• Clinical Research •

Effect of autologous serum after amniotic membrane transplantation for persistent corneal ulcers

Rozaliya Hristova[1], Petya Yankova[2], Georgi Markov[1], Alexander Oscar[1], Yani Zdravkov[1]

[1]Department of Ophthalmology, Medical University Sofia, University Hospital Alexandrovska, Sofia 1431, Bulgaria
[2]Department of Clinical Immunology, Medical University Sofia, University Hospital Alexandrovska, Sofia 1431, Bulgaria
Correspondence to: Rozaliya Hristova. 72A Burel str., Sofia 1408, Bulgaria. alleta@abv.bg
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Abstract

● **AIM:** To investigate the effect of adding autologous serum eye drops to the postoperative regime after amniotic membrane transplantation for severe persistent corneal ulcers.

● **METHODS:** Forty eyes of 40 patients with persistent corneal ulcers were randomly assigned to artificial tears (sodium hyaluronate 0.2%, ATs group, *n*=20) or autologous serum eye drops (ASEDs, *n*=20) following treatment with amniotic membrane transplantation. Digital slit lamp images were acquired from all patients before and 30d post treatment. The area with fibrovascular tissue was calculated using Image J. Central corneal sensitivity was assessed by Cochet-Bonnet aesthesiometry before and one month after treatment. Scar tissue transparency was assessed with a novel optical densitometry.

● **RESULTS:** Mean age of patients was 61.65±16.47y and 57.3±19.11y in the ATs group and ASEDs group, respectively. Twenty-two male and 18 female patients were included in the study. The improvement in visual acuity was significantly greater in the ASEDs group (0.14±0.04) than the ATs (0.08±0.04; *P*=0.00046). Cochet-Bonnet aesthesiometry improved significantly after treatment with a similar rate between groups. There were no statistically significant differences in the area of postoperative fibrovascular tissue between the two groups (*P*=0.082). The success rate in the two groups was similar. The difference in densitometry between the ATs and ASEDs group was statistically significant (*P*=0.042) with greater reduction from baseline in the ASEDS group.

● **CONCLUSION:** Autologous serum eye drops can lead to better visual acuity, more stable results and improved densitometry and should be considered in the postoperative care following amniotic membrane transplantation.

● **KEYWORDS:** autologous serum; amniotic membrane; ocular surface; persistent corneal ulcer
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INTRODUCTION

Corneal disease is a leading cause of blindness worldwide[1]. In addition to low vision, corneal disease decreases the quality of life due to constant pain, tearing, light sensitivity, constant need for lubrication and aesthetic appearance of the affected eye[2].

Amniotic membrane transplantation (AMT) has been widely and successfully used as a treatment of ocular surface disease[3]. It is the innermost avascular layer of the gestational sack and is composed of epithelium, basement membrane and stroma[4]. It has antiseptic, anti-inflammatory, antiangiogenic properties and promotes regeneration[5-6]. However, there are cases in which amniotic membrane (AM) grafting fails to achieve complete epithelialization with recurrence of persistent corneal defects or formation of thick opaque scar tissue[7-8].

An additional benefit could be gained by adding autologous serum eye drops (ASEDs). Autologous serum has been in use for ocular surface disease since 1975[9]. It has been experimentally proven that serum has similar properties to natural tears and is rich in growth factors, antimicrobial and anti-inflammatory substances such as immunoglobulins, lysozyme, cytokines, and vitamins[10]. All of these components promote cell proliferation, migration and differentiation, which could improve the wound healing process. There have been numerous studies on the efficacy of topical autologous serum in concentrations ranging from 20% to 100%, as well as significant differences in the protocols for manufacturing of the product[11]. Most studies were focused on autologous serum application in dry eye and as an adjunct to treatment of neurotrophic keratitis and persistent epithelial defects.

The purpose of this study is to investigate the effect of adding ASEDs to the postoperative regime after AMT for severe persistent corneal ulcers.

