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Biological therapy of anterior ocular surface, a step towards personalized ophthalmology

ABSTRACT

of the dissertation for acquisition of the educational and scientific degree "doctor of scientific specialty "ophthalmology", code 03.01.36

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The dissertation contains 228 pages, including 12 tables and 52 figures. 176 literary sources are cited. Five chapters are presented, corresponding to the aim and tasks set and meeting the requirements for the layout of the dissertation.

The dissertation was discussed and proposed for defense by the departmental council of the Department of Eye Diseases and Vision Sciences at the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna on 14/10/2024 y.

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The official defense of the dissertation will take place on the 15/01/2025 at 08:30 at a meeting of the Scientific Jury at the Department of Eye Diseases and Vision Sciences of the Medical University of Varna. The materials for the defense are available at the Scientific Department of the Medical University of Varna and are published on the website of the Medical University of Varna.

CONTENTS

Note: The numbering of the figures and tables in the abstract does not correspond to the numbering in the dissertation.

Note: The team and the principal investigator declare that they have no financial interest or affiliation with any of the mentioned trademarks of the products, devices or cited websites used in the study.

ABBREVIATIONS

AB Antibiotics

AM Amniotic membrane

ASD/SED Autologeous serum drops

CL Contact lens

PED Persisting epithelial defect

AOS Anterior ocular surface

TCL Therapeutic contact lens

AI Artificial Intelligence

AME Amniotic membrane extract

AMEED Amniotic membrane extract eye drops

AMUC Amniotic membrane and umbilical cord eye drops

AS-OCT Anterior segment optical coherence tomography

CLET Cultivated Limbal Epithelial Transplantation

COMET Cultivated oral mucosal epithelial transplantation

DED Dry eye disease

DSEK Descemet's Stripping Endothelial Keratoplasty

EGF Epidermal growth factor

FDA Food and drug administration

GVHD Graft vs. Host Disease

IVCM In vivo confocal microscopy

LASIK Laser assisted in situ keratomileusis

LSCD Limbal stem cell deficiency

LSCM laser scaning confocal microscopy

MAGUK Membrane-associated guanylate kinases

MSCs Mesenchymal stem cells

NEI National eye institute

NK Neurotrophic keratitis

OCT Optical coherence tomography

OSDI Ocular Surface Disease Index

PACK-CXL Photo active chromophore for Keratitis-Corneal Cross-linking

PK Penetrating keratoplasty

PPCD Posterior polymorphic corneal dystrophy

PRGF Plasma rich in growth factors

PROSE Prostethic Replacement of the Ocular Surface Ecosystem

PRP Plasma rich in platelets

PTK Phototerapeutic keratectomy

RCE Recurrent Corneal Erosion

RCT Randomized clinical trials

SMILE Small Incision Lenticule Extraction

SSCM Slit scanning confocal microscopy

TBUT Tear break up time

TNF-α Tumor necrosis factor α

TSCM Tandem scanning confocal microscopy

UCS/ UCBS Umbilical cord serum

VEGF Vasoendothelial growth factor

VN Vitronectin

ZO Zonula occludens

I. INTRODUCTION

The senses are our contact with the environment and we perceive up to 80% of any information through our vision (1). Thanks to the visual sensory system, a person is able to perceive and distinguish shapes, colors, the orientation and movement of objects in the world around him. The sensitive to light sensory organ of the visual system is the eye, which has a complex structure - its morphology includes three layers: outer, middle and inner. The anterior ocular surface is a structure characterized by great dynamics and sensitivity, which is always in contact with the environment and its normal functionality determines the following functions of visual and blinking comfort, barrier function against external agents, trophic and protective function of the cells of the ocular surface. In case of a breach of any of the underlying structures, difficult-to-treat diseases occur, characterized by progressive inflammatory processes, vascularization and scarring, leading to reduced vision, blindness and impaired quality of life. Part of the outer layer, which is always in contact with the environment, is the cornea. This bradytrophic tissue, consisting of 5 histological layers, maintains the shape of the eye, together with the sclera, has a protective function, participates in the dioptric apparatus of the eye by carrying, refracting and reflecting light, providing about 45 diopters of refractive power. The corneal epithelium is unable to maintain its integrity and is in a constant process of renewal and regeneration. Its abnormal state leads to instability and the occurrence of epithelial defects. Persistent epithelial defects are the result of unsuccessful re-epithelialization and complete healing within two weeks after damage to the corneal epithelium, even after conventional treatment. They represent a challenge for ophthalmologists, as they are often refractory to standard treatment. Patients report heavily expressed subjective complaints, including reduced vision, pain, redness, tearing, irritation, foreign body sensation, which negatively affects their quality of life. Anterior segment disorders are a leading cause of ocular morbidity, with conditions including dry eye syndrome, infections, trauma of various types, inflammatory processes, hereditary diseases and cataracts. While the latter is a disease of the elderly population, blindness caused by corneal opacity occurs in all age groups. In children, it is the third most common cause of blindness, with reduced vision leading to delayed motor, emotional, social and cognitive development with lifelong consequences. In adults, visual impairments are associated with lower levels of employment and higher levels of depression and

anxiety, as well as social isolation. For some of these patients, the conditions are continuously associated with progression and worsening of symptoms. In recent years, awareness of persistent epithelial defects has increased, but the true incidence is currently unknown. Diagnosis is primarily clinical - it can be amplified by highly specialized tests to quantify the area of the defect, its morphology, depth and response to treatment. The global market for the treatment and management of persistent epithelial defects is expected to grow by 18% by 2033 (2). The projected increase is due in part to the fact that, despite the existence of several therapies and the development of an increasing number of new approaches, treatment requires both management of the underlying causal condition and coping with the underlying subjective symptoms. The examination, diagnosis, treatment and follow-up of these patients is a complex process involving a number of medical structures, offices, equipment, units and personnel. In recent years, with the development of regenerative medicine, biological therapy and an individual approach, many new options have been discovered for the treatment of severe, refractory cases that are not affected by standard drug and moisturizing therapy. Among the directions are the use of natural matrix tissues to restore the loss or damage of tissues that have disrupted their integrity and function due to disease, hereditary, inflammatory or age-related processes. Autologous serum drops, amniotic membrane, tissue engineering are new approaches to the arsenal for the treatment of anterior ocular surface diseases (3). They can be considered as the fifth pillar of therapeutic approaches in addition to lubricants, local and systemic immunosuppression, surgical techniques and therapeutic contact lenses. Their moisturizing and epitheliotropic properties show high efficiency in dealing with ocular discomfort, neuropathic pain and corneal epithelial repair. New approaches for storage, distribution and application of this type of therapy are proving to be an indispensable part in the successful treatment of patients unresponsive to standard therapy, harmlessly restoring anatomical and visual function, affecting the subjective symptoms and improving the quality of life and are a step in personalized ophthalmology – a cascade of approaches to tailor healthcare capabilities to the individual needs of the patient, from optimized genetic counseling and conventional therapies to trials of new DNA-based methods. It is the multifaceted nature and etiology of the disease, high-tech research, clinical trials and medical capabilities that stimulated the direction of our scientific interests towards studying the problem.

II. AIM AND OBJECTIVES

2.1. Aim

The aim of this dissertation is to conduct a detailed analysis of a wide range of eye diseases characterized by persistent epithelial defects and to assess the clinical effectiveness of the applied therapeutic approaches.

2.2. Objectives

To meet this aim, we set ourselves the following tasks, namely:

1. To conduct a review of publications in the literature and an assessment of modern diagnostic approaches in conditions associated with persistent epithelial defects and the therapeutic approaches applicable to them.

2. To assess the etiology of diseases characterized by persistent epithelial defects in patients treated at USBOBAL - Varna.

3. To analyze the microstructural changes in the cornea in patients with persistent epithelial defects using in vivo confocal microcopy (Heidelberg Rerina Tomograph II Rostock Cornea Module (HRT II-RCM) and anterior segment optical coherence tomography (Cirrus HD-OCT 5000, Carl Zeiss Meditec, Inc).

4. To compare the results of the effectiveness of the treatment.

5. To assess the subjective symptoms and visual function in patients with persistent epithelial defects before and after treatment.

6. To create a scorecard for assessing the subjective and objective signs of patients with persistent epithelial defects, which will optimize the therapeutic approach.

III. METHOLODY OF THE DISSERTATION

3.1 Object and scope of the study

The present study was conducted at the Department of Eye Diseases and Vision Sciences of the Medical University - Varna on the territory of the University Specialized Hospital for Eye Diseases for Active Treatment USBOBAL - Varna for a period of 4 years - from 01.12.2017 to 01.12.2021. A total of 102 patients treated in hospital and pre-hospital care were included in the study. Criteria for the selection of participants included in the study are:

 Patients with chronic recurrent diseases of the anterior ocular surface;

 Patients over the age of 18;

 Patients from Northeastern Bulgaria.

Criteria for the exclusion of participants from the study:

• Patients under 18 years of age;

• Patients with coagulation status disorders;

• Patients with systemic diseases;

• Patients who have not signed an informed consent form.

All participants were thoroughly informed about the nature of the study and signed an informed consent form for their participation. All examinations were performed under the same conditions by a single examiner.

3.2 Research methodology

3.2.1 Documentary method

Research and analysis of the published scientific literature on:

- Types of diagnostic methods for the assessment of persistent epithelial defects;

- Analysis of the anterior segment of the eye in conditions associated with persistent epithelial defects;
- The method of production, mechanism of action, indications for use and the impact of autologous serum drops in various nosological entities;
- Therapeutic approaches that are applied in patients with PED and new proposals for treatment and diagnosis.

3.3.2 Clinical methods

- Determination of the best corrected visual acuity using an automatic test projector;
- Assessment of subjective signs of irritation, pain, redness and foreign body sensation – based on a questionnaire;
- Biomicroscopy of the anterior ocular surface and assessment of the condition of adjacent structures;

Anterior ocular surface and its structures were assessed using a biomicroscope (Carl Zeiss Meditec AG). Careful examination of both eyes, adjacent structures and systemic evaluation are essential to determine the etiology of PED. Fluorescein staining is used to visualize the defect, for serial monitoring and to measure the size, depth, and location of the epithelial defect using a slit lamp. Deeper PEDs take longer to absorb fluorescein into the stroma and often have gray-white margins. Associated signs of infection, such as changes in opacity, presence of infiltrates and inflammatory cells in the anterior chamber should be actively sought. There may be associated basement membrane dystrophies, nodular degeneration, dendrites, limbal stem cell deficiency, conjunctival injection, features of superior limbal keratoconjunctivitis, allergic conjunctivitis and foreign body. It is essential to assess dry eye using the tear film break-up time (TBUT) test. Rose Bengal stain stains devitalized epithelial cells, suggesting impaired tear function, regardless of tear clearance.

Examination of adjacent ocular structures is also important. The position of the lacrimal puncta, eyelid abnormalities, presence of trichiasis, blepharitis and meibomianitis, etc. should be considered. Periorbital and facial asymmetry should be carefully assessed to rule out facial nerve palsy, along with assessment of corneal sensation to rule out neurotrophic factors.

The diagnosis of PED is primarily clinical. It can be supplemented by tests that help quantify the lesion and monitor its progression.

- Anterior segment optical coherence tomography;

AS-OCT is a noninvasive technique for quantifying epithelial thickness and loss over time, especially in areas of corneal thinning and scarring. A Fourier-domain optical coherence tomography (Cirrus HD-OCT 5000, Carl Zeiss Meditec, Inc.) was used to perform anterior segment optical tomography (AS-OCT). The scanning speed of the device is 27,000-68,000 A-scans per second. The scanning plane is 1024 points along the A-scan axis. The axial resolution is 5 μm and the lateral resolution is 15 μm. The device has a superluminescent diode light source with a wavelength (λ) of 750 nm. The patient places his chin on the chin rest and rests his forehead on the attachment, then directs his gaze to a fixation point in the device to maintain the direction of the rays and image integrity. In the absence of fixation, the examiner should instruct the patient properly. The examiner should select the region of interest and manually select the location of the targeted structure. Anterior segment imaging is performed using an additional attachment - for corneal visualization, in combination with a pachymetric map (HD cornea attachment) or a 16mm white-to-white scan (Anterior Chamber), offering an image that can visualize both angles of the anterior chamber in a single OCT B-scan, which facilitates the assessment of the anterior chamber angle. In addition, tools can be used to manually measure parameters of structures, including the cornea, sclera and chamber angles. After imaging, which lasts a few seconds, the built-in software (Windows® 7, 4th generation i7 Intel® processor) displays high-resolution images of the studied structures. Anterior segment optical tomography was performed in each patient immediately after biomicroscopy.

• In vivo confocal microscopy;

The HRTII-RCM (Heidelberg Retina Tomograph II – Rostock Cornea Module, Heidelberg Engineering GmbH, Germany) was used in this study. The device has the following technical specifications:

• Field of view: 15°x15° (transverse);

• Scanning depth: 1.0 to 4.0 mm;

• Optical resolution: 10 μm /pixel (transverse);

• Digital object size: 2-D image: 384 x 384 pixels;

• 3-D image: up to 384 x 384 x 64 pixels;

• Scanning time: 2-D image: 24 msec;

• 3-D image: 2mm scan depth;

• Focus range: -12 to +12 dsph -6 to +6 dcyl;

• Minimum pupil diameter: ≥ 1 mm;

• Light source: Diode laser λ=670 nm;

• Image alignment/artifact rejection: TruTrack™ software;

• Operating system: Heidelberg Eye Explorer and Windows XP.

Immediately before the examination, the topical anesthetic proxymetacaine hydrochloride (Alcaine 0.5% collyre, Alcon) was placed in the patient's inferior conjunctival sac. A disposable sterile PMMA applanation cap (Tomo-cap) was filled with gel (Corneregel, Bausch & Lomb GmbH, Germany) as a bonding agent between the applanation cap and the objective lens. The patient was positioned on the chin and forehead rest of the apparatus, after which the applanation cap was introduced to the cornea. The position of the eye was monitored with a camera. In confocal microscopes, the illumination optics focus on a single, diffraction-limited spot in the sample, which is the only spot imaged by the detector during confocal scanning. To generate a complete image, the spot must be moved over the sample and data collected point by point. The duration of a single confocal microscopy session was approximately 10 minutes. None of the subjects complained of discomfort or experienced any adverse effects after the study.

3.3 Therapeutic methods

After a detailed history taking, completed questionnaires and a standard ophthalmological examination, the highly specialized tests necessary for the purpose of the dissertation were performed - anterior segment optical coherence tomography and in vivo confocal microscopy. Based on the data, the etiology and size, depth and localization of the epithelial defect of each of the patients included in the study were determined. Depending on the etiology, size and depth of the epithelial defect, the presence/absence of infiltrate, the patients were divided into four randomized groups:

• Group I - Therapeutic contact lens (TCL)

• Group II - TCL+Autologous serum drops (ASD)

• Group III - Amniotic membrane (AM)+TCL+ASD

• Group IV - AM+ASD+TCL+Cross-linking - In the presence of infiltrate

Autologous serum drops were prepared according to the standard protocol described by Geerling et al. (4). The patients were informed in detail about the method of storage and administration of the drops. Surgical treatment was performed in two of the patient groups:

• Transplantation of cryopreserved amniotic membrane (AM) fixed on nitrocellulose paper, provided by the "Center for Translational Medicine and Cell Therapy" at the Medical University of Varna.

• Corneal cross-linking (PACK-CXL) - a minimally invasive intervention that uses ultraviolet A (UVA) rays and riboflavin (vitamin B2) to slow or even stop the progression of corneal ectasia and to treat some corneal pathologies.

