venerdì 18 novembre 2016

Trattamento medico-chirurgico delle patologie della superficie oculare: dai colliri biologici al trapianto di cornea

Salone di Rappresentanza
Azienda Ospedaliera
SS. Antonio e Biagio e Cesare Arrigo
Via Venezia, 16
ALESSANDRIA
La GVHD e le patologie della superficie oculare: colliri biologici, nostra esperienza

Mariarosa Astori
Regenerative medicine is a new resource for the treatment of many diseases. Its aim is the biological regeneration of damaged tissues or organs instead of their replacement. It investigates the properties of tissues constituents, as growth factors or extracellular matrix. In 1984 Fox used autologous serum for the treatment of keratoconjunctivitis sicca. Different conditions associated with decreased tear production and/or decreased corneal sensitivity lead to CEDs. Using autologous serum eye drops rather than natural eye drops is based on the premise that epithelial regeneration factors are both in eye drops and in the serum. Serum eye drops have got pH, osmolarity and bio-mechanical properties which resemble natural eye drops. Furthermore, the serum ones provide growth factors, vitamins and bacteriostatic as lisoizyme IgG and complement, just like them. In addition, they are not only lubricant, but they also have antimicrobical and epitheliotrofical properties that artificial eye drops don’t.
Plasma contains cell adhesion molecules which promote epithelial migration (fibrin, fibronectin, vitronectin). Platelets are big tanks of growth factors. Platelet α-granules contain more than 800 bioactive proteins: vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived AB growth factor (PDGF_AB), insulin-like growth factor (IGF-1). They provide a pool of growth factors which comes out synergistically causing a biological effect higher than the effect of every single factor.
Growth factors released in the wound area produce “Homing”...
Cells proliferation

Fibroblasts

Endothelial cells

- PLT-E 0%
- PLT-E 5%
- PLT-E 10%
- PLT-E 20%
- PLT-E 30%

Cells x 10^3
SEQUENCE OF CELLS STIMULATED WITH PLT LYS

Control group

Pulse with PLT lys
A wound was created on a monolayer of cells...

the distance between the sides of the “wound” was measured in pixels

Platelet lysate stimulates wound repair of HaCaT keratinocytes
E. Ranzato, M. Patrono, L. Mazzucco, B. Burian

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PLATELET GROWTH FACTOR INDUCES CHEMOTAXIS
The rationale for this approach is the capacity of blood components to enhance healing and stimulate tissue regeneration.

Platelet Concentrates have a demonstrable, positive impact, on wound healing by modulating its different phases, especially re-epithelialization and tissue remodeling [17].

The bioactive components relesate of PLATELETS are likely vitamins, growth factors and fibronectin, all of which are required for corneal and conjunctival integrity; modulators of the inflammatory response are possibly also involved [18,19].

Persistent corneal epithelial defects (ECSR) caused by decreased tear production or corneal sensitivity reduction

CAUSES OF CEDs

1. Dry eye
   - keratoconjunctivitis sicca
   - Sjogren's syndrome
   - GVDH
   - Ocular cicatrical pemphigoid
   - Rheumatoid Arthritis
   - dry eye after LASIK

2. Neurotrophic keratitis
   - viral keratitis
   - chemical or thermal burns
   - paralytic lagophthalmos keratitis
   - drug toxicity
   - Multiple Sclerosis
   - diabetes

### REGENERATIVE MEDICINE AMBULATORY

**pt nonresponder**

**diagnosis**

<table>
<thead>
<tr>
<th>DEW Grading Dry EIE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>+</th>
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<td>SEVERE</td>
<td>SEVERE CONSTAN T DISABLING</td>
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<td>SPORADIC</td>
<td>DISABLING</td>
<td>CONSTANT DISABLING</td>
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<td>MODERATE</td>
<td>SEVERE</td>
<td>++</td>
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<td>≤5</td>
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<td>≤10</td>
<td>≤5</td>
<td>≤2</td>
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</table>
MEDICAL RECORD
(BCVA –OSDI-BUT-SHIRMER TEST- OXFORD SCORE)