SUBJECTS AND METHODS

Ethical Approval This was a prospective randomized study approved by the relevant Ethics Committee (2280/28.06.2023). The study began in January 2019 and continued through January 2023. Informed consent was obtained from all individual participants included in the study prior to treatment. Patients were prospectively enrolled by their attending physician. All physicians were informed of the study protocol and inclusion and exclusion criteria, and trained in the enrollment of patients. All procedures performed in studies involving human participants were in accordance with the 2013 Helsinki declaration and its later amendments or comparable ethical standards. Statistical analysis of the data was performed using IBM SPSS v23.

Inclusion criteria were defined as age over 18y, symptoms of impaired vision, tearing, photosensitivity, pain and redness, persistent corneal ulcers, which were defined as epithelial defects, stromal infiltrates, stromal melting for more than 30d unresponsive to conservative measures including preservative-free antibiotics, extensive lubrication and patching. Causes of persistent corneal ulcers were most often toxic keratopathy, neurotrophic keratitis, primary Sjögren syndrome, severe dry eye disease, chemical burns. Exclusion criteria were defined as corneal perforation, active corneal infection, uveitis, systemic autoimmune disease, *e.g.* lupus, rheumatoid arthritis, polyarteritis nodosa, dermatomyositis.

Patients underwent a comprehensive ocular examination including best corrected Snellen visual acuity (VA), intraocular pressure (IOP), biomicroscopy, ocular surface fluorescein staining and ophthalmoscopy (whenever possible). Data from patients with very low vision was quantified using the results described by Schulze-Bonsel *et al*[12]. Infectious etiology was ruled out based on negative microbiological tests, as well as negative systemic serology for hepatitis B and C, syphilis, and human immunodeficiency virus. Due the ongoing corona virus disease (COVID)-19 epidemic only patients with negative serology for SARS-CoV-2 were included in the study.

Forty patients with persistent corneal ulcers were randomly assigned using Microsoft Excel 2016 to either artificial tears (sodium hyaluronate 0.2%, ATs group, n=20) or ASEDs group, (n=20) following treatment with AMT. The same packaging of artificial tears was used in both groups with the serum added to the original bottle using the manufacturer's filtering system.

Central corneal sensitivity was assessed by Cochet-Bonnet aesthesiometry before and one month after treatment. Sensitivity threshold was determined as the length of the filament inducing a blink reflex by 50% of the stimuli (3 out of 5).

Digital slit lamp images were acquired from all patients before treatment and 30d postoperatively. Images were analyzed by an observer masked to the treatment and time point. A scale in pixels was set based on corneal diameter. The area with fibrovascular scar tissue was assessed and calculated automatically by software Image J v.1.53e (USA; Figure 1).

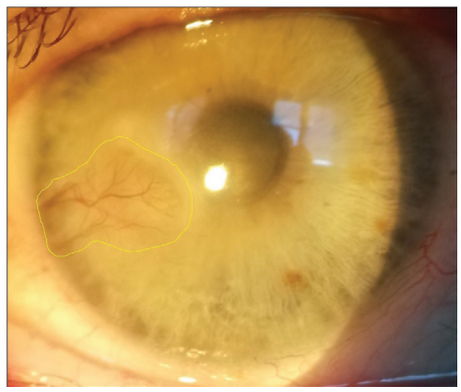

Figure 1 Digital slit lamp image of a patient with selected area of fibrovascular corneal tissue After setting a scale in pixels based on corneal diameter the Image J software automatically calculated the selected area.

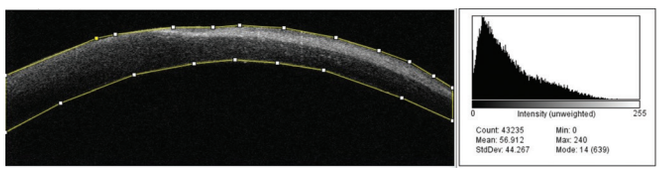

Figure 2 Digital image analysis of anterior segment OCT scan with gray scale measurement of the selected area The method resembles Scheimpflug densitometry, which is based on scattered light from the cornea. To validate the method the mean corneal gray scale of two independent visits of 44 healthy controls were compared. OCT: Optical coherence tomography.