3.3.1 Algorithm for production and storage of autologous serum drops

Before undertaking the production of ASD, it is necessary to obtain informed consent from the patient and establish negative serological results: hepatitis B, hepatitis C, syphilis and HIV. The required amount of blood (10 ml) is aspirated under sterile conditions from the cubital vein and stored in special vacutainers. They are left to stand in a vertical position (stand) for 30 min. at +4°C temperature to allow complete clotting. The next step in the production process is centrifugation, 3000g for 15 min., which leads to the separation of the serum from the other blood elements. The blood serum is aspirated under sterile conditions into 10 cc syringes and diluted in a ratio of 1:5 with saline (0.9 % NaCl). The resulting 20% ASD are distributed in sterile 1 ml containers, which are stored at -4°C temperature. The patient receives full instructions on how to store and use ASE.

The production parameters had a significant impact on the quality of the blood product. It was found that centrifugation at 3000g for 15 min resulted in good separation of the blood serum without inducing hemolysis.

3.2.4. Statystical methods

The statistical software package IBM SPSS for Windows, v.20.0 was used for data processing. Other statistical methods included:

- Analysis of variance (ANOVA) to assess the statistical significance of the given factors.
- Analysis of variance to study the quantitative characteristics of the indicators.
- Correlation analysis to assess the dependence between the studied indicators. The assessment of the strength of the relationship between variables is based on the results of the Pearson (r) and Spearman (ρ) coefficients:

 $0 < r (\rho) < 0.3$ – weak correlation

 $0.3 < r (\rho) < 0.5$ – moderate correlation

 $0.5 < r (\rho) < 0.7$ – significant correlation

 $0.7 < r (\rho) < 0.9$ – high correlation

 $0.9 < r (\rho) < 1$ - very high correlation

- Regression analysis to assess possible functional dependencies between the studied indicators.

Investigation of cause-and-effect relationships

 Comparative analysis (hypothesis assessment) – χ 2, Student's t-test for comparing quantitative and qualitative indicators and examining the difference between them;

 Graphical and tabular method of displaying the obtained results.

In all analyses, an acceptable level of significance of $p<0.05$, $p<0.01$, $p<0.001$ with a confidence interval of 95% is assumed.

IV. RESULTS

Over a period of 4 years (2017-2021), 102 patients with PED have underwent treatment at USBOBAL-Varna. The results show that the male gender predominates (62.7%). The average age of patients with PED is 40.5, with the lowest being 18 years of age and the highest being 75 years. In terms of the underlying etiology, the leading cause of PED among patients who have passed through USBOBAL-Varna are trauma and burns, followed by inflammatory diseases of the cornea and foreign body ingress. The proportion of patients with bullous keratopathy, corneal dystrophy and postherpetic neuropathy is smaller. In males, the leading causes of PED are the presence of a foreign body in the cornea and keratitis, followed by trauma and burns. In females, the leading etiological factor is trauma and burns, followed by corneal dystrophies.

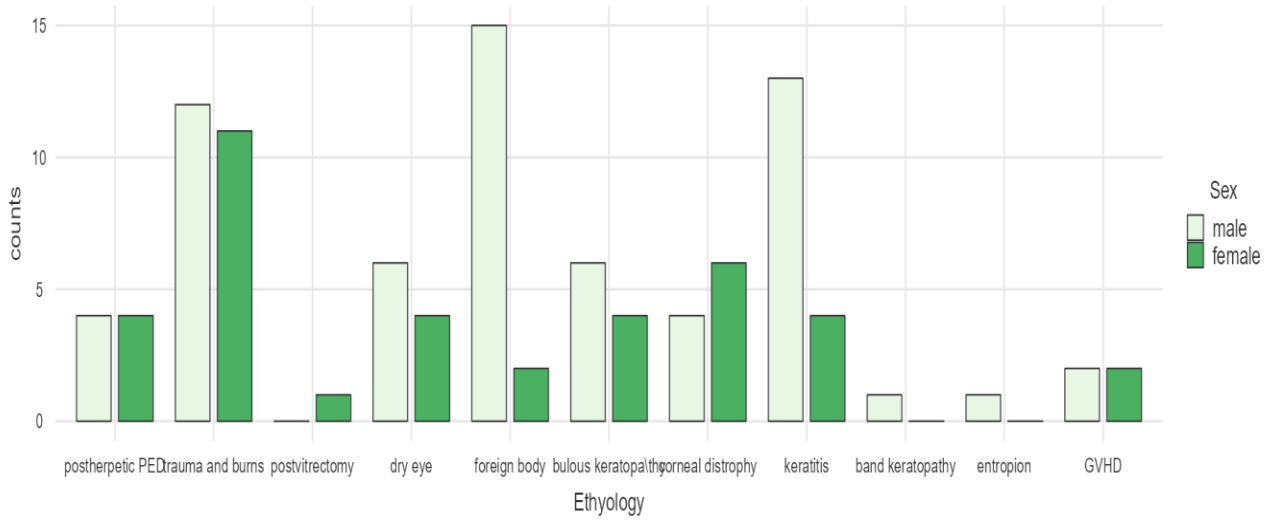


Figure 1. Distribution of study participants by etiology and gender.

4.1 Assessment of subjective symptoms in patients with PED before and after therapy

In this study of patients with PED, the following indicators were used to assess subjective symptoms: pain, redness and foreign body sensation. They were monitored before starting therapy, on the 1st, 2nd week and 30 days after treatment. The results show that 92.2% of patients experience pain before starting treatment. A significant response to the pain syndrome was observed already in the first week, with 63.7% of patients having no pain syndrome, and in the

first month - in 86.3% of them. A statistically significant difference was found in the perception of pain in patients with different diseases before treatment and in the first week after treatment ($p<0.001$), before treatment and in the first month after treatment ($p<0.001$), as well as in the first week and in the first month after treatment ($p<0.001$). The analysis of the results shows that before starting the respective therapy, 33.3% of the patients reported severe and 43.1% reported prominent pain symptoms. A significant reduction in pain was found already in the first week, with only 1% of the studied patients having severe and 8.8% having pronounced pain symptoms.

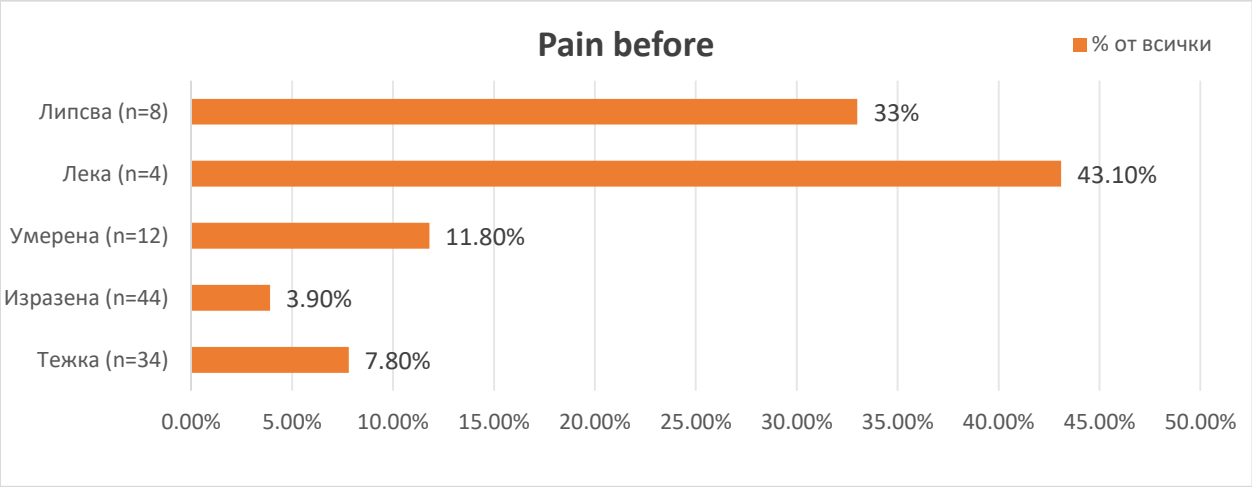


Figure 2. Subjective pain sensation before starting the prescribed therapy.

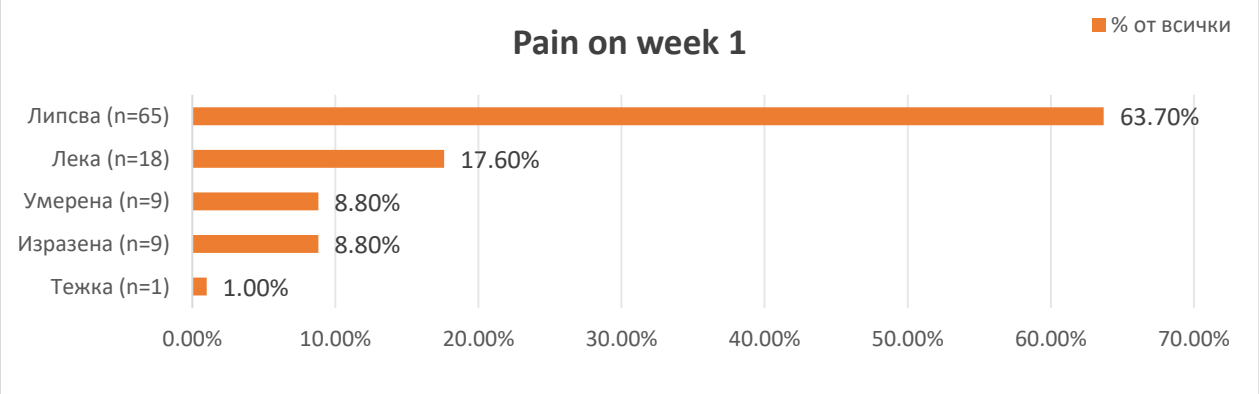


Figure 3. Subjective pain sensation on the first week of therapy.

A statistically significant difference in pain perception was found in patients with different underlying etiologies leading to PED.

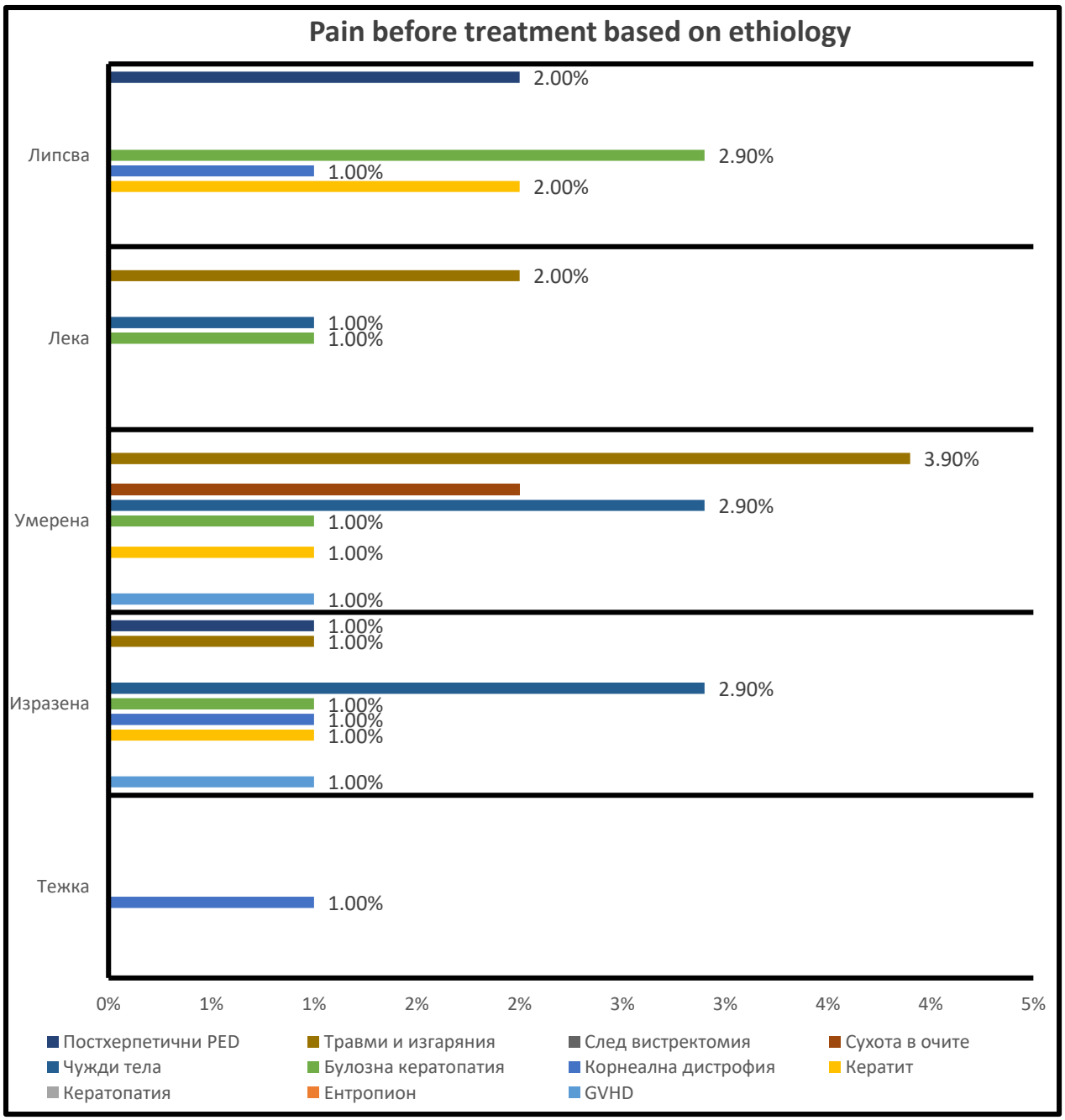


Figure 4. Subjective pain perception before treatment by diagnosis.

The most prominent pain symptoms (severe and pronounced) before starting treatment are those of patients with inflammatory diseases (keratitis), trauma, burns and the presence of a foreign body.

