A NEW BIOLOGICAL COLORATION FOR CORNEAL AND CONJUNCTIVAL EVALUATION OF EPITHELIAL INJURIES.

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Purpose
Our purpose was to study the effectiveness of a new biological stain, based on riboflavin (Droptest©), for the biomicroscopic evaluation of corneal and conjunctival injuries, compared with standard colorations (fluorescein and lyssamine green) in patients suffering from bilateral ocular surface diseases, having possible epithelial damage. The secondary endpoint was to evaluate both stains' tolerability, using a preference based survey.

Setting/Venue
Salerno Hospital University, Department of Ophthalmology, Salerno (Italy); Vecchio Pellegrini Hospital, Eye Bank, Napoli (Italy).

Methods
Corneal and conjunctival colorations were evaluated after administration of a fluorescein based solution (Fluorofta©) and a lyssamine green strip (F+L), and then Droptest© solution (D), putting a yellow filter prior to the oculars. Stain sequence was F+L/D in an eye, and D/F+L in the other eye, with a random assignment for each eye. The two colorations were made with 30 minutes distance, after three soakings with physiological solution (NaCl 0.9%), every 10 minutes; each coloration was evaluated in double blind by microscopy. After staining, a tolerability survey was compiled for each eye.

Results
48 eyes from 24 patients were examined, affected by inflammatory, post-traumatic or dry eye disease, with average age of 49±9 years. The average corneal score, based on Lemp scheme, was 3.82 for F+L and 3.84 for D, whereas the average conjunctival score was 5.12 for F+L and 5.04 for D, without statistically significant difference between the two groups. In 42 of 48 cases, Droptest© coloration was subjectively more tolerated than F+L, with statistically significant difference ($p<0.02$). There were no reported cases of intolerance to the cited products.

Conclusions
Staining with the new solution based on Vitamin B2, combined with the anteposition of a wratten 12 filter in front of the oculars, was equally effective in the detection of corneal and conjunctival suffering areas, compared to the combined use of sodium fluorescein, specific for the corneal epithelium, and lyssamine green, specific for the conjunctival injuries. The riboflavin solution was preferred by 87.5% of the patients, with a better patient compliance and reduction of evaluation time, compared with the evaluation using the white light for lyssamine and cobalt blue for fluorescein stain.

Pre-treatment therapy and protocol

Three days before treatment:

- preservative-free norfloxacin 0.3% eye drop, one drop every 6 hours.

Starting twenty minutes before treatment:

- Local anesthesia (oxybuprocaine hydrochloride 0,2% two drop every 5 minutes);
- Antibiotic prophylaxis (one drop of norfloxacin 0.3% every three minutes);
- Protection of posterior ocular structures with miosis (two eyedrops of 1% pilocarpin);
- Disinfection of periocular skin with iodine povidone 10% solution.

Treatment protocol

- Silicone ring (12 mm. diameter, 3 mm. height) placed on the corneo- scleral limbus;
- Riboflavin solution (riboflavin-dextran 0.1 g/100 g, D-alpha-tocopheryl polyethylene-glycol 1000 succinate (vitamin E TPGS) 500 mg/100 ml, coenzyme Q 100 mg/100 ml);

- The ring filled with the riboflavin solution was maintained for 15 minutes; further drops added if necessary to maintain the soaking of corneal tissue. - UV-A radiation was then applied after the positioning of a speculum, without any epithelium removal;
- VEGA CBM X-Linker® (CSO, Italy) as UV-A emitter (370 nm. wavelength); - the energy was lowered with partially absorbing UV-filters, to achieve 1.5 mW/cm2, measured with UV-meter (Peak Tech 5085);
- Treatment diameter: 8 mm, at 5 cm. distance from corneal apex;
- Two irradiation steps of 5 minutes were performed.
- No further riboflavin solution applied during UV-A exposure;
- Washing of the corneal surface with balanced salt solution (BSS) to remove the superficial riboflavin film before irradiation;
- Delivering of BSS drops on the corneal surface during UV exposure to maintain an adequate moisture.
- Norfloxacin 0,3% eyedrops administered at the end of the treatment.

Post-treatment therapy

- Topical antibiotic and lubricant therapy (norfloxacin 0.3% and hyaluronate 0.15% eye drops every six hours).

