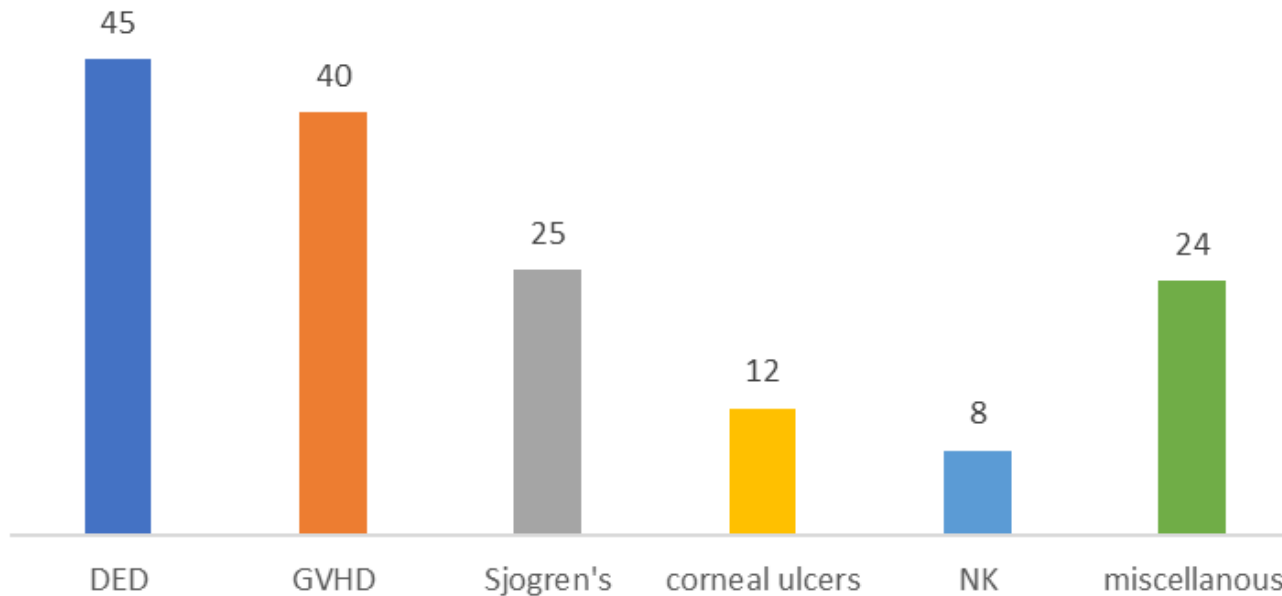
## My eyes feel refreshed longer than expected, when I use eye drops