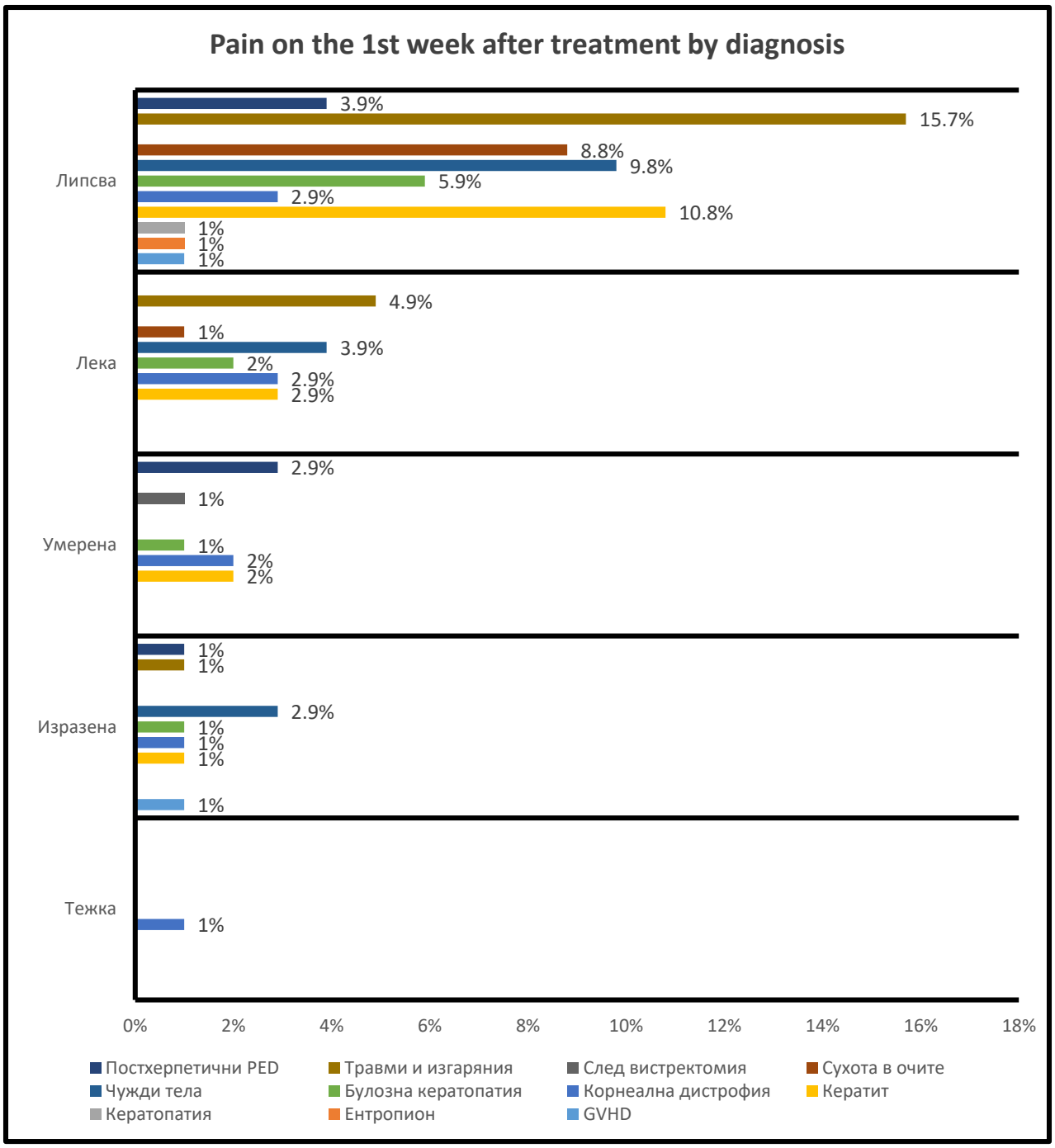


Figure 5. Subjective pain perception in the first week of the applied therapy by diagnosis.

The results display a significant improvement in the pain syndrome, with 63.7% of patients experiencing no pain. The most severe pain syndrome was observed in patients with inflammatory

diseases of the cornea (keratitis), followed by those with injuries and burns of the AOS. Redness is another indicator for subjective assessment, which was studied in patients with PED. The results show that before starting therapy, 96.1% of patients reported a "red eye". Fig. 6 shows that the proportion of patients with prominent redness prevails - 42.2%, followed by those with moderate - 30.4%. Fig. 6 and 7 present the data obtained when assessing the subjective indicator of redness before and after treatment. The figure shows that the largest percentage of patients (42.2%) reported pronounced redness, followed by those with moderate - 30.4%. Of those included in the study, 17.6% reported severe flushing before starting treatment.

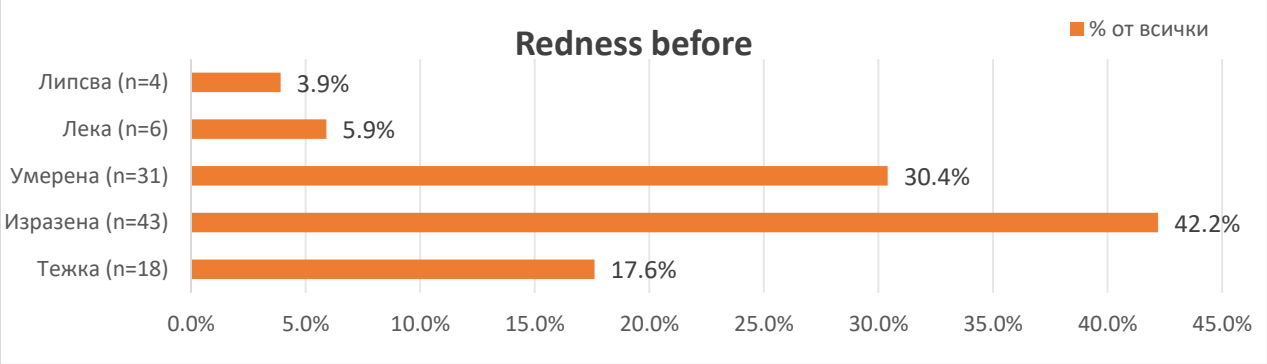


Figure 6. Subjective assessment of redness before starting the prescribed therapy.

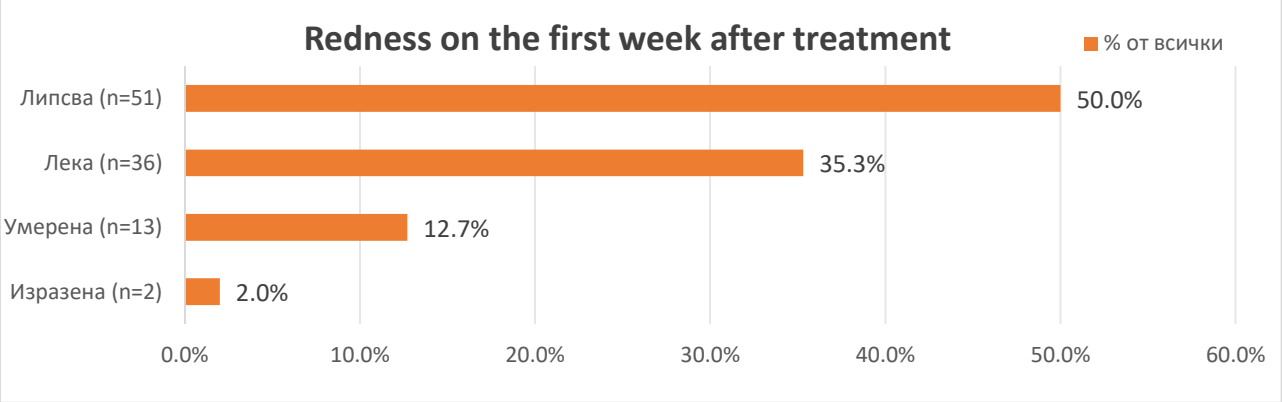


Figure 7. Subjective assessment of redness during the first week after starting therapy.

A significant impact on this subjective indicator is observed already in the first week. The figure shows that in 50% of patients there is no redness, in 35.3% there is mild, in 12.7% - moderate and only in 2% - prominent redness. The absence of patients with severe redness is impressive already

in the 1st week from the start of therapy. The analysis of the data shows that the most commonly presented prominent redness before the start of therapy is observed in patients with keratitis, followed by patients with injuries and burns and due to the presence of a foreign body.

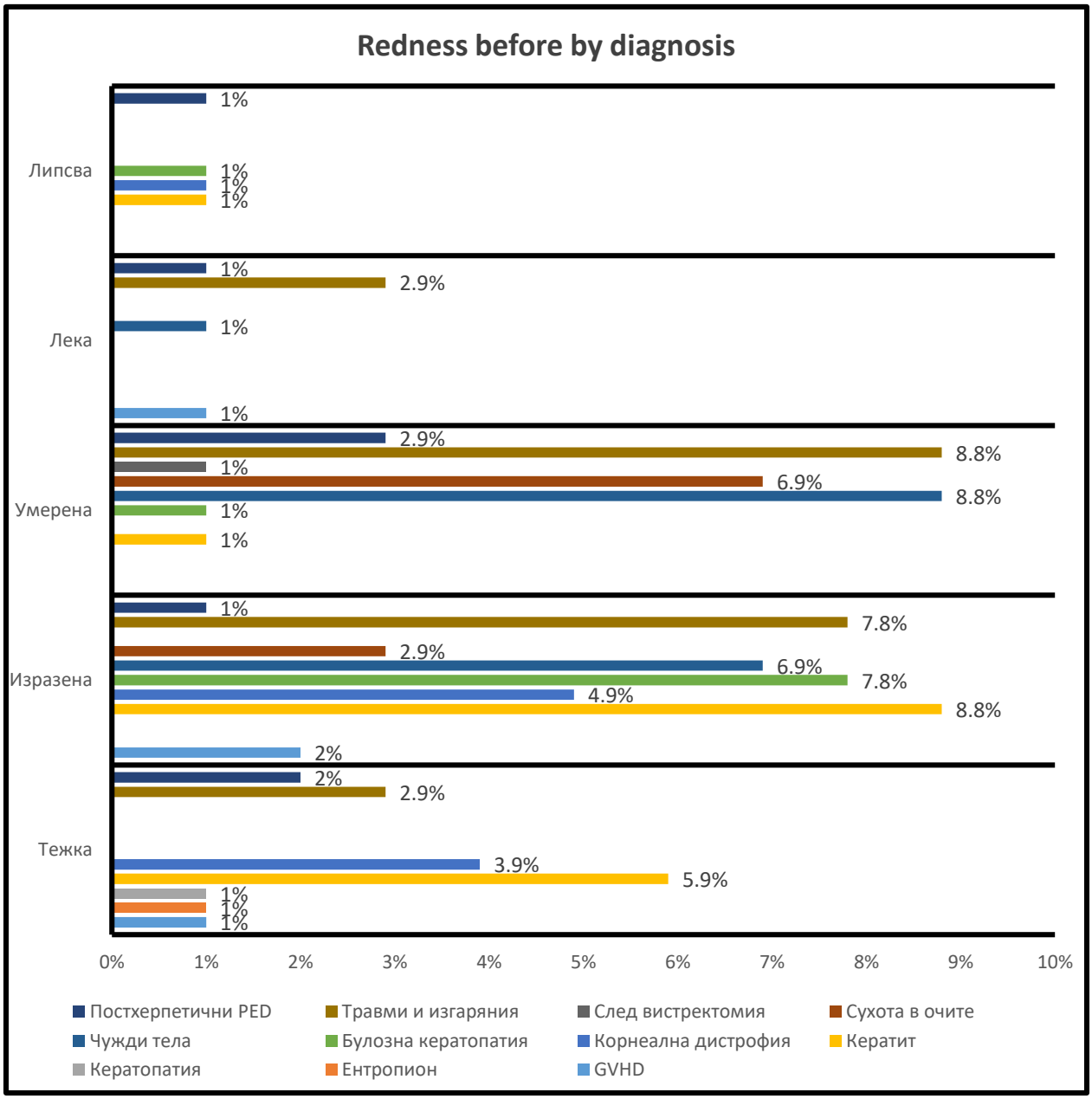


Figure 8. Subjective assessment of redness before initiating therapy by etiology.

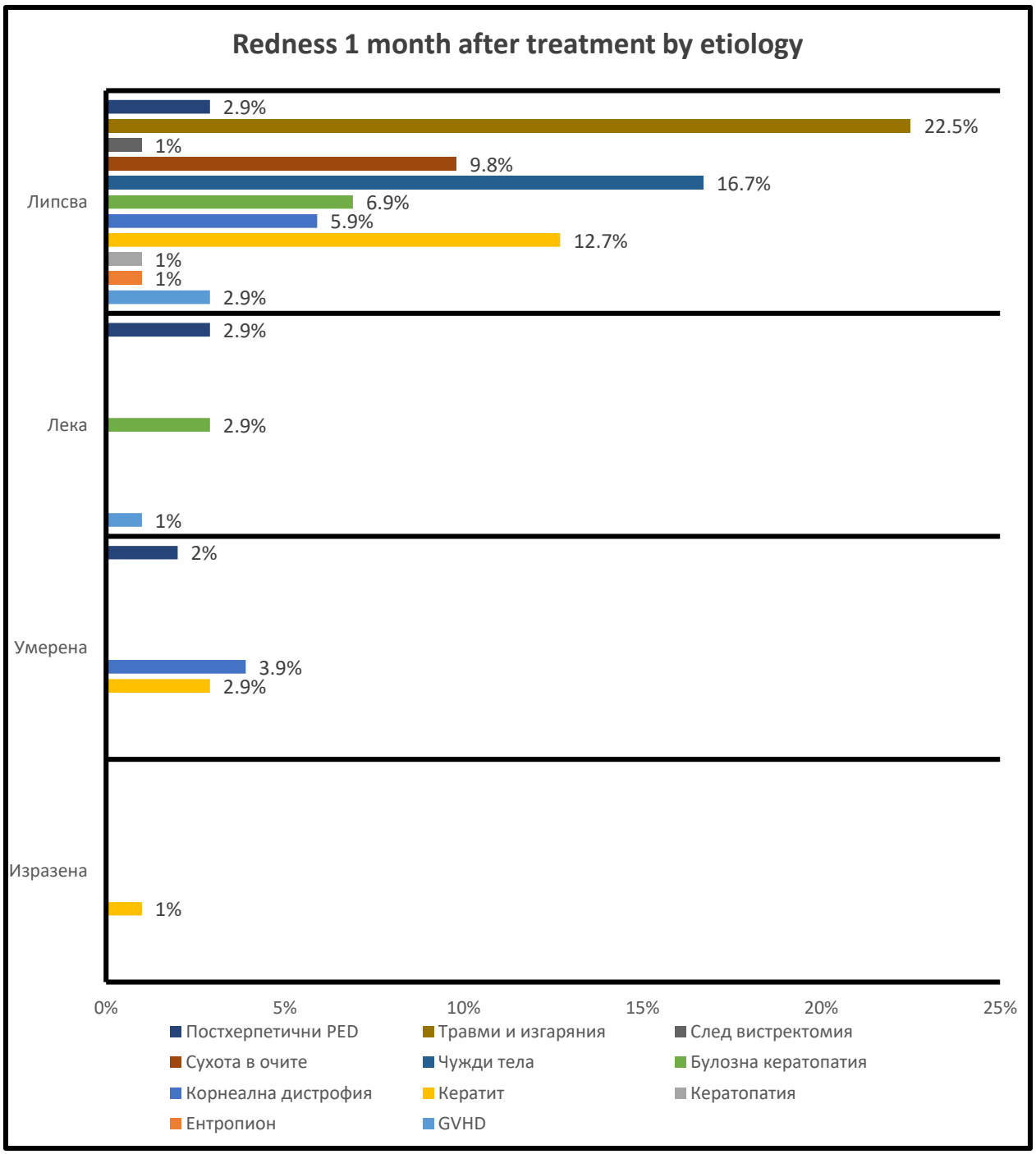


Figure 9. Subjective assessment of redness on the first month of the assigned therapy by etiology.

In the first month after starting treatment, 83.3% of the patients reported no redness, 8.8% reported moderate redness and 6.9% reported mild redness.

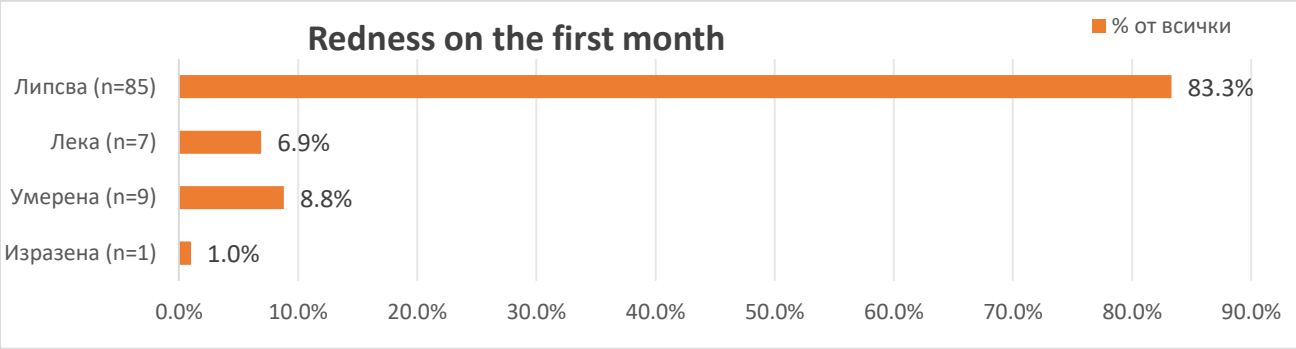


Figure 10. Subjective assessment of redness on the first month of treatment.