Results

- No severe side effects were observed during follow-up;
- One case of corneal haze and two cases of stromal oedema (managed with topical

corticosteroid therapy, with complete clinical resolution after few weeks);

- A little epithelial oedema, giving a superficial greyish aspect, was present sometimes soon after the treatment, and reverted in a few days;
- A stabilization of corneal and refractive parameters was observed in all cases, throughout the observation period;
- No endothelial cell loss was detected.
- A statistically significant ($P<0.05$) improvement of some corneal, refractive, and visual acuity parameters was evident after treatments; specifically:

	After 6 months	After 12 months	After 24 months
average UCVA (logMAR)		0.56 vs 0.87	
average BSCVA (logMAR)	0.01 versus 0.103	0.002 vs 0.103	0.01 vs 0.103
average CYL (diopters)		-2.30 vs -3.25	-1.90 vs -3.25

- A significant ($P<0.05$) decrease of the average MEAN K (MEAN K DECREASE) respect to pre-treatment values:
  - At 12 months: 0.58 diopters;
  - At 24 months: 0.59 diopters;

- A significant ($P<0.05$) decrease of the average sphero-equivalent correction (SE DECREASE) respect to pre-treatment values:
  - At 6 months: -0.50 diopters;
  - At 12 months: -0.60 diopters;
  - At 24 months: -0.60 diopters;

Visante® images showed an hyper-reflective stromal area extending from the outer surface to the inner layers; the central depth of this area was always more than 300 microns.

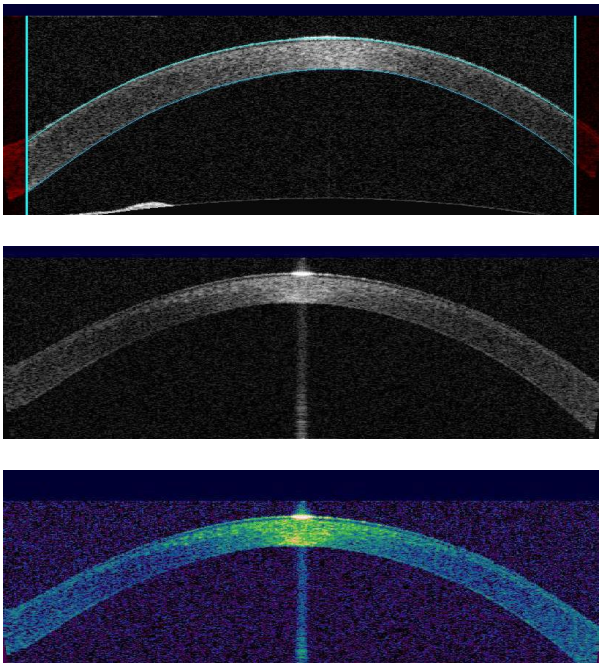

Figure 1 – Details of the stromal area.

Graphic results

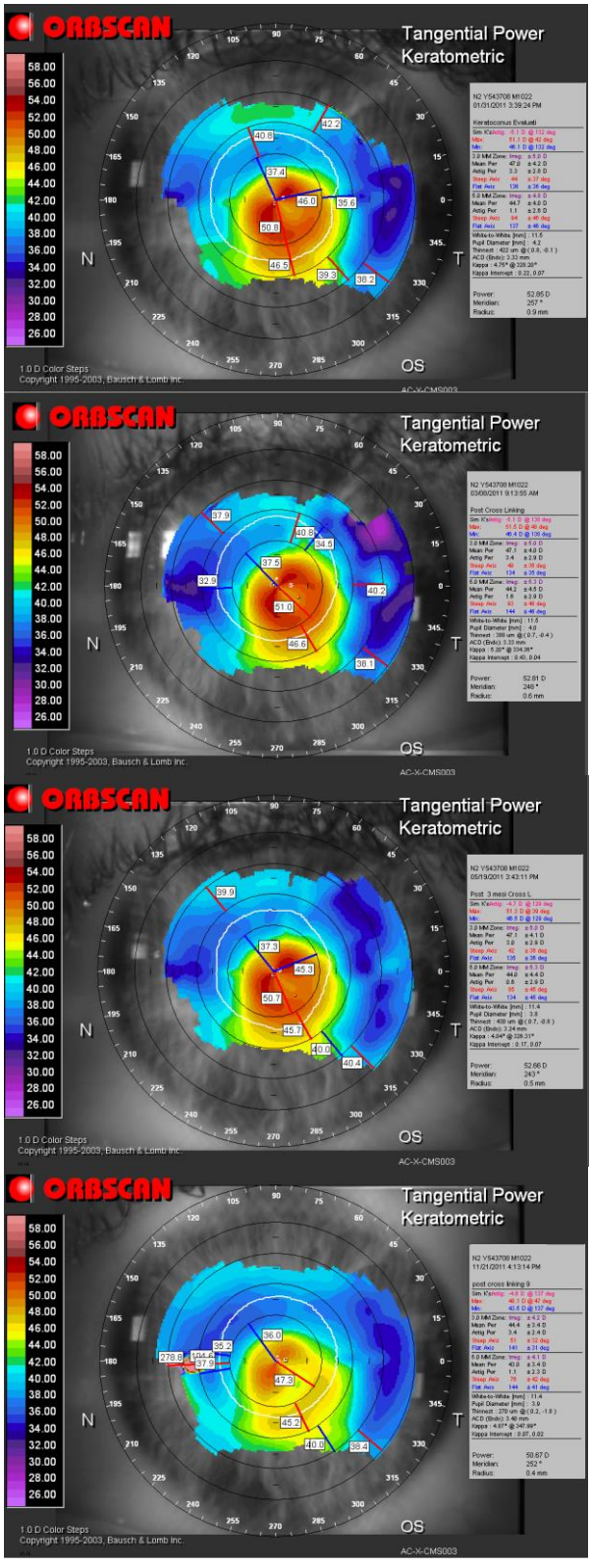

Figure 2 – Example of one case. Sequence: pre, post 3 months, post 6 months, post 12 months.