## scraping cytology



# CBS treatments 156 patients / disease

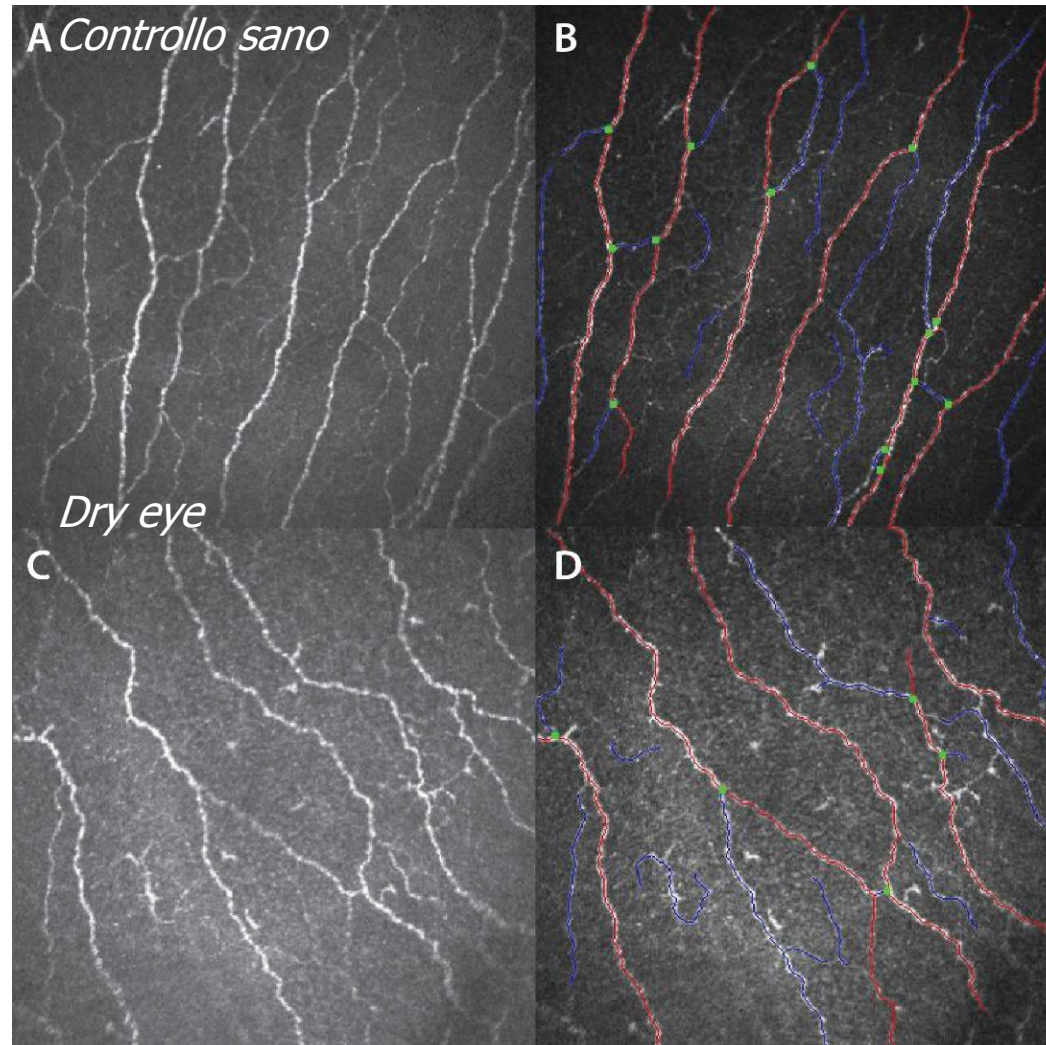


Updated Nov 2019

dilution	AS eyedrops	CBS eyedrops
20%	2.27	3.0
Estimated costs ( € / day treatment )		

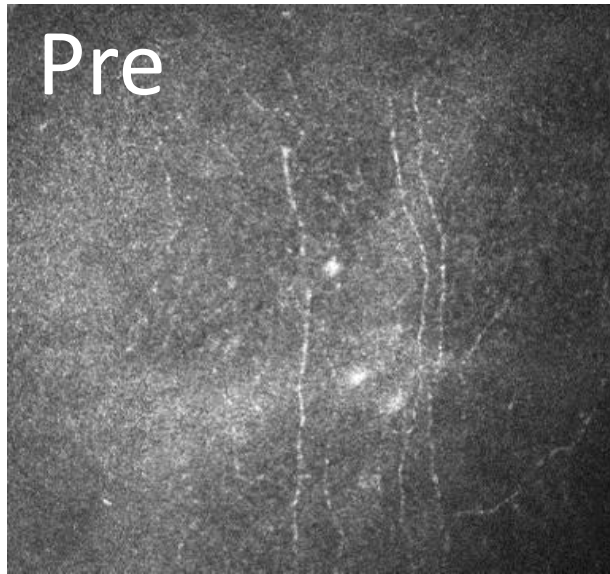
4 cycles / year

# Sistema di analisi machine-learning

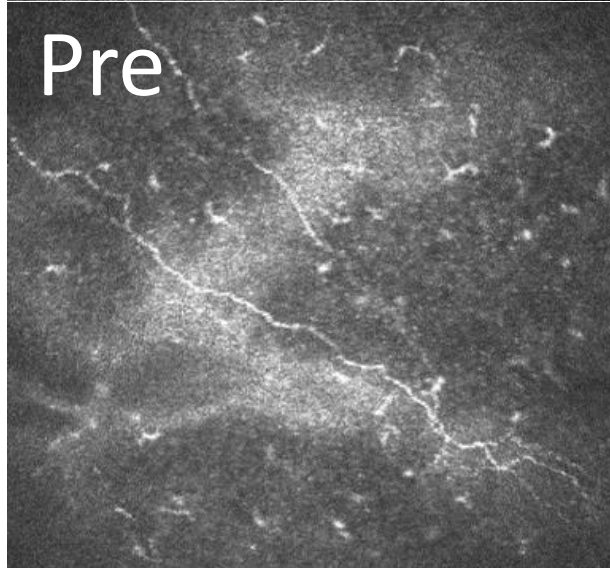
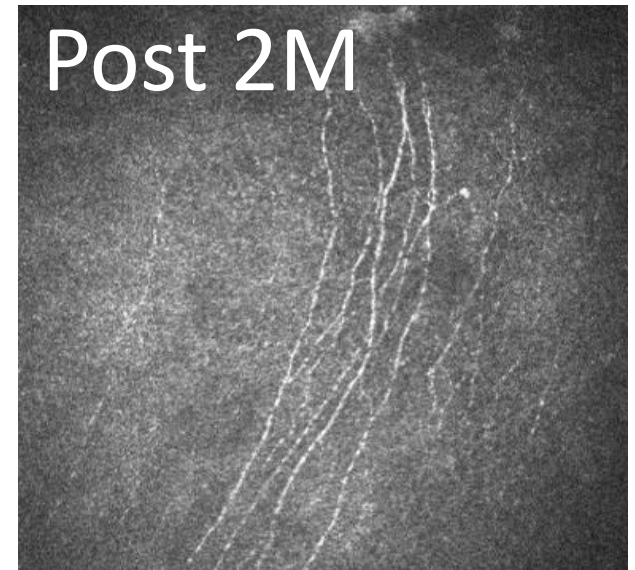


- 1) Densità nervi ( $n/mm^2$ )
- 2) Ramificazione nervi ( $n/mm^2$ )
- 3) Lunghezza nervi ( $mm/mm^2$ )
- 4) Ramificazioni tot nervi ( $n/mm^2$ )
- 5) Area nervi ( $mm^2/mm^2$ )
- 6) Larghezza nervi ( $mm/mm^2$ )
- 7) Dimensione frattale