Radial anterior segment optical coherence tomography (OCT) scans were analyzed using Image J software to determine the mean gray scale of all pixels comprising the corneal tissue before and after treatment (Figure 2).

The rate of epithelialization was assessed using biomicroscopy with fluorescein staining on days 1, 14, and 30. Human AM tissue was collected from full-term placental tissue, treated with antibiotics and prepared according to international standards after donor screening for transmissible infections. An only technique for AMT was used. The membrane was fixed to the ocular surface epithelial side up with 10/0 nylon interrupted limbal suture. Postoperative regimen included topical combination of antibiotic and steroid for 1wk for all patients.

A sterile phlebotomy was performed in all cases to keep the study blinded. Preparation of autologous serum eye drops included inserting the 10 mL blood sample in a centrifuge for 15min at 3000 g, dilution with sterile artificial tears (sodium hyaluronate 0.2%) to 25% using the manufacturer's filtering system, stored at 4 degrees Celsius. The ASEDs were aliquoted in the original artificial tears bottle of 10 mL. The ASEDs were

started within 24h of the AMT. Patients were instructed to use the drops four times a day. The procedure was repeated after two weeks and the solution was replaced.

Success criteria were defined as complete epithelialization of the corneal surface and improvement in VA. Secondary outcome measures included partial or complete reduction of neovascularization and achieving improved corneal transparency, thickness and reflex. Assessment for adverse reactions was made including infection, progressive corneal thinning, stromal immune deposits, scleritis with or without vasculitis.

RESULTS

Mean age of patients was 61.65±16.47y in the ATs group and 57.3±19.11y in the ASEDs group. Twenty-two male and 18 female patients were included in the study. Baseline VA was similar in the two groups with an average of 0.04±0.10 and 0.10±0.12 in the ATs and ASEDs groups, respectively (P=0.169). There was statistically significant improvement in VA in both groups with postoperative mean VA 0.12±0.14 (P=0.002) and 0.24±0.16 (P=0.001). Mann-Whitney test demonstrated greater improvement of VA in the ASEDs group (P=0.00046, z=-3.503; Figure 3).

Mean aesthesiometry in the ATs group was 141.15±78.01 and 125.7±93.37 in the ASEDs group. Baseline aesthesiometry was comparable in the two groups (P=0.474) and improved significantly after treatment (84.3±51.6, P<0.01 in the ATs group, 93.37±40.51, P=0.05 in the ASEDs group). The rate of improvement was similar between the two groups (P=0.576).

Mean area of the corneal fibrovascular tissue in the ATs group was 40.45±21.30 and 27.84±16.04 in the ASEDs group (P=0.068). In all cases statistically significant improvement was achieved with reduction of the area with fibrovascular tissue (ATs -21.79±18.16, P=0.001; ASEDs -16.26±15.81, P=0.005). Kolmogorov-Smirnov Z test did not demonstrate statistically significant differences in the area of postoperative fibrovascular tissue between the two groups (P=0.082; Figure 4).

Mean gray scale values of the corneal OCT densitometry of the 44 healthy controls were 39.81±8.62 and 40.73±8.50 at Visit 1 and Visit 2, respectively. The paired samples t-test did not show statistically significant differences between the two visits (P=0.497). After validating the test, we proceeded to compare the pre- and post-treatment values for each group as well as the difference between the two groups (Table 1). The difference in densitometry between the ATs and ASEDs group was statistically significant (P=0.042) with greater reduction from baseline in the ASEDs group.

Both the ATs and ASEDs group demonstrated statistically significant differences from the controls (P<0.001 and P=0.003, respectively). The baseline densitometry values of ATs and ASEDs groups, 55.80±20.56 and 59.30±18.72, respectively, were similar (P=0.682).