A significant impact on this subjective indicator was observed already in the first week, with 50% of patients experiencing no redness. The effect was even more pronounced in the first month, with 83.3% of patients reporting a “white eye”. A statistically significant difference was found in the perceptions of redness in patients with various diseases before treatment and in the first week after treatment ($p<0.001$), before treatment and in the first month after treatment ($p<0.001$), as well as in the first week and in the first month after treatment ($p<0.001$). A severe degree of redness was observed in patients with keratitis, trauma and burns, as well as in those with bullous keratopathy. It is impressive that in the first month, 80.4% of patients experienced no redness, while before starting therapy, only 4% did.

Foreign body sensation is the last subjective indicator that we assessed in our sample of patients with PED. The data from the comparative analysis in the period before the start of treatment and at the 1st week showed a significant difference ($p<0.001$). The largest percentage of patients - 43.1% reported a prominent foreign body sensation, in 27.5% the symptomatology was severe, and moderately pronounced - in 22.5%. The rapid response of the patients to the applied treatment is impressive.

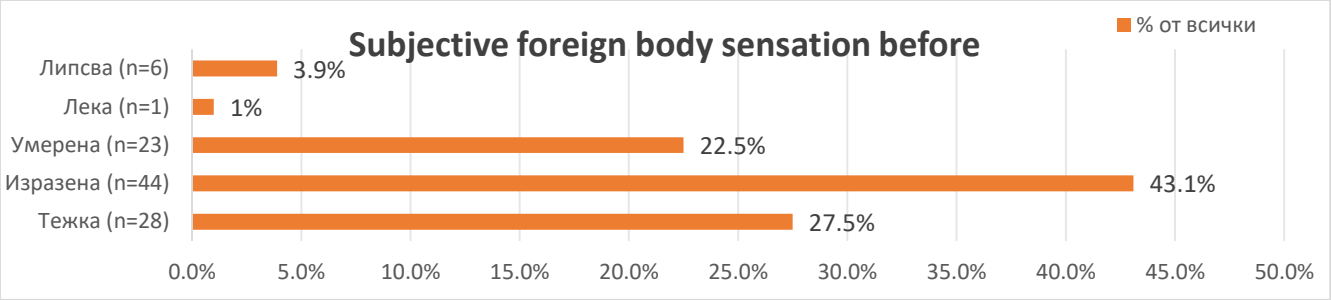


Figure 11. Subjective foreign body sensation before starting treatment.

Fig. 12 shows that at week 1 only 5.9% had severe foreign body sensation, 8.8% had moderate symptoms and 18.6% had mild symptoms. 65.7% of the patients studied had no foreign body sensation even at week 1 after starting therapy.

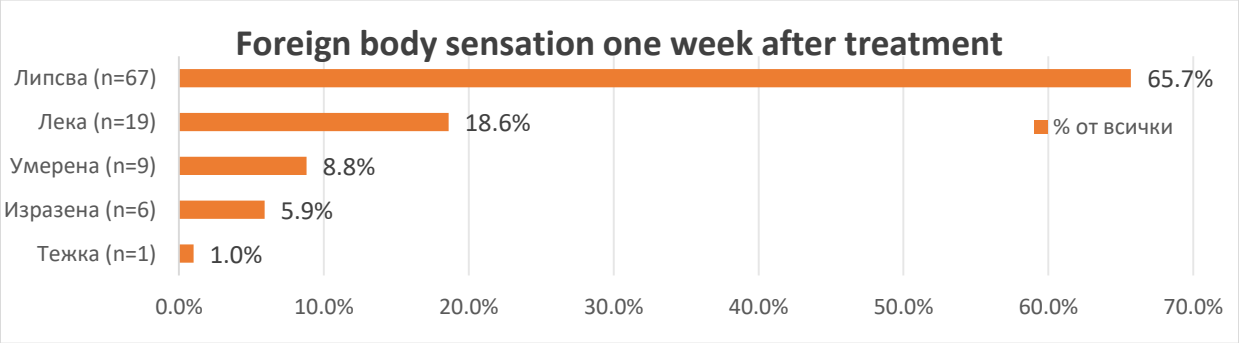


Figure 12. Subjective foreign body sensation in the first week of therapy.

Fig. 13 displays that this percentage is even higher in the first month, with 89.2% of patients lacking foreign body sensation.

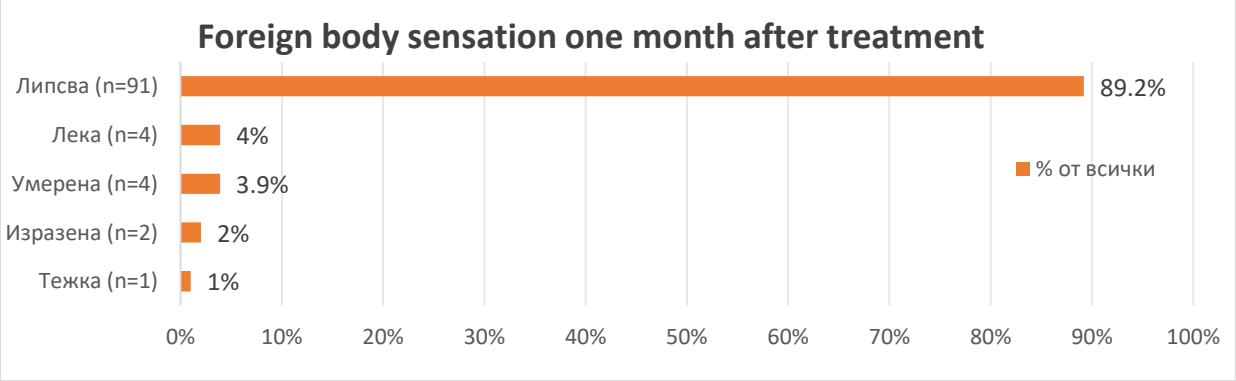


Figure 13. Subjective foreign body sensation during the first month of treatment.

In terms of underlying etiology, the most prominent foreign body sensation (severe) is observed in patients with trauma and burns, followed by patients with inflammatory diseases (keratitis).

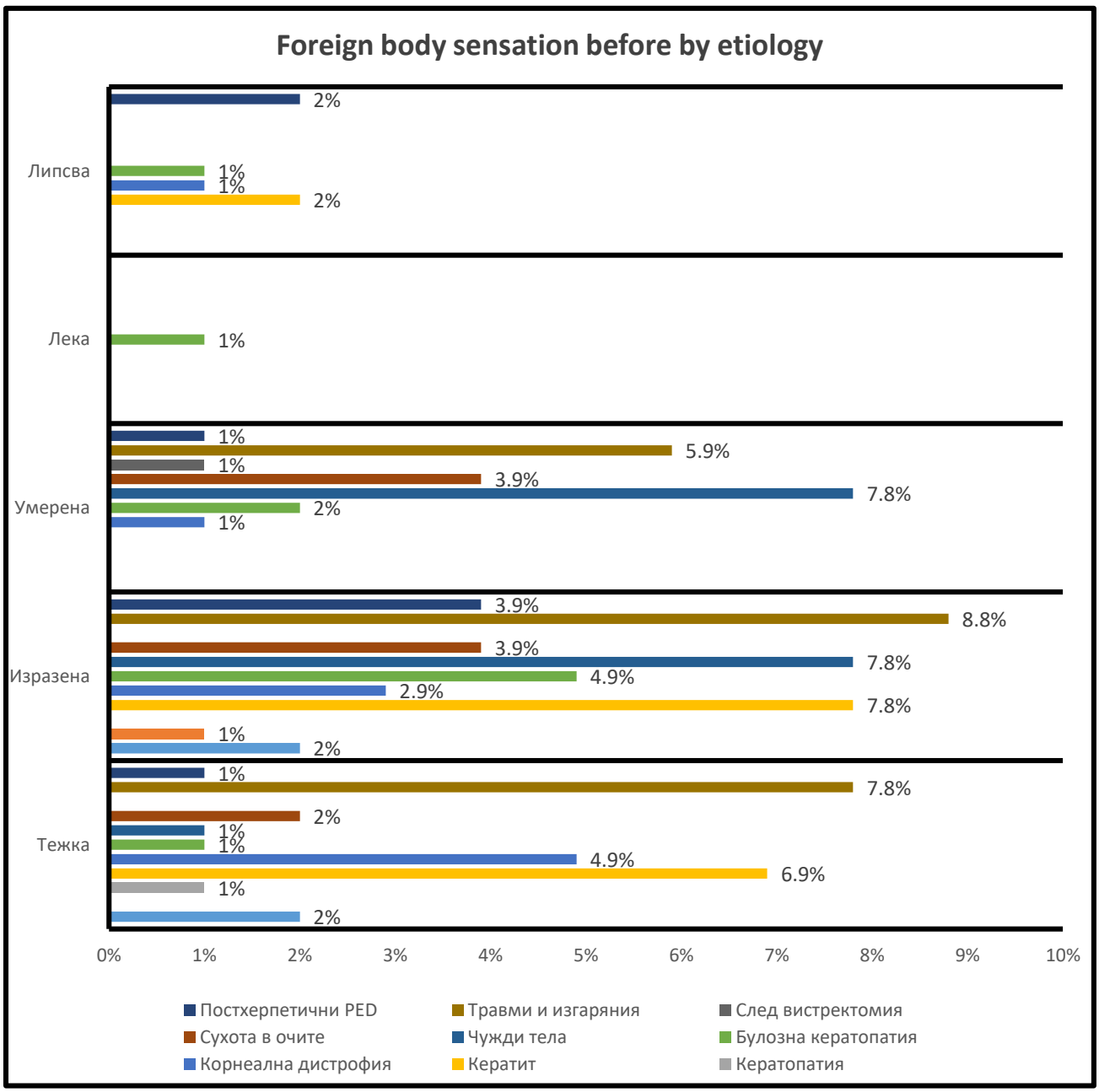


Figure 14. Subjective foreign body sensation according to the etiology of PED before starting therapy.

The results portray a significant improvement in symptoms already in the 1st week, with 65.7% of patients lacking FBS and in the 1st month the results are even more notable - 89.2% lacking FBS.

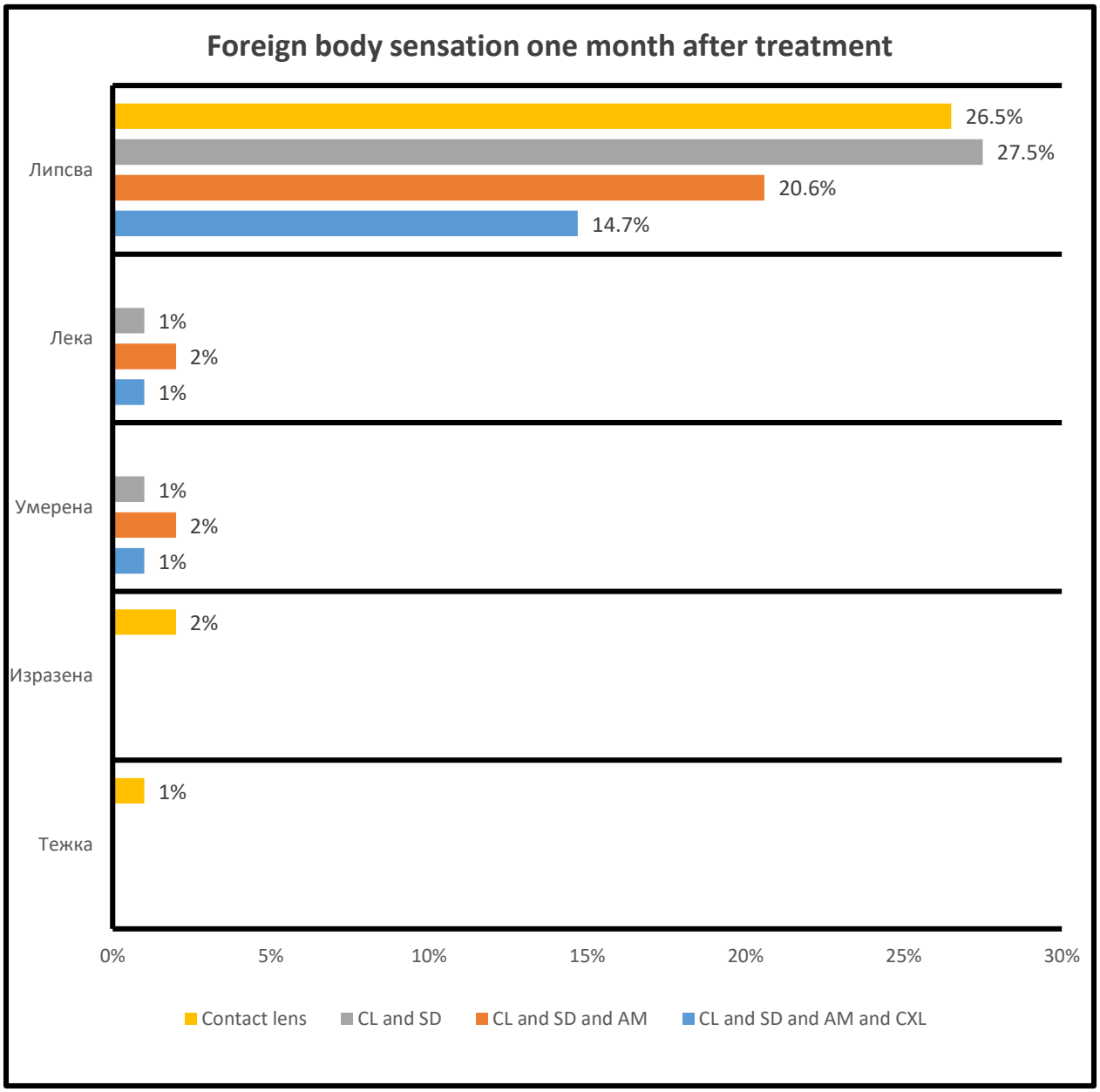


Figure 15. Subjective foreign body sensation according to therapy performed in the first month of treatment.

4.2 Assessment of objective signs in patients with PED

The size of the epithelial defect is the first objective sign that we monitored in our sample of patients and is also used as a criterion for the effectiveness of the applied therapy. A significant difference in the size of the epithelial defect was observed between the individual groups before the start of therapy. The highest percentage of epithelial defects with a size between 3-5 mm was observed in the group with injuries and burns - 11.8%, and of epithelial defects with a size < 5 mm - in the group with keratitis. At the first month, 22.5% of the patients with injuries and burns had epithelialization (no epithelial defect), followed by patients with a foreign body - 16.7% and keratitis - 12.7%. Fig. 16 displays that before the start of treatment, the largest percentage of patients (34.3%) had an epithelial defect with a size of 3-5 mm, followed by those with a size of 2-3 mm - 26.5% and >5 mm - 24.5%.