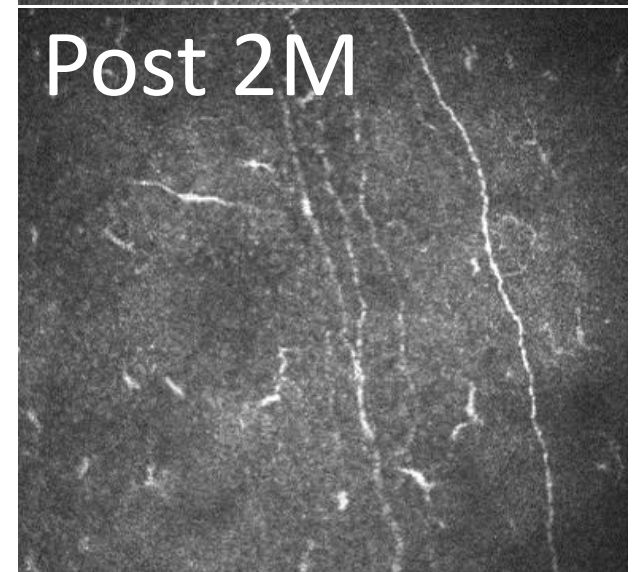
# CBS e microscopia confocale in vivo



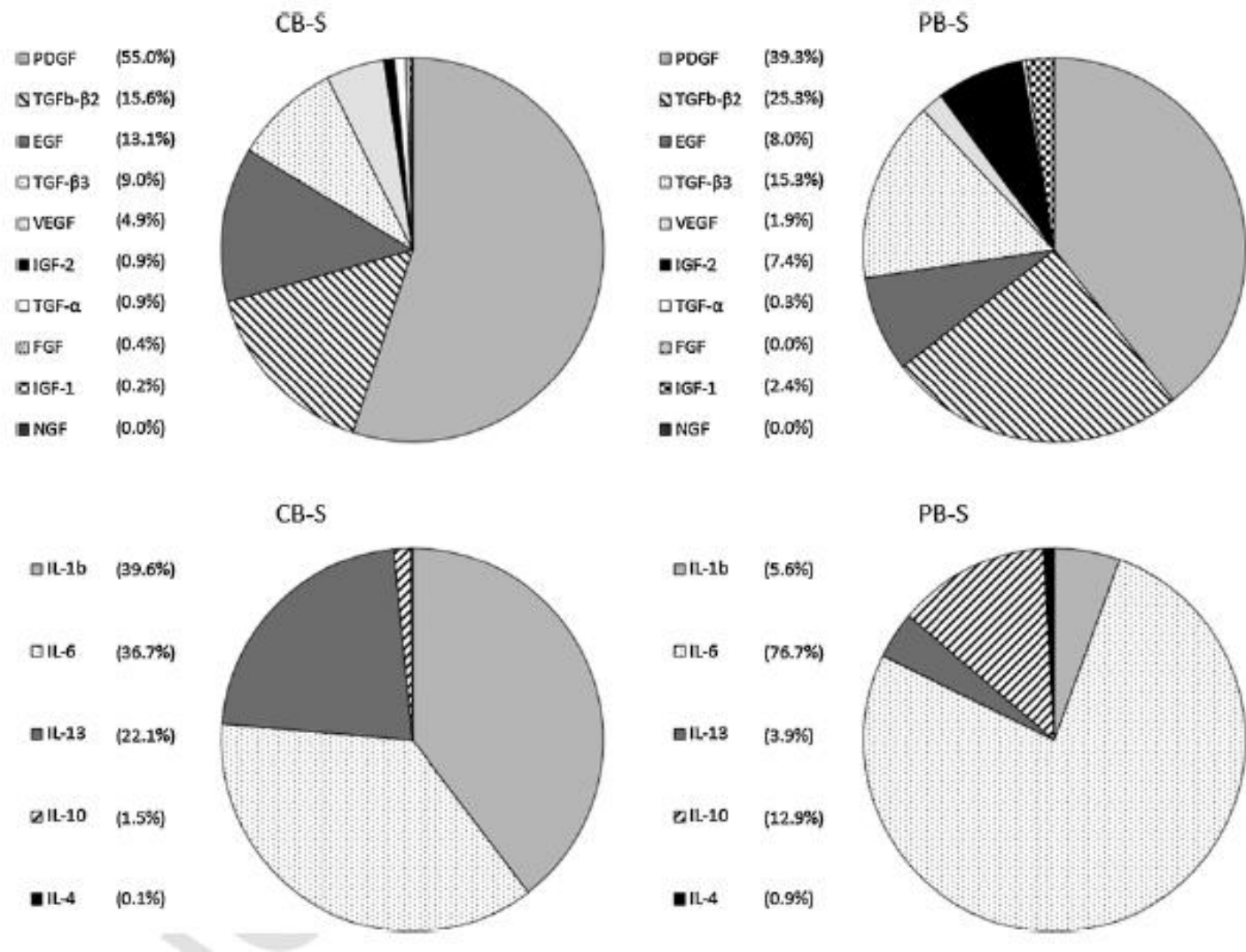
↑ Densità

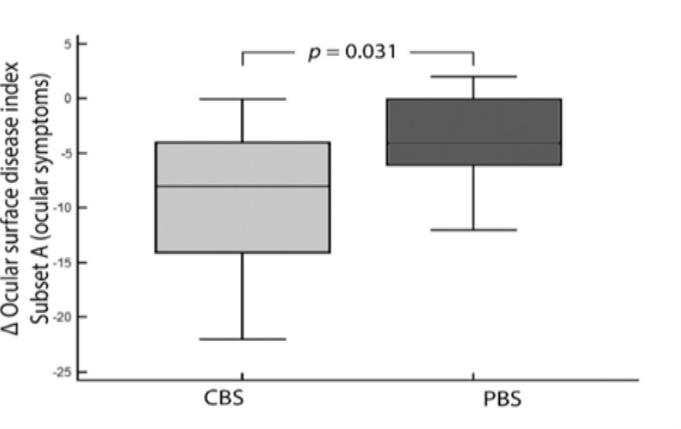


↓ Tortuosità

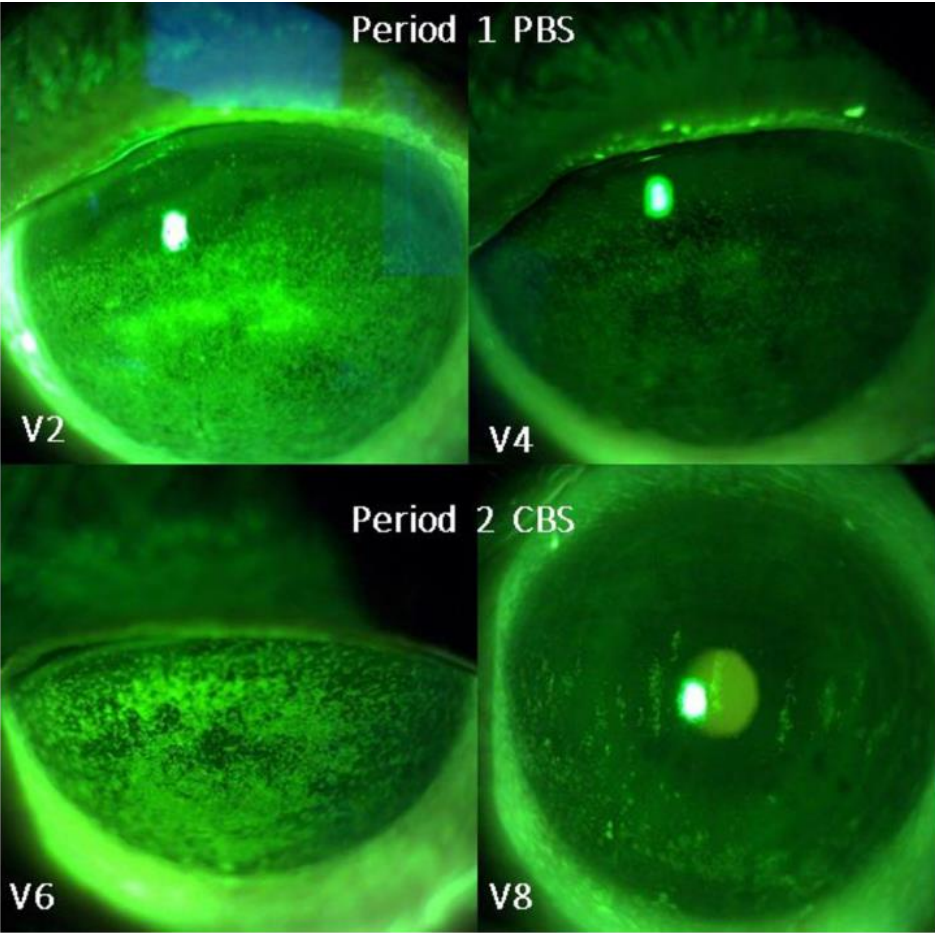


Comparison of growth factors and interleukin content of adult peripheral blood and cord blood serum eye drops for corneal and ocular surface diseases.  
Buzzi M et al. Transfus Apher Sci 2018