1 Automatic serum
2 CPlys
3-4 PATCH-gel PLT and/or CPlys

Six time /day lasting one month

Control depending on clinical situation
but everybody control after 2 months

pt-tailored therapy
B.E. Sjogren’s syndrome
F.D. Sjogren’s syndrome
F.C. Stevens-Johnson syndrome
P.T.: aseptic corneal ulcer
P.T.: aseptic corneal ulcer
P.T.: few days after platelet lysate treatment: no more ulcer!
P.T.: few days after platelet lysate treatment: no more ulcer!
C.S.: O.D. Platelet membrane application in corneo-conjunctival caustication
C.S.: O.D. corneal conjunctivalization
C.S.: O.S. after platelet lysate application for corneo-conjunctival caustication
CS.: OD. 25 months after corneo-conjunctival caustication
C.S.: O.D. 25 months after caustication and limbus transplant
A Single-Center Phase II Study of Topical Application of Platelet-Derived Eye Drops for patients with Ocular Chronic Graft-Versus-Host Disease

SS Antonio & Biagio and C. Arrigo Hospital, Alessandria Italy
Ophthalmologic Department (head physician dr. D.Dolcino)

Francesco Zallio (Hematology Department), Laura Mazzucco (Transfusion and Regeneration Medicine), Mariarosa Astori (Ophthalmologic Department), Daniela Dolcino (Ophthalmologic Department), Roberto Guaschino (Transfusion and Regeneration Medicine), Marco Ladetto (Hematology Department)
Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative approach for several hematologic neoplasms [1].

**Graft-Versus-Host Disease** (GVHD) remains the most important cause of morbidity and mortality after transplantation.

**Cronic GVHD** (cGVHD) is the most common, long-term complication, and occurs in 30% to 70% of adults that survive for more than 100 days after receiving their transplant [3].

Ocular manifestations of acute GVHD, ranging from conjunctival hyperemia to pseudomembranous conjunctivitis, are uncommon but reported in literature because they might be associated with irreversible ocular damage and very poor prognosis [8];

ocular complications are more frequently associated with the occurrence of systemic cGVHD and arise in up to 60% of such cases [9,10].

This pathology results from the infiltration of the lacrimal gland by fibroblasts and T lymphocytes; these infiltrates provoke impaired secretory function, and corneal damage [11,12].

Common symptoms of ocular cGVHD include inflammation, and ocular discomfort such as: photophobia, pain, foreign body sensation, and dry eyes (xerophtalmia).

Standard treatment, such as steroids and artificial tears (…), has not shown a real effectiveness.

Based on these unsatisfactory, alternative treatments have been developed, focusing on autoimmunity control, inflammation suppression, and tissue regeneration promotion.

NEW strategy: In this scenario we introduce a solution that could reverse the underlying pathological processes of cGVHD:

the topical application of AUTOLOGOUS PLATELET CONCENTRATED LYSED (CPlys) and then reconstituted as eyes drops [14,16].

We therefore designed a phase-II clinical study to offer CPlys eye drops to allografted patients diagnosed with ocular cGVHD.

The results of this trial with a focus on:

1) the characteristics of patients with ocular cGVHD in terms of general clinical outcome;

2) feasibility and efficacy of PClys eye drops for the treatment of ocular cGVHD.
Patients

This study was conducted at the “SS Antonio e Biagio e Cesare Arrigo” Hospital, Alessandria, by a consortium of the Hematology, Ophthalmology and Transfusion Divisions.

Since 2005, follow-up had been provided by local ambulatory services for patients that survived for more than 100 days after allo-SCT. Follow-up involved systematic cGVHD screening, based on the Seattle [23], and National Institutes of Health (NIH) criteria [24] (the latter assigned retrospectively).

A subjective “Activities of Daily Living” (ADL) score was also collated. All patients receiving allogeneic HSCT for a hematologic malignancy were requested to complete a self-administered, subjective, questionnaire about their ocular disability (the Ocular Surface Disease Index (OSDI)) [25]. Patients scoring greater than 15 were referred to an ophthalmologist for a formal diagnosis and assessment for study eligibility.

Treatment with the PClys eye drops: from January 2007 to January 2014.

**Eligibility criteria for enrollment**

a) recent diagnosis of ocular chronic GVHD;
b) no active systemic or ophthalmic disease other than cGVHD;
c) absence of major systemic comorbidity related other to systemic cGVHD;
d) control of primary hematological neoplasm with life expectancy > 3 months,
e) platelet count (PLT) in excess of $100 \times 10^3/\mu l$.

all patients underwent a scheduled ophthalmic evaluation at days +30, +180 and +360.
Ophthalmic analysis:

**Subjective symptoms**
pain, photophobia and eye-dryness were assessed according to the NIH Eye Score and OSDI questionnaire.