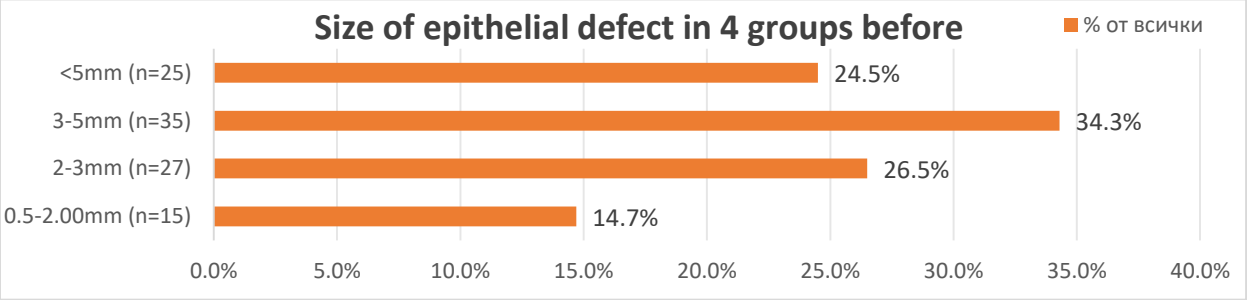


Figure 16. Size of the epithelial defect in four groups before starting treatment.

The rapid epithelialization process, which we observed in the first week after implementing the appropriate therapeutic plan, is impressive.

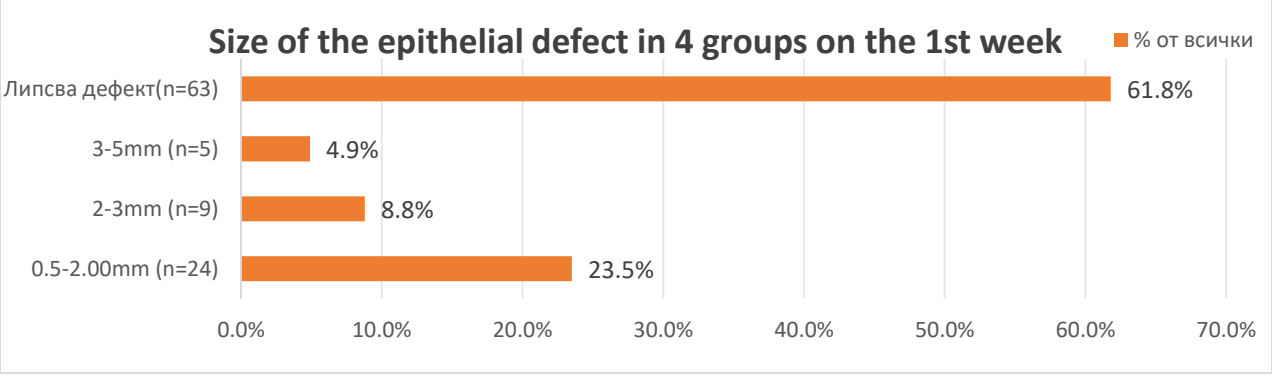


Figure 17. Size of the epithelial defect in four groups on the first week of treatment initiation.

In 61.8% of patients, complete restoration of corneal epithelial integrity was observed. In most of the remaining patients, a significant reduction in the size of the PED was observed.

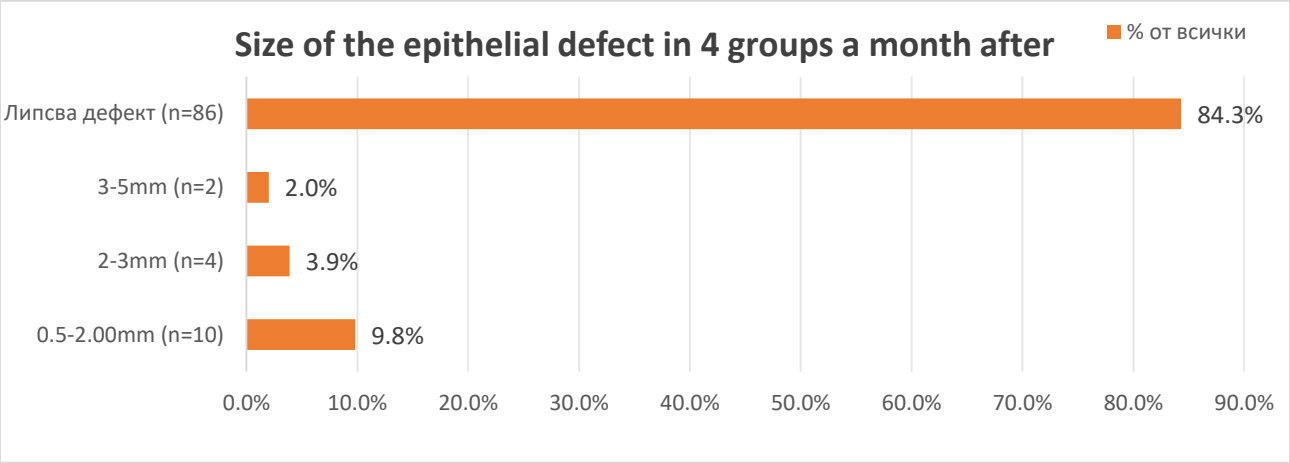


Figure 18. Size of the epithelial defect in four groups one month after the start of treatment.

Fig. 18 presents the data from the assessment of the size of the epithelial defect in the first month of the assigned therapy. The figure shows that in 84.3% of the patients there is no epithelial defect, in 3.9% we observed persistence of a defect with a size of 2-3 mm, in 2% with a size of 3-5 mm, and in 9.8% the size is 0.5-2.0 mm. A statistically significant difference in the size of the epithelial defect was found in the patients with different underlying etiology. The largest size of the epithelial defect > 5mm was observed in the group of patients with inflammatory diseases (keratitis) - 11%, followed by those with corneal dystrophies - 5.9%. Among patients with trauma and burns, PED with a size of 3-5mm prevailed - 11.8%. Fig. 19 shows that in patients after a foreign body enters the cornea, defects with a size of 2-3 mm prevail - 8.9%, and in those with severe dry syndrome with a size of 2-3 mm - 4.9%, followed by those with a size of 0.50-2.00 mm.

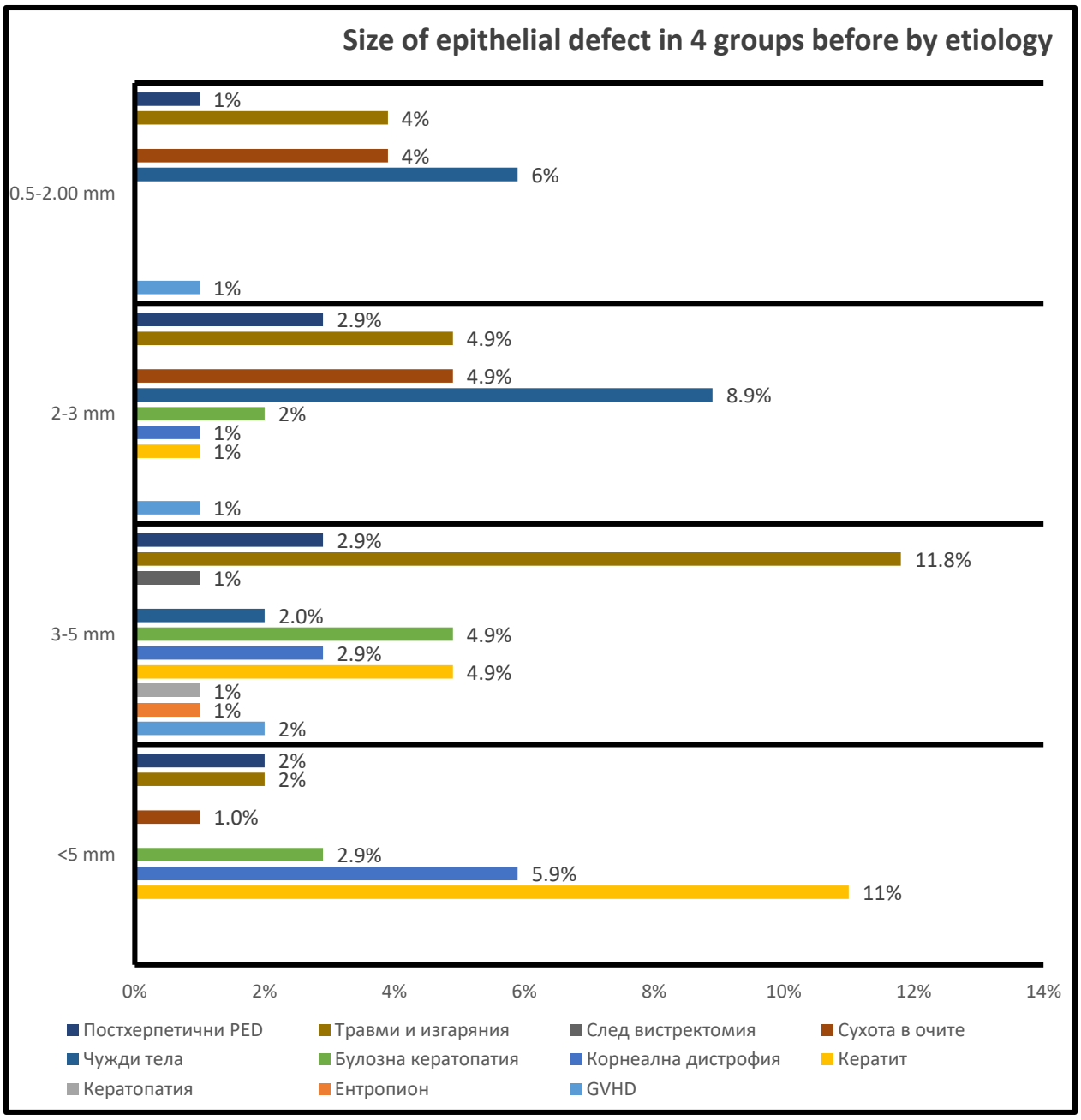


Figure 19. Size of the epithelial defect in four groups before starting treatment according to etiology.

The analysis of the data obtained during the follow-up of our patients in the 1st week displays an extremely good response (absence of epithelial defect) of patients with trauma and burn (18.6%), presence of a foreign body in the cornea (12.7%) and severe dry syndrome (9.8%).

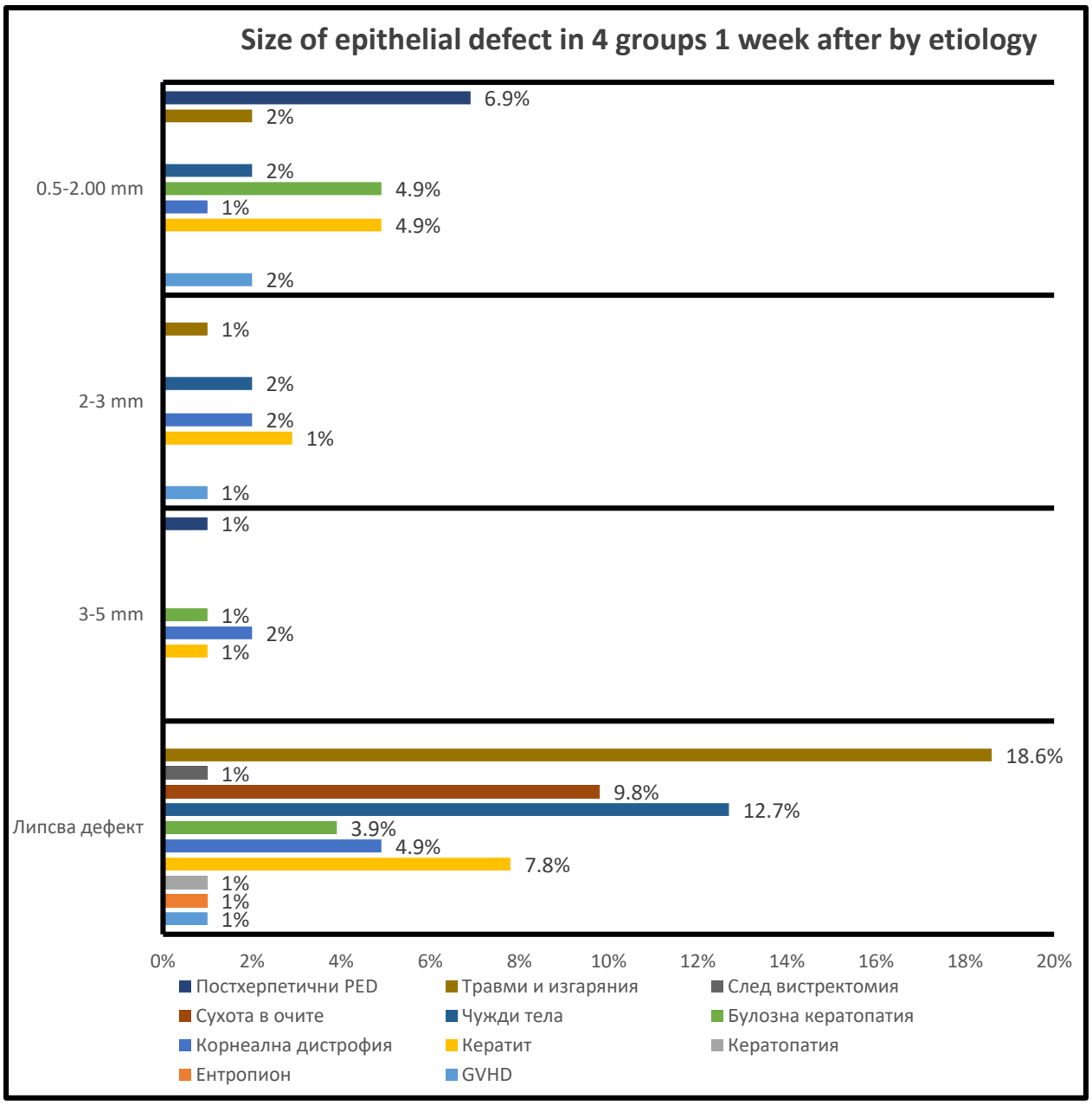


Figure 20. Size of the epithelial defect in four groups on the first week of treatment according to etiology.

We observed a weaker effect of the treatment for this indicator in patients with postherpetic keratitis, bullous keratopathy and inflammatory diseases (keratitis). In them, we found persistence of the epithelial defect, but with a smaller size - 0.50-2.0 mm.