- 1. occhi sensibili alla luce
  - 2. sensazione di sabbia negli occhi
  - 3. dolore o irritazione negli occhi
  - 4. visione annebbiata
  - 5. visione insufficiente
- OSDI subset A



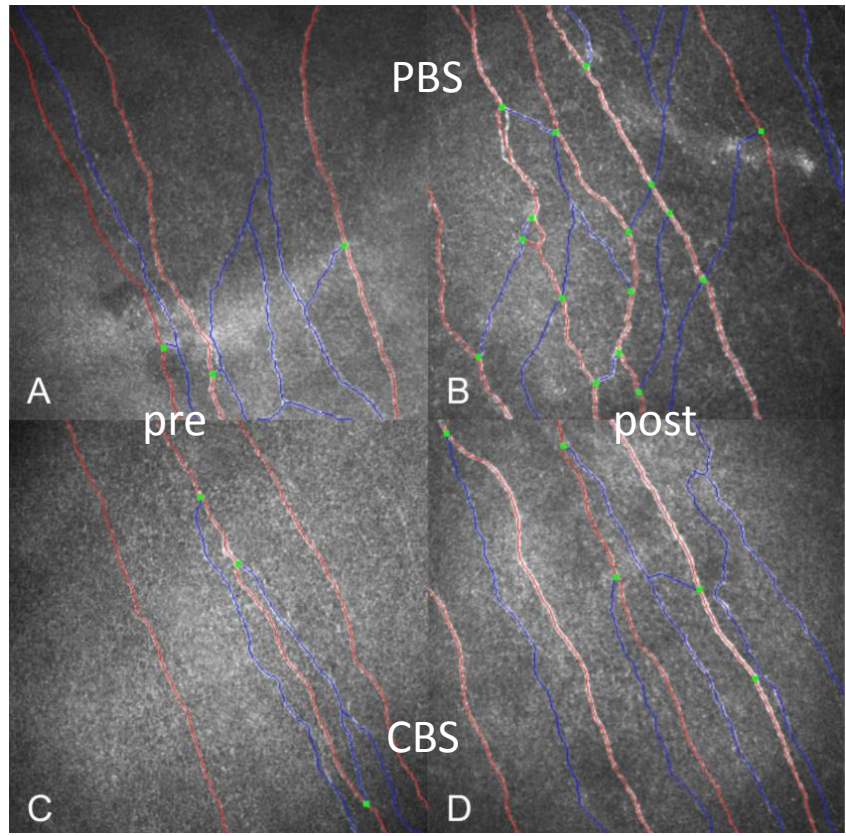
CB-S eye drops were more effective in decreasing symptoms and keratoepitheliopathy in severe dry eye syndrome (including SS and oGVHD) as compared to PB-S eye drops.