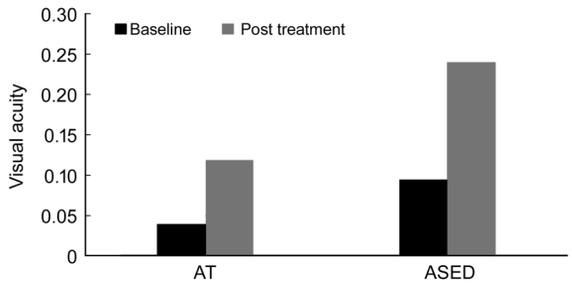

Figure 3 Change in the mean visual acuity of the two) groups before and after treatment AT: Artificial tears; ASED: Autologous serum eye drops.

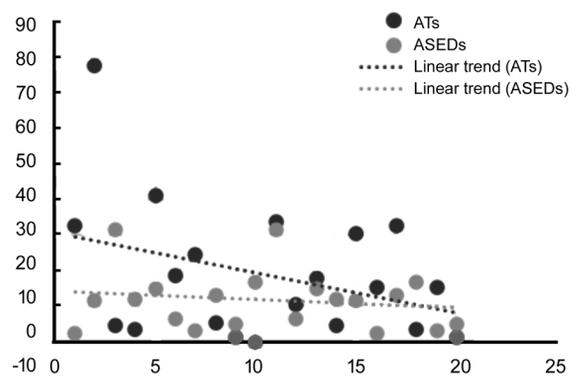

Figure 4 Graphic representation of the difference in fibrovascular tissue area before and after treatment in ATs and ASEDs group Linear trend analysis demonstrating stable results in the ASEDs group. ATs: Artificial tears; ASEDs: Autologous serum eye drops.

Table 1 Comparison between mean values of OCT densitometry in the healthy controls and between the controls and the groups

Group	V1/pretreatment	V2/post-treatment	P
Healthy controls	39.81±8.62	40.73±8.50	0.497
ATs	55.80±20.56	51.99±12.42	0.334
ASEDs	59.30±18.72	53.93±21.67	0.036

ATs: Artificial tears; ASEDs: Autologous serum eye drops; V1: Visit one; V2: Visit two.

There was statistically significant improvement in densitometry values only in the ASEDs group before and after treatment (Table 1). There was significant difference in the rate of improvement between the two groups with more favorable results in ASEDs group.

The success criteria were met in 17 out of 20 cases in the ATs group and in all patients in the ASEDs group. Fisher's exact test demonstrated similar success rate (P=0.231) in the two groups. Chi-square test did not show significant differences in the number of patients with complete epithelialization at day 1, 14, and 30 [χ^2(1, n=37)=0.76, P=0.68]. The three patients from the ATs group that did not meet the success criteria were followed after the end of the study and their corneas eventually epithelialized completely. No adverse reactions as defined previously were recorded.

DISCUSSION

The management of non-healing corneal ulcers is challenging. Autologous serum has been useful in mild cases of persistent epithelial defects, neurotrophic keratopathy and dry eye[4]. Its effects on corneal wound healing and scar tissue formation, however, have not been studied in detail. AMT, on the other hand, has become a gold standard in the treatment of persistent corneal ulcers with numerous studies on its applications[4].

Our initial hypothesis was that autologous serum eye drops would have additional beneficial effect on corneal wound healing, since they promote regeneration.

The ASEDs group demonstrated greater increase in VA, which could be explained by improved composition and stability of the tear film compared to standard sodium hyaluronate. It is known that tear film integrity is of utmost importance for normal refraction and epithelium function[13]. ASEDs have been shown to have immunoregulatory effects as well[14], which could also benefit VA by reducing cellular infiltrates and edema. There were studies reporting superior composition and effect of ASEDs compared to artificial tears in dry eye disease, but the results of Meta-analyses were inconsistent[15].