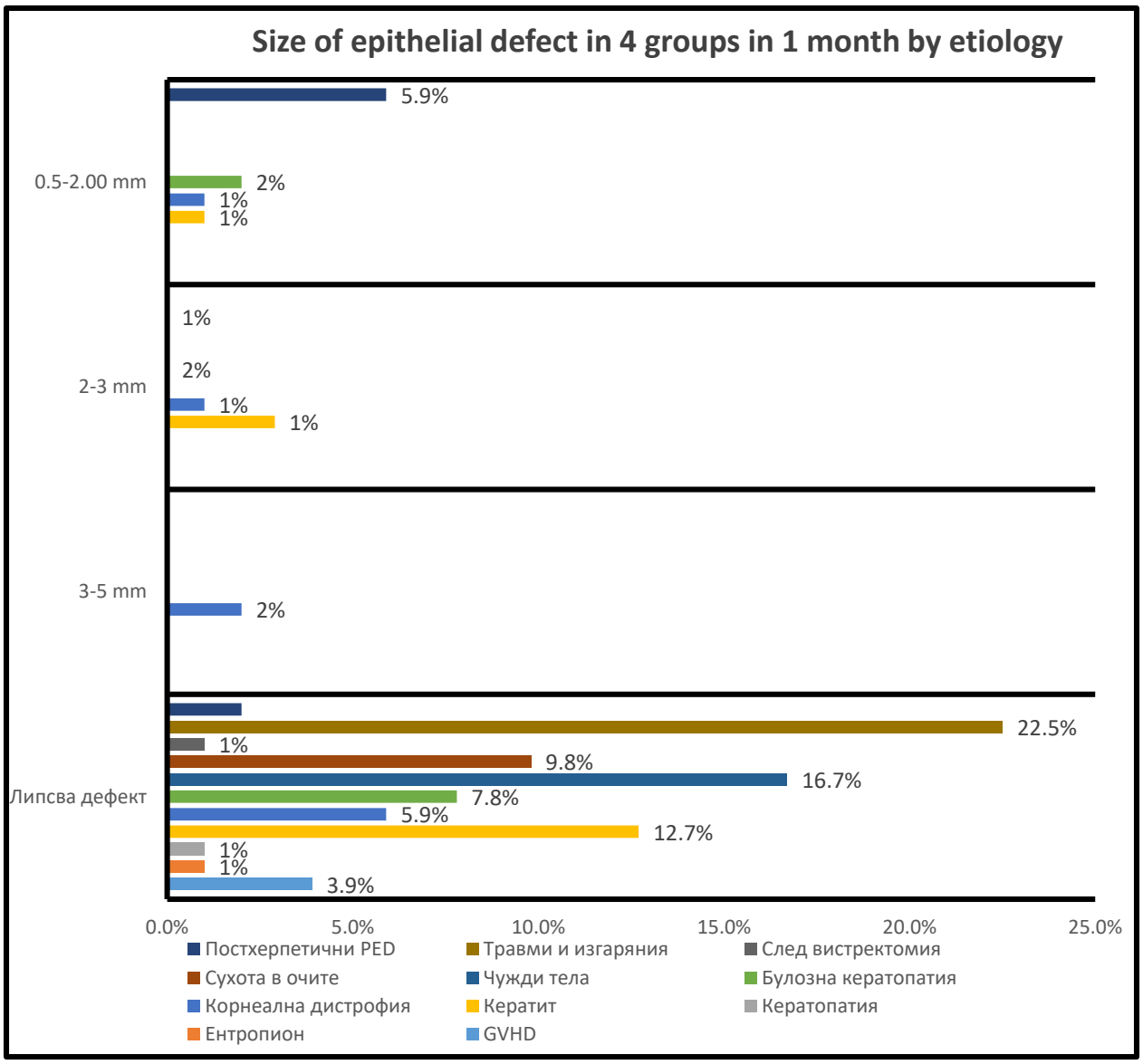


Figure 21. Size of the epithelial defect in four groups on the first month of treatment according to etiology.

Injection is another objective indicator that we monitored in our patients with PED. The results for injection in the period before starting therapy, presented on Fig. 22, show that the proportion of patients with ciliary injection in 2-3 quadrants prevails - 51%, followed by those with injection along the entire circumference - 33.3%. A significant improvement in this indicator is observed already in the 1st week, as in 36.3% of patients injection is absent, while in the period before

starting therapy this percentage was only 2%. The increase in the proportion of patients with conjunctival injection in the 1st week is impressive - 35.3% (compared to - 2.9% before therapy), which is associated with the invasive therapeutic approach (placement of AM) in some patients. In the 1st month, the proportion of patients in whom injection is absent reaches 81.4%. The proportion of patients with conjunctival injection remains higher – 11.8%. This phenomenon is observed only in the groups of patients undergoing invasive treatment.

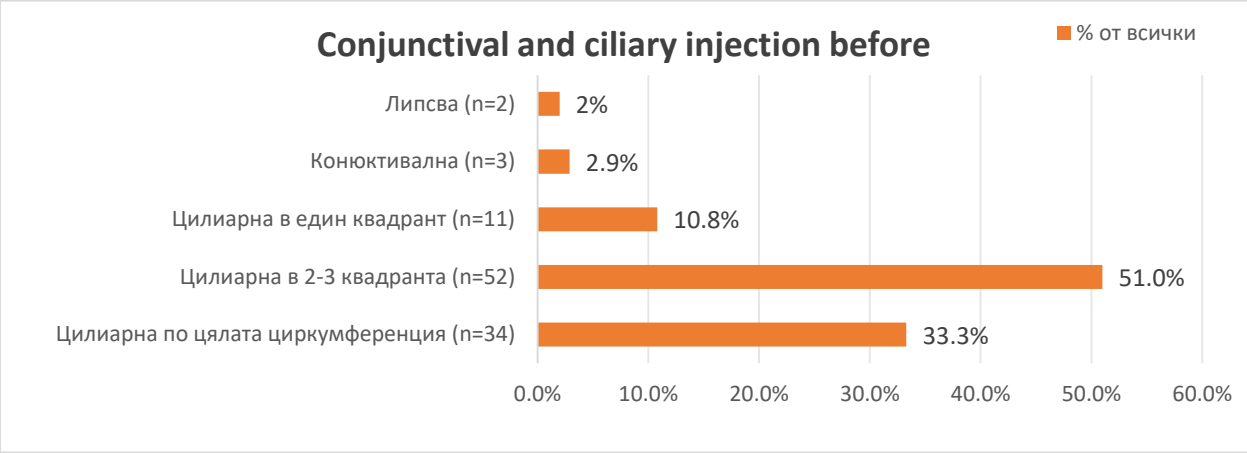


Figure 22. Conjunctival and ciliary injection before starting the prescribed therapy.

Analysis of data during the 1st week after starting treatment shows a significant improvement in this objective indicator.

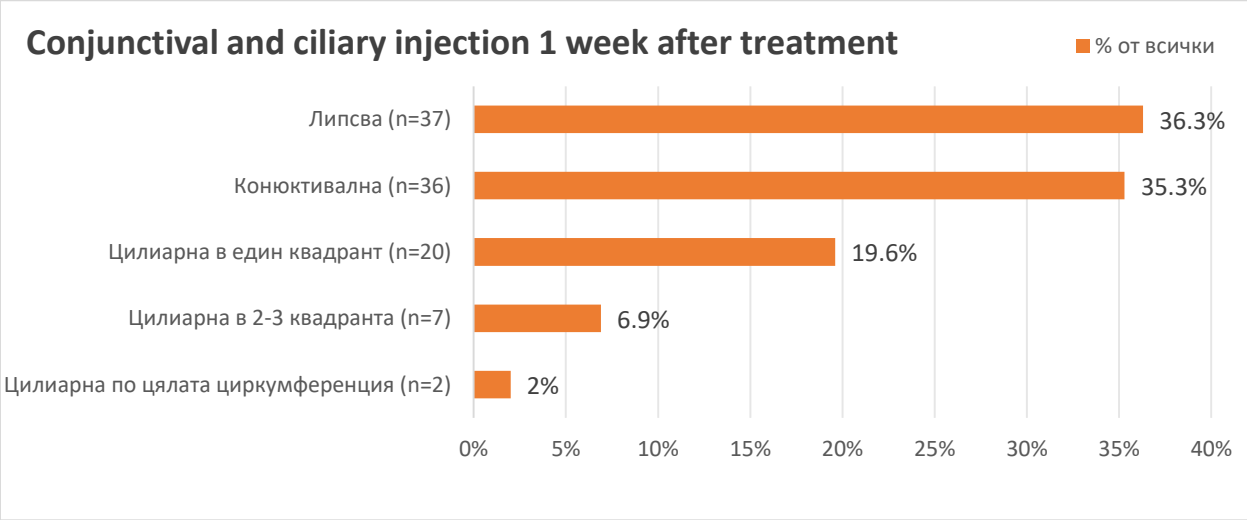


Figure 23. Conjunctival and ciliary injection one week after starting the prescribed therapy.

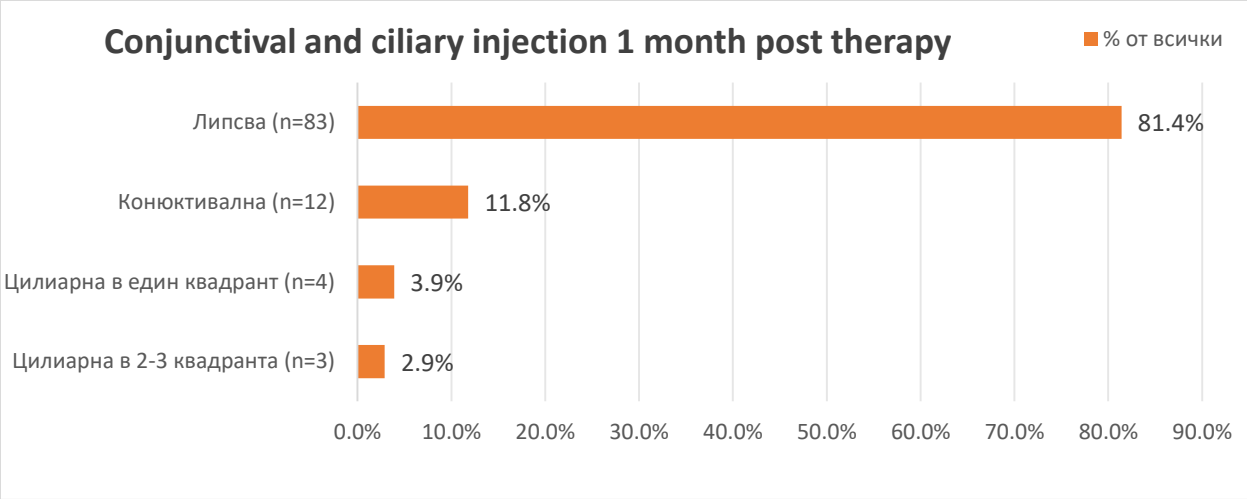


Figure 24. Conjunctival and ciliary injection in the first month after starting the prescribed therapy.

The last objective sign that we examined and included in our study is visual acuity. The results show a significant improvement of this indicator in all groups of patients. The best results were reported in patients with a foreign body in the cornea, with inflammatory diseases (keratitis), including those with postherpetic keratitis. We observed a weaker impact on this indicator in patients with GVHD and those with bullous keratopathy. We also reported good results in patients with trauma and burns. The majority of the patients included in this dissertation had a moderate degree of burn, which explains the results observed in this group.

4.3 Analysis of the results for the effectiveness of the treatment performed

Patients were randomized into four groups based on subjective symptomatology and objective findings. Patients with superficial epithelial defects without corneal stroma involvement were treated conservatively (58.40%). In patients in whom we found the presence of an ulcer (stromal involvement without the presence of infiltrate), we performed AMT and placed TCL, in combination with 20% ASD. In 16.7% of the patients included in this dissertation, we found the presence of corneal infiltrate. In them, we performed PACK-CXL, then placed AM and TCL, and after 1 week we assigned 20% ASD. Of the subjects in our clinical sample, 58.8% underwent conservative treatment and 42.1% required surgical treatment.

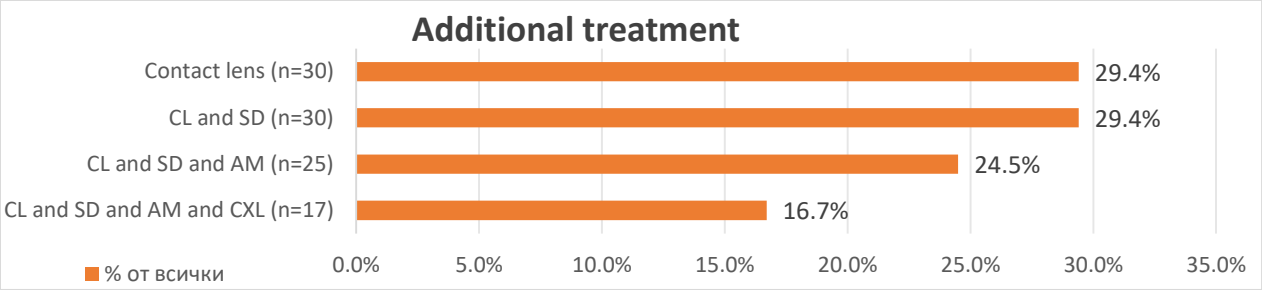


Figure 25. Additional treatment performed among patients with persistent epithelial defects.

Regarding pain, a subjective symptom that we studied in our sample of patients with PED, it is striking that already in the first week we observed a significant reduction in symptoms, with only 1% of patients experiencing severe pain.

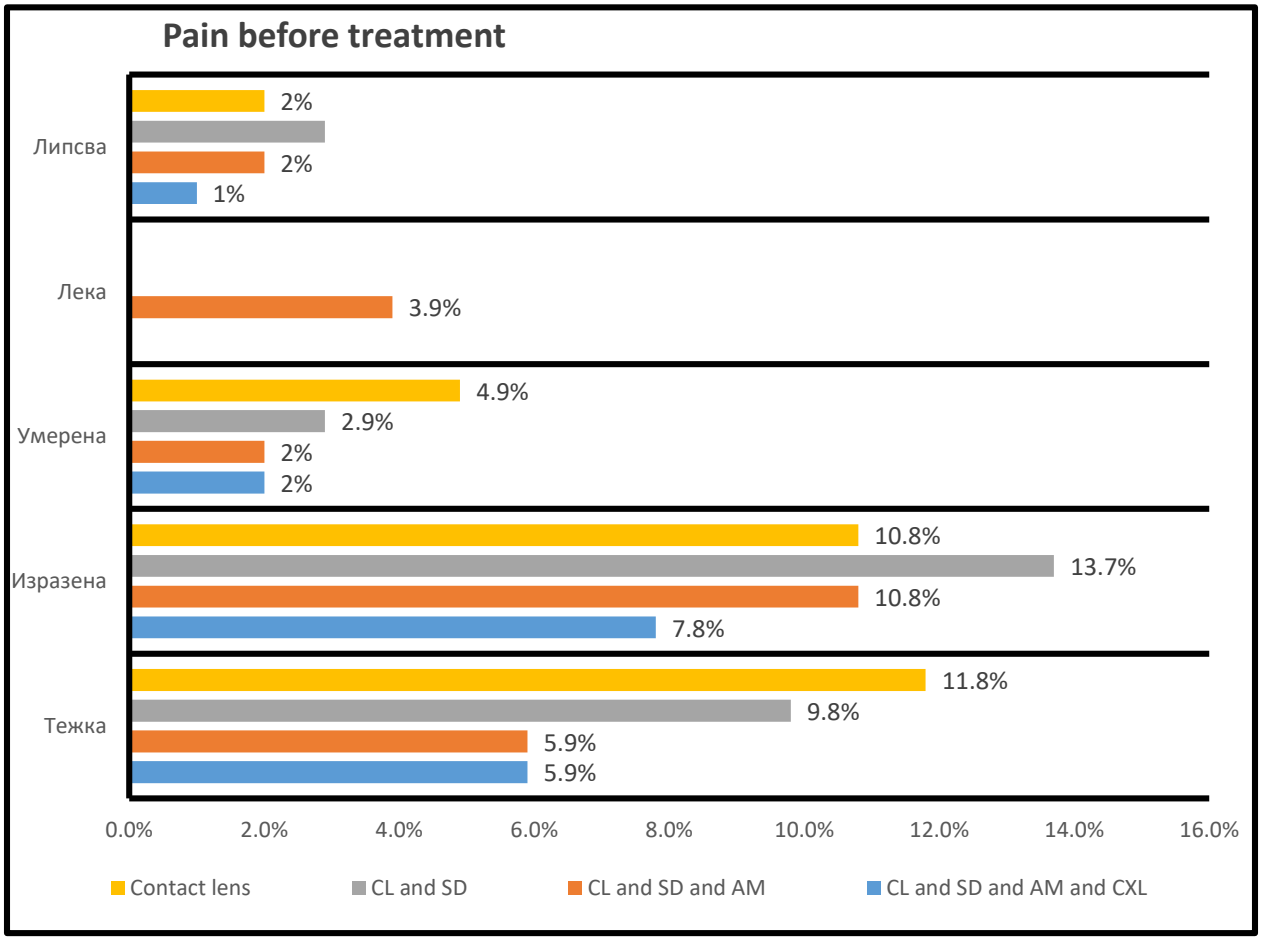


Figure 26. Pain symptoms before the treatment by groups before starting therapy.