Buzzi M et al, Transfus Apher Sci. 2018; 57: 549-555  
Giannaccare G et al. Transfus Apher Sci. 2017; 56:595-604.  
Giannaccare G et al. Cornea. 2017; 36: 915-921  
Versura P et al. Blood Transfus. 2016; 14: 145-51.  
Versura P et al. Blood Transfus. 2014 Jan;12 Suppl 1:s44-50  
Versura P et al. Cornea. 2013; 32: 412-8.

# In Vivo Confocal Microscopy Automated Morphometric Analysis of Corneal Subbasal Nerve Plexus in Patients With Dry Eye Treated With Different Sources of Homologous Serum Eye Drops

Giuseppe Giannaccare, MD, PhD,\* Marco Pellegrini, MD,\* Federico Bernabei, MD,\*  
Fabiana Moscardelli, CO,\* Marina Buzzi, BSD,† Piera Versura, BSD,\* and Emilio C Campos, MD\*

(*Cornea* 2019;38:1412–1417)



**TABLE 3.** IVCM Metrics of Corneal SNP Before and After Treatment With Allo-PBS (Group 1) and CBS (Group 2) Eye Drops

Parameter	Group 1		Group 2	
	V1	V2	V1	V2
CNFD (n/mm <sup>2</sup> )	21.2 ± 11.5	21.4 ± 9.6	14.5 ± 7.8	19.6 ± 6.3
CNFL (mm/mm <sup>2</sup> )	13.5 ± 5.6	14.1 ± 4.3	10.8 ± 4.5	13.0 ± 3.7
CNFW (mm/mm <sup>2</sup> )	0.023 ± 0.002	0.022 ± 0.002	0.023 ± 0.002	0.022 ± 0.002
CNFrD	1.479 ± 0.050	1.481 ± 0.035	1.455 ± 0.041	1.471 ± 0.030

## TABELLA DELLE INDICAZIONI CON GRADO DI RACCOMANDAZIONE

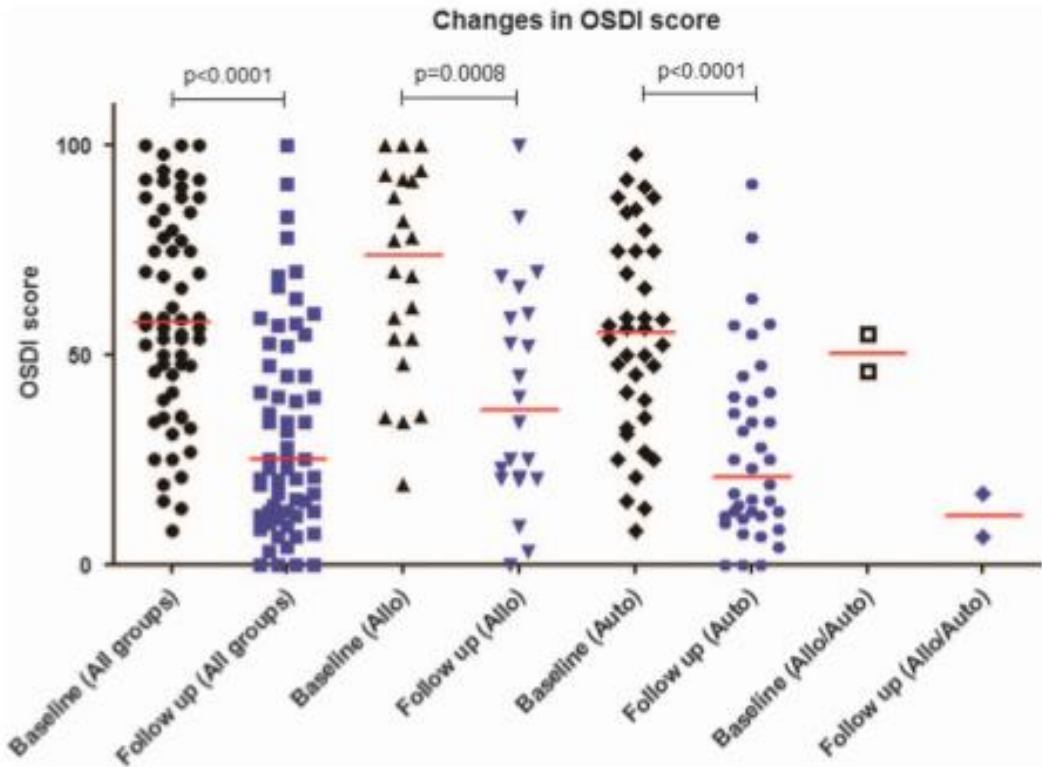
PATOLOGIA	GRADO DI RACCOMANDAZIONE
ULCERE DIABETICHE (per ciclo di trattamento corrispondente a 12 applicazioni)	1B
ULCERE E FERITE DI DIFFICILE GUARIGIONE (per ciclo di trattamento corrispondente a 12 applicazioni)	1B
TRATTAMENTO DELLE OSTEOARTROSI (per ciclo di trattamento corrispondente a 3 applicazioni)	1B
RICOSTRUZIONE TENDINE CROCIATO ANTERIORE	2B
TRATTAMENTO DELLA PSEUDOARTROSI	2B
TRATTAMENTO DELLA TENDINOPATIA ROTULEA	2B
TRATTAMENTO INFILTRATIVO DELLE EPICONDILITI	2B
TRATTAMENTO DELLE LESIONI DEL LEGAMENTO CROCIATO ANTERIORE	2B
TRATTAMENTO DELLE LESIONI DEL TENDINE DI ACHILLE	2B
ALTRE PATOLOGIE OSTEO-MUSCOLARI LIGAMENTOSE	2B