**Objective tests**

- **Schirmer’s test** (15mm of a paper strip was considered our reference (normal) value and a 5mm increase was rated as “improved”)
- **Tear Film Break Up Time test** (>10 seconds was the reference time and a 5 second increase was considered “improved”)
- **Best Visual Acuity** (score)

Finally there was an assessment of corneal damage, which comprised fluorescein score and lissamine score.

All the patients have been taken in a photograph at day 0 and +180

**Enrollment** was allowed for seven years with the aim of enrolling at least 29 patients with ocular cGvHD.
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<td>I</td>
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<td>C</td>
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<td>III</td>
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<td>IV</td>
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<tr>
<td>&gt;E</td>
<td>V</td>
<td>Greater than panel E</td>
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</table>
Patient Characteristics and Outcome

From March 2005 until August 2014, a total of 127 patients underwent an allogeneic transplant at our institution for a hematologic neoplasm; their median follow-up was 654 days (10-3486).

Occurrence of Ocular Chronic GVHD

Twenty-nine patients (23% of the HSCT population) developed ocular cGVHD. Median time of onset of ocular GVHD was 218 days (range 90-1750 days). Clinical characteristics of the 29 patients who developed ocular cGVHD
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>DISEASE</th>
<th>GENDER</th>
<th>AGE (YEARS)</th>
<th>SOURCE OF STEM CELLS</th>
<th>TYPE OF DONOR</th>
<th>cGVHD OTHER THAN EYES</th>
<th>OCULAR NIH score</th>
<th>GLOBAL SCORING of GVHD</th>
<th>cGVHD AS THE SOLE MANIFESTATION of EXTENSIVE cGVHD</th>
<th>DAYS FROM TRANSPLANT to GVHD OCULAR</th>
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Abbreviations: cGVHD = chronic Graft-versus-Host Disease; AML = Acute Myeloid Leukemia; RAEB = Refractory Anemia with Excess Blasts; NHL = Non-Hodgkin Lymphoma; CML = Chronic Myeloid Leukemia; MI = Myelofibrosis Idiopathic; CLL = Chronic Lymphocytic Leukemia; MM = Multiple Myeloma; BPDNC = Blastic Plasmacytoid Dendritic Cell Neoplasm; ALL = Acute Lymphoblastic Leukemia; MDS = Myelodysplastic Syndrome; PB = Peripheral blood; BM = Bone Marrow.
29 patients with ocular cGVHD
Platelet Lysate Eyedrops in ocular GvHD Protocol

Patient submitted to Allo-SCT (n=127) → No ocular GvHD (n=98)

Enrollment

Assessed for eligibility (n=29)

Excluded (n=5)
- Screening failure (n=1)
- Development of extensive GvHD requiring systemic therapy (n=1)
- Early death due to disease progression (n=1)

Enrolled (n=26)

Treatment interruption (n=2)
- Poor compliance (n=1)
- Poor tolerance to therapy (n=1)

Received allocated intervention (n=24)

Lost to follow-up (death before end of protocol) (n=0)

Follow-Up

Analysis

Suitable for final analysis (n=24)

Figure 1. CONSORT Flow Diagram of study protocol.
26 patients with ocular cGVHD

- Classified as NIH score 1: 19%
- Score 3: 12%
- Score 2: 69%
Classified as NIH score 1 59%

Score 2 14%

Score 0 27%
### Table 3. Subjective and Objective Results over 26 Patients Treated with Platelet-Derived Eye Drops