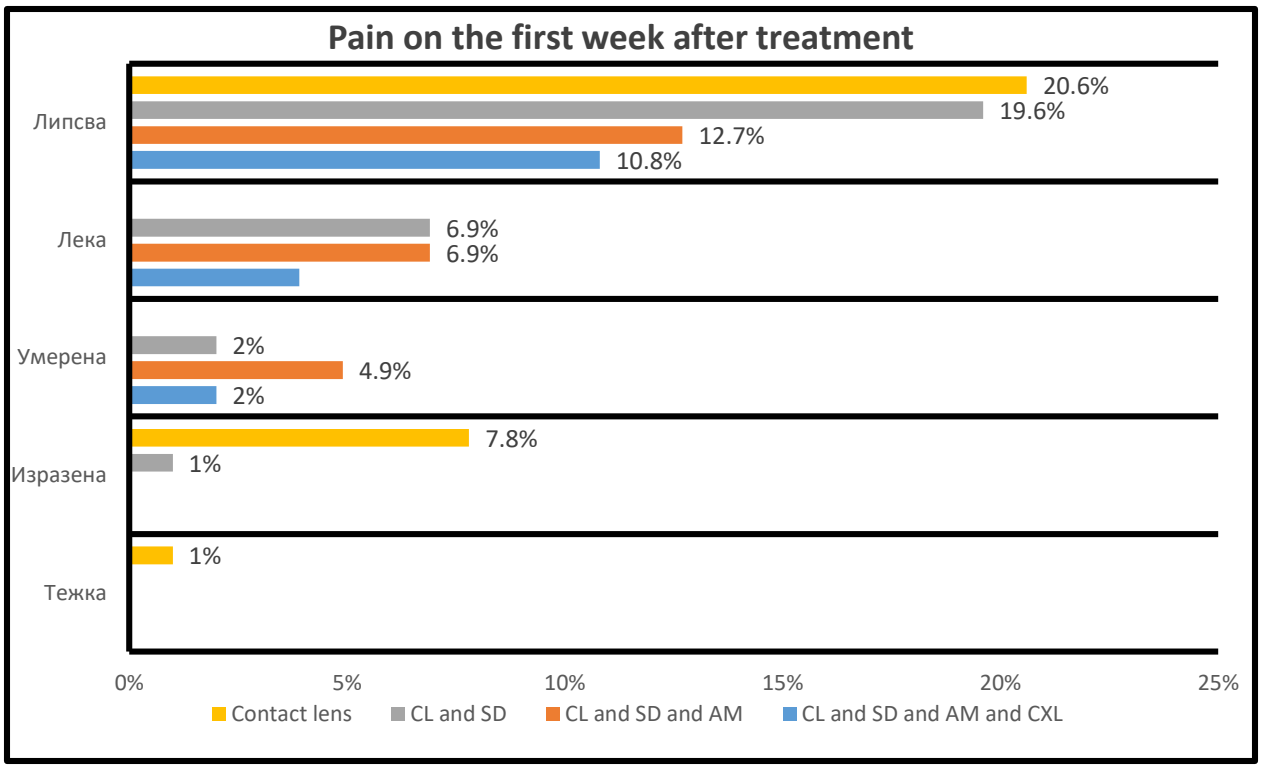


Figure 27. Pain symptoms 1 week after treatment depending on the therapy.

Foreign body sensation is another subjective symptom that we monitored. Fig. 29 shows that again, already at week 1, the symptomatology disappears in a large part of the patients from the four groups, with only 1% persisting with a severe foreign body sensation.

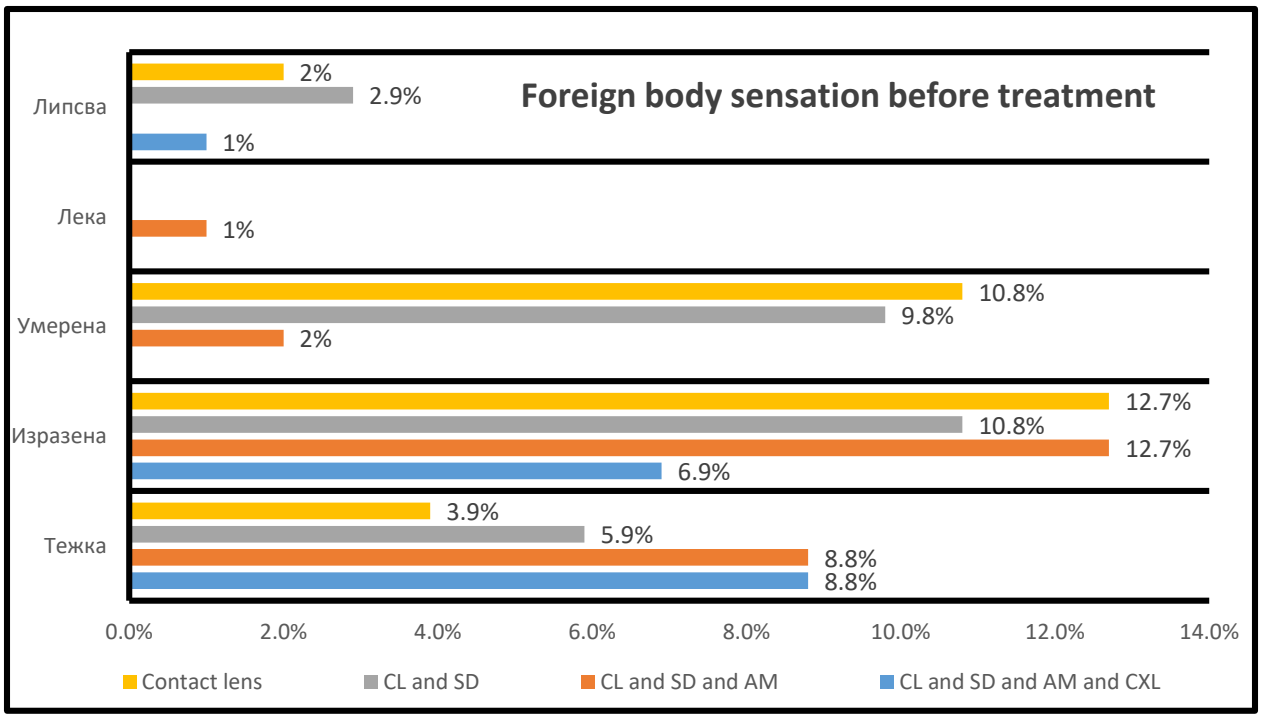


Figure 28. Subjective foreign body sensation according to the type of therapy administered before starting treatment.

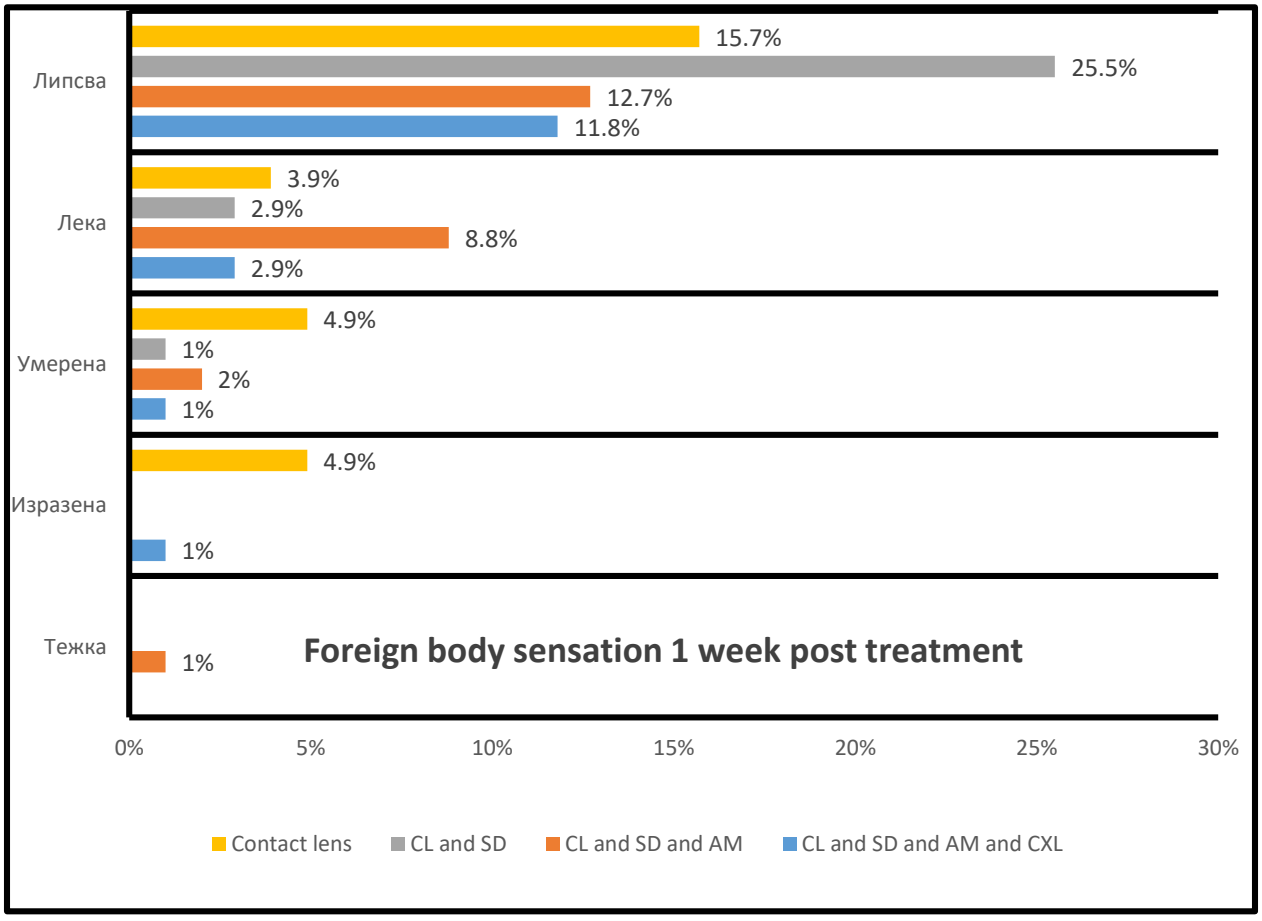


Figure 29. Subjective foreign body sensation according to the type of therapy administered in the first week of treatment.

The size of the epithelial defect is the main objective sign that we monitored in the patients included in this dissertation. At week 1, we found the absence of a defect in a significant proportion of patients from the four groups, and in others we observed a decrease in the size of the defect**.**

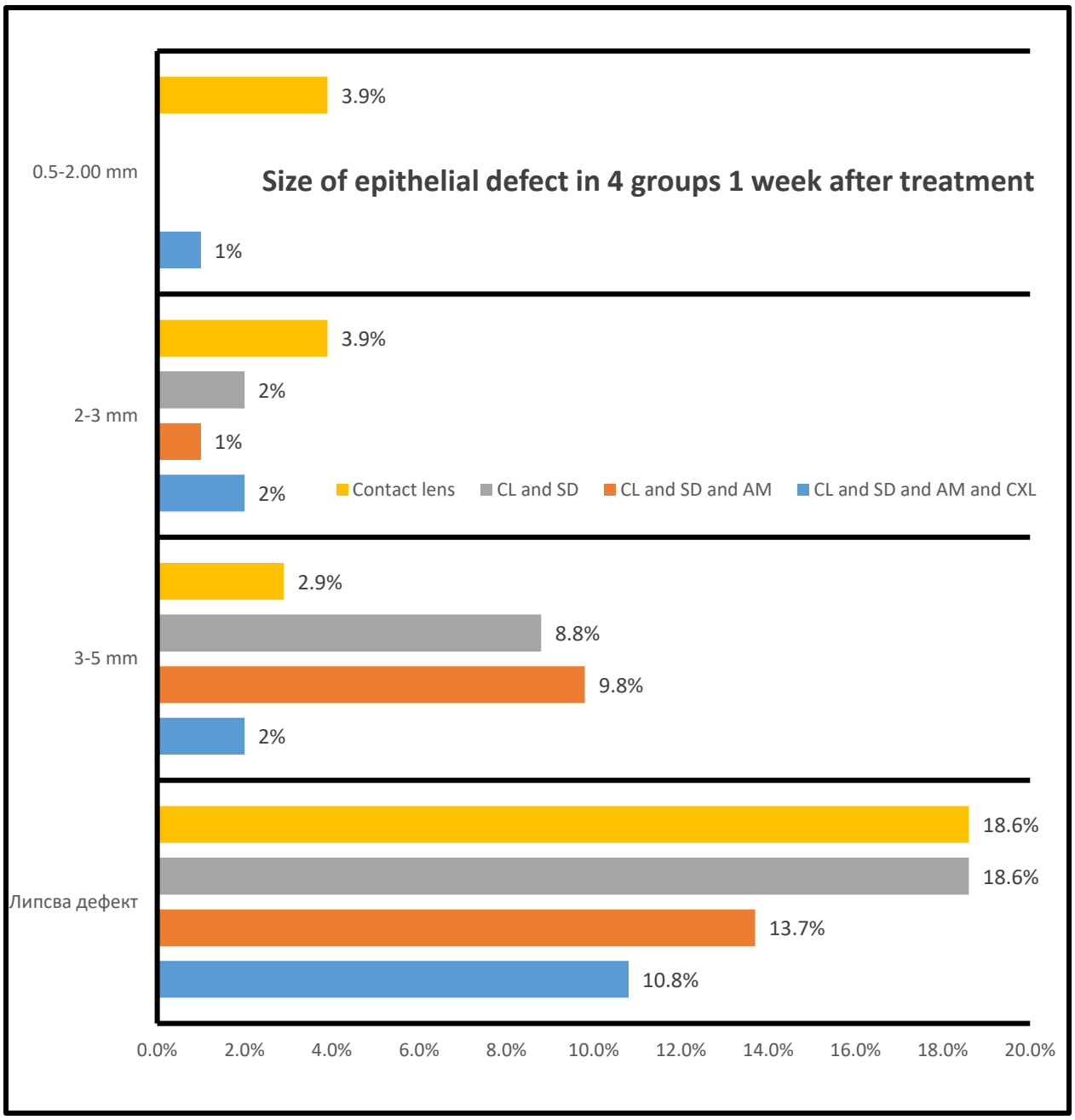


Figure 30. Size of the epithelial defect in four groups according to the type of therapy 1 week after the start of treatment.

The results of the study show a positive impact on all indicators.

4.4 Evaluation of data, obtained from the conducted highly specialized tests

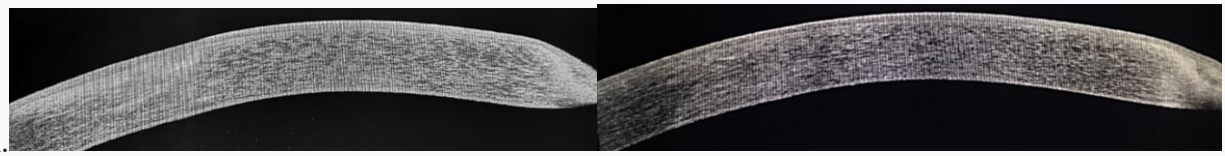
The data from the conducted study are presented on Table 1.