Our original assumption was that due to the content of different growth factors [*e.g.* epidermal growth factor (EGF), transforming growth factor (TGF)-β, platelet-derived growth factors (PDGF), neurotrophic factors, vitamin A, vitamin E, *etc.*] and anti-inflammatory cytokines[16], ASEDs would have a modulatory effect on stromal keratocytes activation and would lead to less cicatricial changes in the stroma. There were studies that autologous serum modifies TGF-β action on human keratocytes *in vitro* and *in vivo*, although it had been found inferior to other blood products like plasma rich in growth factors[17-18]. Conversely, studies on the effect of autologous serum on corneal stroma in dry eye disease did not show notable changes in keratocytes, immune cells or nerve fibers[19]. Our findings suggested that ASEDs had a significant effect on the area of fibrovascular tissue. It would be pertinent to use other imaging techniques *e.g.* confocal *in vivo* microscopy or corneal densitometry to assess if there is a difference in the transparency and composition of the fibrovascular tissue. The observed change in densitometry values could signify increased transparency and could explain the greater improvement in VA observed in the ASEDs group of our study. Confocal microscopy could also reveal if nerve regeneration was influenced by the addition of ASEDs. There were some reports of improvement in sub-basal nerve plexus structural indices in eyes treated with autologous serum products compared to artificial tears[20]. In our study, however, functional testing using Cochet-Bonnet aesthesiometry did not show significant differences between the ATs and ASEDs group. It is our personal observation that patients in the ASEDs group reported less discomfort, redness, pain and tearing, however evaluation of subjective symptoms was beyond the scope of this study.

The results of the present study demonstrate that ASEDs could influence stromal scar tissue formation with improved mean gray scale values of the corneal OCT scans. Previous studies implementing Scheimpflug densitometry in cases of keratitis demonstrate that infiltrates have much higher density[21] than that of residual scars and there was no correlation between pachymetry and densitometry values. However, there was very limited data on scar tissue formation in general and especially following AMT and ASEDs use[22]. Cox *et al*[23] found a positive correlation between densitometry values and AMT. Kam *et al*[24] found that densitometry was significantly reduced following primary pterygium excision with adjuvant topical mitomycin-C application. Scheimpflug densitometry has the advantage of using readily available software, which can be used for standardized measurements at certain depths and zones[25]. The OCT technique used in the current study utilizes an approach measuring all of the corneal tissue in 12 linear scans, which could lead to errors of omission[26]. However, we found that the measurements were consistent between visits in healthy controls and only changed in the groups with ASEDs. Further studies are needed to compare the two different approaches to corneal densitometry and ascertain the applicability of OCT.

There were scientific reports that autologous serum accelerates epithelialization compared to artificial tears[27-28]. Although Chi-square test did not demonstrate statistically significant differences in the time for epithelialization in our study, the linear trend analysis indicates that adding ASEDs leads to more stable results in time.

Published studies on a limited number of participants, animal models and cell cultures demonstrated the positive effects of autologous serum in corneal wound healing[29-30]. Although our data did not fully support previous findings, we achieved significantly higher VA in the ASEDs group which ultimately has greatest significance for the patient.

The main limitation of our study is the small sample size and the lack of multimodal corneal imaging techniques that could give us more insight on the effects of ASEDs in the treatment of persistent ulcers, especially Scheimpflug imaging and *in vivo* confocal microscopy. Our results could be biased by the different etiologies which led to the persistent corneal ulcers as well as the short-comings of the conservative measures taken before presentation to our clinic. However, our study adds to current knowledge especially in terms of confirming a benefit of adding ASEDs to the postoperative regime after AMT and introducing the novel optical densitometry.

In conclusion, autologous blood-derived products for ocular surface disease are under constant development with numerous reports of their beneficial effects. Adding ASEDs in the postoperative care following AMT could lead to better VA, more stable results and improved densitometry.

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Conflicts of Interest: Hristova R, None; **Yankova P,** None; **Markov G,** None; **Oscar A,** None; **Zdravkov Y,** None.

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