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<th>Baseline</th>
<th></th>
<th>30 Days</th>
<th></th>
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<td>Score</td>
<td>Score</td>
<td>Difference with Baseline</td>
<td>P-value</td>
<td>Score</td>
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<tr>
<td>OSDI score</td>
<td>69 (range 27-93)</td>
<td>51 (range 81-15)</td>
<td>-18 (-3 to +12)</td>
<td>0.074</td>
<td>21 (range 72-12)</td>
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<td>1 (range 0-3)</td>
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<td>0.0001</td>
<td>1 (range 0-2)</td>
<td>-1 (-2 to 0)</td>
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<td><strong>Test</strong></td>
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<td>13 (range 6-26)</td>
<td>+6 (-10 to +22)</td>
<td>0.091</td>
<td>10 (range 3-30)</td>
<td>+3 (-13 to +27)</td>
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<td>7 (range 3-15)</td>
<td>+3 (+1 to +9)</td>
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<td>0 (range 0-2)</td>
<td>-1 (-1 to 0)</td>
<td>0.003</td>
<td>0 (range 0-1)</td>
<td>-1 (-2 to 0)</td>
</tr>
<tr>
<td>Lissamine score</td>
<td>1 (range 0-3)</td>
<td>0 (range 0-2)</td>
<td>-1 (-2 to 0)</td>
<td>0.004</td>
<td>0 (range 0-1)</td>
<td>-1 (-2 to 0)</td>
</tr>
</tbody>
</table>

Abbreviation: OSDI = Ocular Surface Disease Index; TBUT = Tear Break Up Time; BCVA = Best-Corrected Visual Acuity.
Values are expressed as medians.
Values: OSDI score: minimum = 0, maximum = 100; NIH score: minimum = 0, maximum = 4; Schirmer Test: normal values >15mm/5min; TBUT Test: normal values >10sec; BCVA: minimum 0/10, maximum 10/10; Flurescin/Lissamine score: Oxford scheme (0 = absence of conjunctival damage; 5 = maximum conjunctival damage)
Figure 2. Ophthalmic Improvement during study protocol
Treatment with PClys Eye Drops

Of 29 patients diagnosed with ocular cGVHD, 26 were eligible for our study.

For a third patient, use of an antibiotic eyewash rapidly improved their ocular symptoms to a state where inclusion was no longer appropriate.

Five of 26 patients (19%) were classified as NIH score 1, 18 patients (69%) scored 2, and 3 patients (12%) scored 3.

The median age of our cohort at enrollment was 60 (range 24-67), and their median time from transplant to enrollment was 258 days (range 97-1750).

Ocular cGVHD arose in 24 patients experiencing contemporary involvement of other organs; these patients re-started or increased immunosuppressive medications with the addition of PClys eye drops;

2/26 patients, developed only ocular cGVHD and PClys eye drops were used as a stand-alone treatment.

For nine patients, ocular cGVHD was the sole manifestation defining extensive cGVHD.

1 patients stopped his participation in the first few days because of treatment-associated ocular pain, burning and conjunctival hyperemia; the episode resolved in few days with discontinuation of the eye drops.
Among patients who underwent the planned treatment the following **results** were observed:

at day +30, 91% had an improvement of symptoms (S), 32% showed an improvement of objective criteria (T), and 86% demonstrated a remission of corneal damage. From a hematological point of view, 73% had an improved NIH score, with 8% attaining a zero score.

positive results were also confirmed at +180 days: 86% now reported continued subjective benefits (S), 59% experienced improved objective function (T), and 86% had remission of corneal damage. A further 8% improved their NIH score; 27% were now graded zero.

at +360 days these findings were confirmed.

None of the cohort had to further increase their systemic immunosuppressive therapy. It is worthwhile noting that during their eye drop therapy, 5 of the 9 patients manifesting ocular-only symptoms of extensive cGVHD (55%), were able to progressively taper and ultimately cease their systemic immunosuppressive therapies.

After one year of treatment, only one patient continued to use the eye drops because of persistent kerato-conjunctivitis.
Our results indicate that CPlys eye drops were a safe, feasible and well-tolerated therapeutic option.

Ocular symptoms develop in a substantial percentage of patients after allo-HSCT and ocular cGVHD often resulting in decreased quality of life and restriction of daily activities.

The eye drop formulation (CPlys) was standardized for platelet number [32], and prepared using a controlled (i.e. sterile) and certified procedure.

Feasibility of use, and tolerability, were excellent.

Only one patient experienced a local reaction to their eye drops of sufficient magnitude to interrupt treatment. Most notably, we observed no ocular infections. Our results confirm the efficacy of the treatment, with an improvement of all ocular cGVHD-related symptoms in almost 80% of patients.

At follow-up, only one persistent case of ocular cGVHD was seen, suggesting the long-term benefit of using the CPlys eye drops. Based on the excellent toxicity profile, and rapid response achieved, we are now investigating whether a shortened program might also allow similar results, without increased risk of late relapse.

Before treatment

After treatment
Before treatment

After treatment
Before treatment

After treatment