<b>SINDROME DELL'OCCHIO SECCO</b>	<b>2B</b>
<b>LESIONI, ULCERE DELLA SUPERFICIE CORNEALE</b>	<b>2B</b>
<b>USTIONI DELLA SUPERFICIE OCULARE</b>	<b>2B</b>
TRATTAMENTO COADIUVANTE LA GUARIGIONE DELL'ALVEOLO POSTESTRATTIVO	2B
TRATTAMENTO COADIUVANTE I PROCESSI DI GUARIGIONE DOPO CHIRURGIA ESTRATTIVA E IMPLANTARE NEI PAZIENTI CON PATOLOGIE SISTEMICHE	2B
INTERVENTO DI CHIRURGIA ORALE (ESTRAZIONE DENTI INCLUSI, EXERESI LESIONI CISTICHE) PER PROMUOVERE L'EPITELIZZAZIONE DELLE FERITE E ACCELERARE LA FORMAZIONE DEL SIGILLO MUCOSO	2B
INTERVENTI DI CHIRURGIA ORALE IN PAZIENTI IN TERAPIA CON BIFOSFONATI ENDOVENA ED ANTIANGIOGENETICI	2B
EXERESI CHIRURGICA DI MRONJ	2B
INTERVENTI DI IMPLANTOLOGIA	2B
INTERVENTI DI INNESTI OSSEI E RIGENERAZIONE COME SUPPORTO ALLA GUARIGIONE DEI TESSUTI MOLLI E COADIUVANTE DEI MATERIALI DA INNESTO	2B
TRATTAMENTO DI CICATRICI PATOLOGICHE	2B
TRATTAMENTO DELL'ALOPECIA ANDROGENETICA IN FASE INIZIALE	2B
TRATTAMENTO DELL'ALOPECIA AREATA IN FASE INIZIALE	2B
RIGENERAZIONE DEL DISCO INTERVERTEBRALE	2C
TRATTAMENTO DEGLI ESITI DELLE CICATRICI DA ACNE	2C
TRATTAMENTO DEL LICHEN GENITALE MASCHILE E FEMMINILE	2C

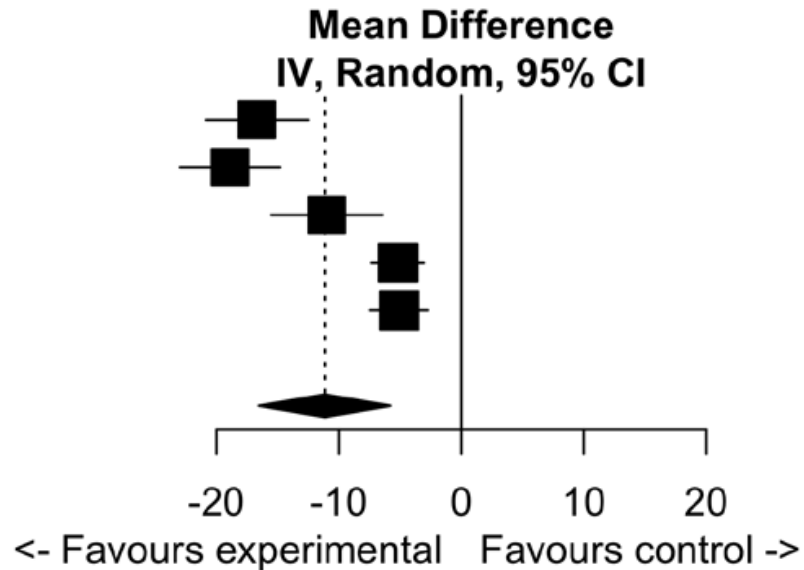
**Table 3** Similarities of key constituents in whole tears and serum (reproduced from Rauz and Saw)<sup>7</sup>