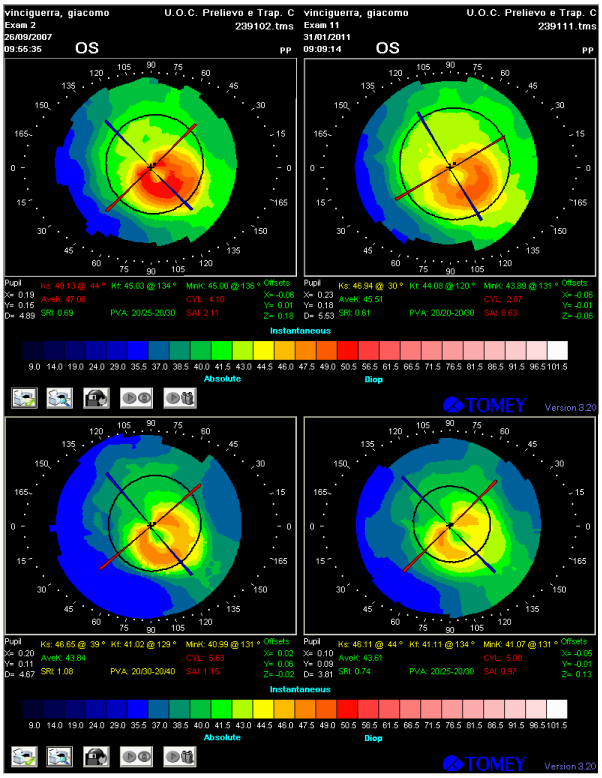

Figure 3 – Pre & post 24 months cases.

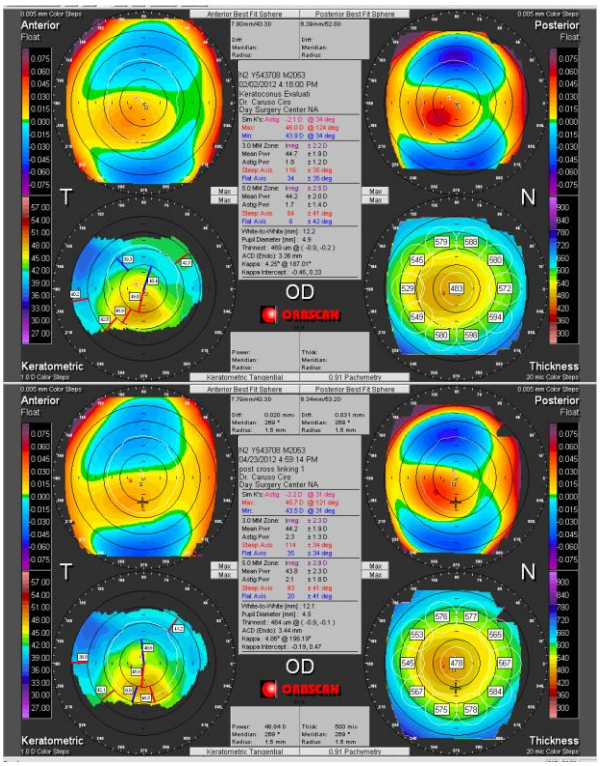

Figure 4 – Pre & post 2 months case.

Discussion

The results of this clinical trial are encouraging:

- The clinical stabilization obtained in all cases, lasting for 24 months, without remarkable side effects.
- Remarkable benefits respect to the traditional TE-CXL protocol:
  a) Reduction of the treatment time respect to traditional protocols (25 minutes versus 60 minutes);
     - More patient compliance;
     - Greater efficiency in doctor and treatment room time;
  b) Less delivery of UV-A radiation:
     - Less risk of damage of the ocular tissues;
  c) More depth of the cross-linking effect in the corneal stroma:
     - Greater lamellar involvement respect to other TE-CXL treatments.

The riboflavin solution for TE CXL is adapted to stop the progression of corneal ectasia, and to improve corneal curvatures and visual acuity. These results were confirmed after two years from treatments, continuing the first year follow-up [2]. Further studies with larger groups of samples are needed, to confirm our results;
Should our results be confirmed in further clinical studies, this solution will be a valid alternative to other solutions used in trans-epithelial cross-linking treatments[3-8].

Conclusions

References

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