Parameter	Whole tears	Serum
pH	7.4	7.4
Osmolality	298	296
EGF (ng/ml)	0.2–3.0	0.5
TGF- $\beta$ (ng/ml)	2–10	6–33
NGF (pg/ml)	468.3	54.0
IGF (ng/ml)	0.31	105
PDGF (ng/ml)	1.33	15.4
Albumin (mg/ml)	0.023	53
Substance P (pg/ml)	157	70.9
Vitamin A (mg/ml)	0.02	46
Lysozyme (mg/ml)	1.4	6
Surface IgA ( $\mu$ g/ml)	1190	2
Fibronectin ( $\mu$ g/ml)	21	205
Lactoferrin (ng/ml)	1650	266

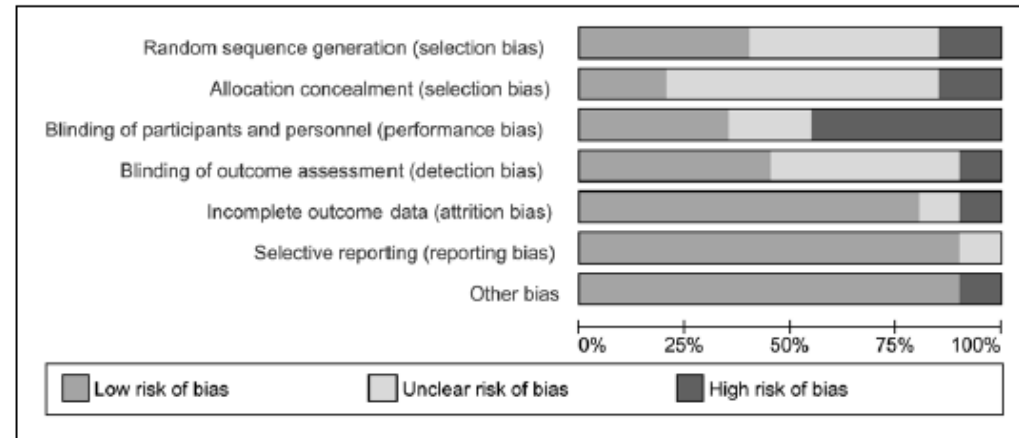
Rauz S et al. The Royal College of Ophthalmologists guidelines on serum eye drops for the treatment of severe ocular surface disease: full report. Eye, 2017



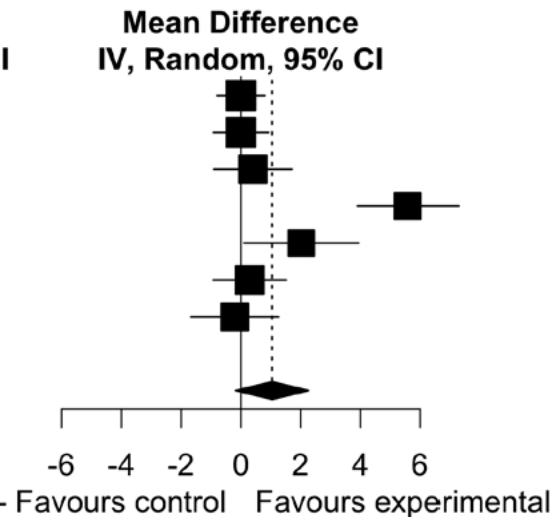
Dry Eye, OSDI, 2-6 weeks



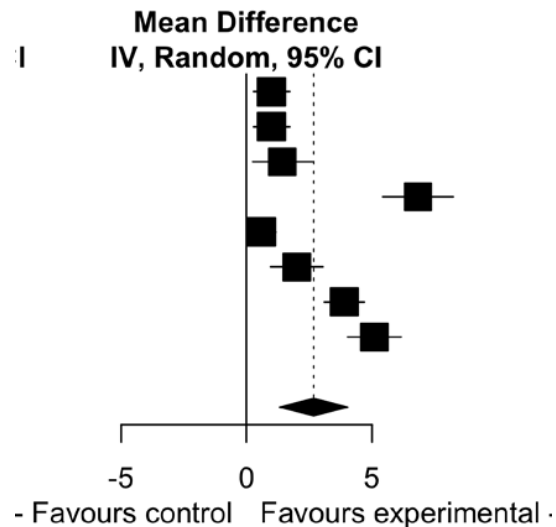
Franchini M et al. Serum eye drops for the treatment of ocular surface diseases: a systematic review and meta-analysis. Blood Transf 2019



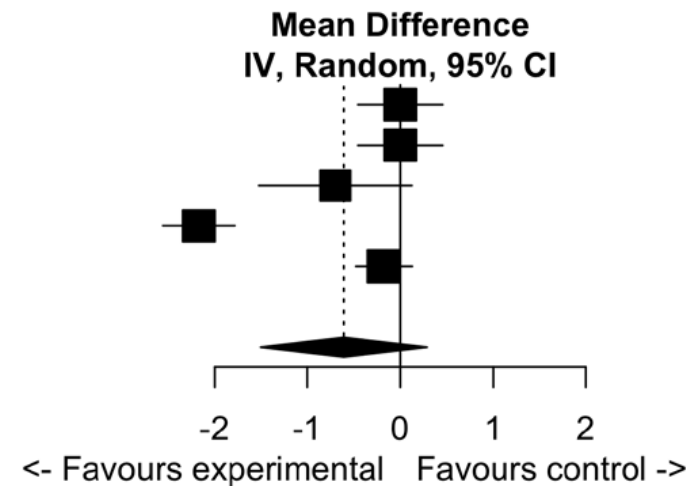
Dry Eye, Schirmer test, 2-6 weeks



Dry Eye, TBUT, 2-6 weeks



Dry Eye, Fluorescein Staining, 2-6 weeks



product name	Product type	Method of production	storage/dosage recommendation	FDA regulated	action/applications
<b>Genesis: Amniotic Cytokine Extract (ACE)<sup>TM</sup>;</b>	AME - Ocular Science, Inc. Palm Beach, CA, USA	Proprietary cryopreservation to extract cytokines from AM; titrated to 10,000 pg/mL	Arrives on dry ice; store in freezer; 30-day treatment; twice daily drops	NO	dry eye disease; <b>active cytokines, growth factors and anti-inflammatory molecules</b> ; prostaglandin E2 promotes wound healing
<b>Keera Lyophilized AME;</b>	AME - Veneto Tissue Bank; Treviso, Italy	Amnion-derived eye drops; reconstituted with saline	Non-preserved; refrigerate and use within 72 hours	NO Internationally available	Use in place of, or following AMT; use in PED secondary to herpes simplex or zoster, trigeminal nerve defect or diabetes (multicenter European clinical trials)
<b>Barcelona Tissue Bank</b>	AME - (Banc de Sang i Teixits); Catalonia, Spain	Freeze-dried product from AM; vial is reconstituted in 4 mL of sterilized water	Treatment is 30 days (6 vials of extract)	NO internationally available	dry eye disease, epithelial defects of the cornea, dry eye secondary to autoimmune syndrome, corneal ulcers, adjuvant in cornea transplant, pterygium and symblepharon excision

product name	Product type	Method of production	storage/dosage recommendation	FDA regulated	action/applications
<b>Amnion-derived Cellular Cytokine Solution (ACCS)</b>	AME - Noveome Biotherapeutics	Proteins secreted by amnion-derived multipotent progenitor cells	Four times a day dosage	Clinical trial ongoing	wound healing, dry eye disease
<b>Regener-Eyes™; Regenerative Processing Plant</b>	AF - Palm Beach, FL, USA	Amniocleanse patent pending process (placental-derived biomaterials); sterile and acellular amniotic fluid drops	Cooler packed box; 30-day supply droppers; apply 1–4 times per day	N; under Section 361 of Public Health Service Act; no clearance, or pre-market approval needed	Increases cytokines, growth factors, hyaluronic acid; no human studies; multiple animal studies
<b>PDA-AF™; Cryoactiv Regenerative Processing Plant</b>	AF - Palm Harbor, FL, USA	Fluid harvested close to 40 weeks gestation; hyaluronic acid mainly	AF eye drops	N; under Section 361 of Public Health Service Act	Use for dry eye
<b>Optiserum™</b>	UC only - Next Biosciences; Johannesburg, South Africa	Umbilical cord blood serum; non-preserved; frozen to –80°C	5 mL eye dropper bottles; use within 7 days	N; internationally available	severe dry eye syndromes, chemical burns, corneal ulcers and erosions, neurotrophic keratitis, post LASEK surgery, and post corneal transplant

## I COLLIRI A BASE DI EMOCOMPONENTI PER LA CURA DELLE MALATTIE DELL'OCCHIO: FACCIAMO IL PUNTO

Aula Polo Murri  
Policlinico S Orsola Malpighi, Bologna  
Padiglione 25, piano 1\*

sabato, 20 ottobre 2018



Società Oftalmologica Italiana

17° CONGRESSO INTERNAZIONALE

24<sup>th</sup> Annual Meeting on Cataract and Refractive Surgery  
da mercoledì 22 a sabato 25 maggio 2019 - RCC La Nuvoletta

**CORSO 118** - Livello base

**Dalla ricerca alla clinica: i colliri a base di emocomponenti**

Direttore: P. Versura

Moderatore: E. Campos

Istruttori: L. Fontana, G. Giannaccare, R. Mencucci, P. Versura

Visita oculistica

Prescrizione



Visita filtro presso  
UO Oftalmologia  
Aziende ospedaliere



Immunoematologia e  
Trasfusionale  
Centro di produzione

- Richiesta SSN
- Consenso informato
- Moduli di erogazione interni del servizio

Codice tariffario

Preparazione-fialettatura  
etichettatura-erogazione

Il paziente si reca al servizio  
e viene istruito alla  
somministrazione

Per quanto?  
E quanto?  
E poi?

Gruppo di opinione interdisciplinare  
Utilizzo emocomponenti ad uso non trasfusionale  
Colliri



## The 5 Ws (and 2Hs) for blood-based eye drops

Who is the patient to be treated, in terms of disease type, severity, and stage?

Why is a blood-based treatment needed, in terms of a target indication?

When is it appropriate to prescribe blood-based therapy, as too late is not always a good option?

Where are the products dispensed? Is a national/regional program a feasible solution to optimize resources?

What is the product of choice? Which source and preparation are targeted for a given patient? Is a patient self-report enough, or should the clinician who prescribed the product report the course, as surgeons do in organ transplants?

How is the product standardized in terms of processing to ensure optimal dilution, solvent, dispenser, and storage time?

How is treatment delivered to the ocular surface, in terms of posology, dose-size modulation, length of treatment, and number of cycles?

**Can pain relief be included as a target indication ?**



grazie

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