Table 1. Etiological characteristics of the patients included in the study by etiology and findings observed on AS-OCT scan.

Etiology	Number of patients	Findings, observed during anterior segment optical coherence tomography
Postherpetic persistent epithelial defects	8	Lenticular/spindle-shaped hyperreflective area in the stroma, which may be diffuse or localized
Trauma and burns **Burns** **Trauma**	23	Epithelial bullae and corneal opacity, Descemetocele, anterior chamber inflammation, with hyper- and hyporeflective stromal cysts, expansive edema. A disorder in the uppermost hyperreflective layer, extending deep into the stroma. The infiltrate is a hyperreflective area with ill-defined borders.
Post vitrectomy	1	Disruption in the uppermost hyperreflective layer.
Dry eye syndrome	10	Disruption in the uppermost hyperreflective layer in normal areas.
Foreign body	17	Disruption in the uppermost hyperreflective layer that remains in the superficial zone or continues deep into the stroma.
Bullous keratopathy	10	Increased corneal thickness in some cases, mild anterior stromal opacity and irregularity.
Corneal dystrophy **Map-dot-fingerprint dystrophy** **Salzmann nodular degeneration**	10	 Multilaminar, linear and curvilinear subepithelial hyperreflective lines. Nodules are visualized as an intraepithelial hyperreflective area.
Keratitis **Dry eye keratitis** **Herpetic keratitis**	17	 Intraepithelial hyperreflectivity, not epithelial defect. Epithelial irregularities and defects. During follow-up, the ulcers healed by bringing their edges closer together.

Filamentary keratitis		Intraepithelial hyperreflectivity temporarily replaced the ulcer before it fully healed. Filaments appear as linear, highly hyperreflective deposits on the corneal surface with posterior shadowing.
„Band“ keratopathy	1	Whitish deposits consistent with intraepithelial deposits with strong hyperreflectivity and posterior shadowing.
Entropion	10	Disruption in the uppermost hyperreflective layer of the cornea.
Graft vs Host Disease(GvHD)	4	Assessment of the graft-host interface and lateral connection; disruption in the uppermost hyperreflective layer with or without shadowing.

A.



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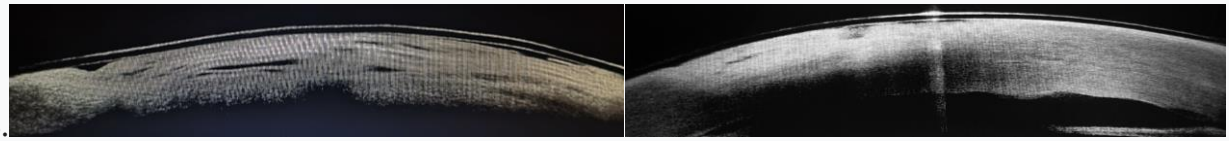

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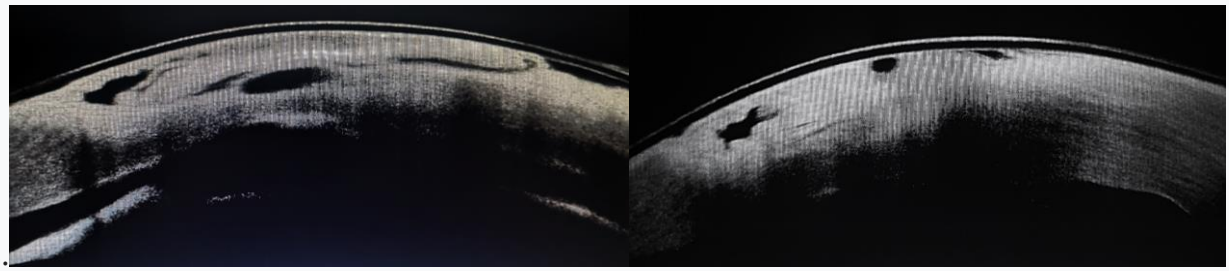


Figure 31. Results from the anterior segment optical coherence tomography of patients with PED before starting treatment and 1 week after the prescribed therapy – (A) Therapeutic contact lens in combination with serum drops; (B) Therapeutic contact lens, serum drops and amniotic membrane; (C) CXL, AMT and ASD

When examining the patients included in the study, we found the following results, presented in Table 2.

Table 2. Characteristics of patients included in the study by etiology and findings observed with in vivo confocal microscopy.

Etiology	Number of patients	Features observed when performing in vivo confocal microscopy
Postherpetic persistent epithelial defects	8	Damaged epithelial cells, partial vacuolar degeneration. No neovascularization or inflammatory cells in the stroma; No damage to endothelial cells
Trauma and burns **Burns** **Trauma**	23	 Irregular features with hyperreflective cytoplasm and hyporeflective nuclei in the epithelium; Small hyperreflective inflammatory cells beneath the superficial epithelium; stromal cell morphology difficult to distinguish. Disorder in the uppermost hyperreflective layer extending deep into the stroma Round, hyperreflective nuclei of epithelial cells; Inflammation in basal cells and Bowman's membrane; Activated keratocytes and hyperreflective extracellular matrix.
Post vitrectomy	1	Reduced cell density, polymegatism and pleomorphism; droplets adherent to the endothelium. Hyperreflective deposits of various shapes in the stroma.
Dry eye syndrome	10	Areas of partial loss of surface epithelial cells. Inflammatory cells - round hyperreflective bodies with a mean conjunctival inflammatory cell density of 421 ± 436 cells/mm2.
Foreign body	17	The basal corneal epithelium appears normal, there is no subbasal corneal nerve plexus, as well as stromal nerves;
Bullous keratopathy	10	Stroma - highly reflective keratocyte nuclei and disorganized architecture. Highly reflective material is observed around the keratocyte nuclei.
Corneal dystrophy **Map-dot-fingerprint dystrophy**	10	Deposits in basal epithelial cells, subbasal microfolds and streaks, damaged subbasal nerves/altered morphology of the anterior stroma. Variously shaped (linear, curvilinear, annular, geographic) highly reflective deposits in the basal epithelial cell layer; In lesions - multiple linear and curvilinear hyporeflective lines;

Salzmann nodular degeneration		Irregular network of highly reflective structures (keratocytes); Unstructured areas of increased reflectivity associated with irregularly arranged collagen fibers and hyaline deposits; Epithelial cells appear atypically shaped and elongated.
Keratitis	17	Infiltrate - hyperreflective area with ill-defined borders.
Dry eye keratitis		Mean density of epithelial cells in the central cornea is lower; Peripheral epithelium is thinned; Subbasal nerves - reduced density and irregular branching patterns.
Herpetic keratitis		Reduced mean density of subbasal nerve plexus (1.7±0.8 mm/mm2); reduced mean density of endothelial cells (2577±576 cells/mm2); hyperreflective desquamating surface epithelial cells are present
Filamentary keratitis		Ulcers are observed, overlapping fungal strands in the center of the ulcer; cysts in chains and groups.
„Band“ keratopathy	1	Hyperreflective areas in basal epithelial cells; Deposits and stromal microdots.
Entropion	10	Multiple hypo- and hyperreflective spherical lesions in the anterior corneal stroma and Bowman's layer measuring 40 ± 230 μm.
Graft vs Host Disease(GvHD)	4	Reduced surface epithelial cell density – 102 ± 606 cells/mm2; reduced pterygoid cell density 983.2 ± 4879.3 cells/mm2; Visible networks of activated keratocytes are observed in the anterior stroma.

Table 3 displays the thickness of the limbal epithelium in four quadrants according to etiology. Results show that a significant difference in the studied quadrants was observed in trauma, keratitis, keratopathies, entropion and foreign bodies. The smallest thickness of the limbal epithelium was measured in patients with corneal dystrophies, and the largest - in patients with dry eye.

Table 3. Analysis of limbal epithelial thickness in four quadrants according to the etiology of persistent epithelial defects. Data from in vivo confocal microscopy.

Etiology	Upper quadrant	Lower quadrant	Nasal quadrant	Temporal quadrant
Postherpetic PED (n=8)	65,7 ± 7,2	60,5 ± 7,2	53,4 ± 7,8	56,9 ± 7,8
Trauma and burns (n=23)	55,3 ± 6,5	58,1 ± 9,7	50,2 ± 6,8	51,7 ± 7,2
Trauma				
Burns	52,6 ± 6,3	52,8 ± 7,2	46,7 ± 8,1	47,9 ± 7,5
Post vitectomy (n=1)	57,3	56,6	51,9	54,5
Dry eye syndrome (n=10)	65,8 ± 5,5	65,2 ± 5,5	58,5 ± 4,8	58,0 ± 4,4
Foreign body (n=17)	61,8 ± 5,8	63,3 ± 7,0	54,5 ± 3,0	55,2 ± 3,5
Bullous keratopathy (n=10)	49,5 ± 4,3	48,3 ± 4,5	46,7 ± 4,8	46,5 ± 4,2
Corneal dystrophies (n=10)	56,00 ± 2,2	43,00 ± 3,1	53,00 ± 1,5	42,00 ± 2,3
Keratitis (n=17)	57,3 ± 5,5	55,0 ± 6,7	50,9 ± 3,9	52,3 ± 4,5
Band keratopathy (n=1)	49,2	48,3	47,7	45,5
Entropion (n=10)	63,9 ± 2,9	57,1 ± 2,8	49,5 ± 3,3	49,6 ± 3,8
GVDH (n=4)	58,5 ± 9,0	61,8 ± 8,7	56,5 ± 9,2	57,8 ± 8,6

Table 4 presents the average density of the long nerves and subbasal nerve plexus according to the etiology of PED. The results show that the lowest density of long nerves is in patients with bullous keratopathy, keratitis and GVHD. The lowest density of subbasal nerve plexus was recorded among patients with foreign body, bullous keratopathy and GVHD. The highest density of both indicators was recorded among patients with corneal dystrophies.

Table 4. Density of long nerves and subbasal nerve plexus according to etiology. Data from in vivo confocal microscopy.

Etiology	Average density of the long nerves	Average density of subbasal nerve plexus
Postherpetic PED (n=8)	11,79 ± 9,87	35,60 ± 17,80
Trauma and burns (n=23) Trauma Burns	 11,73 ± 9,54 7,96 ± 9,33	 40,21 ± 19,78 32,98 ± 20,32
Post vitectomy (n=1)	6,57	39,87
Dry eye syndrome (n=10)	14,70 ± 12,56	50,13 ± 19,78
Foreign body (n=17)	61,8 ± 5,8	28,54 ± 16,98
Bullous keratopathy (n=10)	3,56 ± 4,21	21,90 ± 10,01
Corneal dystrophies (n=10)	19,10 ± 10,32	55,12 ± 12,32
Keratitis (n=17)	3,45 ± 7,45	28,67 ± 18,97
Band keratopathy (n=1)	4,78	28,17
Entropion (n=10)	13,01 ± 5,95	29,56 ± 22,73
GVDH (n=4)	3,03 ± 1,11	26,45 ± 2,35

V. DISCUSSION

Persistent epithelial defects (PED) are corneal epithelial defects that fail to heal within 2 weeks. This is a condition that carries a high morbidity and is becoming increasingly common, so more efforts are needed to establish reliable treatment methods (5). Despite the availability of multiple therapies, the treatment of AOS diseases can be a great challenge and the outcome - unsatisfactory. The aim of this dissertation is to evaluate the effectiveness of different types of therapeutic approaches in patients with impaired anterior ocular surface, to pay attention to the correct selection when choosing a therapeutic approach and to propose a simplified chart for taking anamnesis of patients with PED, both for subjective and objective signs. The set goal is the main driving force in the tasks set, the results of which prove the need to conduct the current study and orient us in the right direction for the future.

The literature review demonstrates that slit-lamp biomicroscopy is an indispensable tool in the ophthalmological office as an in vivo examination of the cornea. In combination with the use of various dyes, such as fluorescein and lissamine green, it can help assess the overall health and integrity of the corneal epithelium. However, its main disadvantage is the low magnification, only up to 40x or 100x, which does not allow visualization and study of the cellular architectonics of the cornea and the subbasal nerve plexus. In the present study, biomicroscopy was performed on all studied patients using a biomicroscope (Carl Zeiss, Meditec AG) to assess the anterior ocular surface and its structures, visualize the defect, and measure its size, depth, and location. With the growing interest in noninvasive, high-resolution techniques for studying the physiology of corneal tissue, the invention and application of IVCM has enabled ophthalmologists to study the cornea at the cellular level. As a rapid and noninvasive technique, IVCM has many advantages and can provide images almost comparable to in vitro histochemical methods. Over the years, IVCM has been established as a useful tool for assessing the cellular architectonics and morphology of nerve fibers, both in healthy individuals and in a number of ocular diseases, such as dry eye syndrome, neurotrophic keratopathy, keratitis, etc. We used HRTII-RCM to study our patients with PED and found the data presented in Tables 2, 3 and 4. High-resolution OCT is a useful tool for visualizing corneal structures. AS-OCT allows morphological differentiation of different dystrophies, improved assessment of structural changes and helps to assess the depth of stromal infiltration,

corneal thickness and many ulcer characteristics. A Fourier-domain optical coherence tomography (Cirrus HD-OCT 5000, Carl Zeiss Meditec, Inc.) was used to perform AS-OCT in the study, and the results are presented in Table 1.

The use of autologous serum eye drops as a treatment for severe ocular surface disorders is increasingly common in clinical practice (6). Serum drops can be manufactured without preservatives, are non-allergenic, and their biomechanical and biochemical properties are similar to normal tears (6). However, the protocols for the preparation and use of autologous serum drops vary considerably between studies. Since this may result in different biochemical properties, variations in the protocol may also affect the epitheliotropic effect of the product. Biological therapy - serum drops is a step towards personalized ophthalmology and an alternative approach to the treatment of patients suffering from AOS diseases. Serum drops restore balance, stimulate re-epithelialization and support the health and homeostasis of the anterior ocular surface. Other blood products such as: allogeneic serum, umbilical cord blood serum (UCB), and platelet-rich plasma (PRP) can also be used in the treatment of patients with PED who do not respond to conventional therapy. The results published in the scientific literature are encouraging (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17). However, the emergence of legal problems in the preparation of these products significantly limits their use, both in our country and worldwide. In recent years, a new strategy for the treatment of PED has been reported in the scientific literature: a combination of TCL and ASD. A number of scientific publications prove that the combination of the epithelotrophic effect of ASD and the mechanical protective effect of TCL leads to a positive synergistic effect on the process of corneal epithelium restoration. In cases where conservative treatment methods (intensive moisturizing therapy, occlusion of the lacrimal puncta, placement of TCL and ASD) of AOS diseases, in particular PED, are ineffective, transplantation of AM application is a rapid and effective method that promotes the epithelialization process. In the treatment of AOS diseases, AM can be applied in three different forms. In cases of pure corneal epithelial defects, AM is placed in the form of a “patch” and acts as a biological CL (18). It is usually sutured to the ocular surface with 10-0 nylon, and a TCL is placed on top of it (18). In cases of corneal ulceration, a “graft” technique is used, in which one of several layers of AM is placed in the ulcer and the most superficial layer is sutured (18). In severe AOS defects, the two

approaches are combined, this is the so-called “sandwich” technique (18). AMEED has been used to date in the treatment of patients with “dry syndrome”, PED, chemical burns, partial LSCD, cicatricial diseases of the ocular surface, bullous keratopathy and corneal ulcer with reported positive results (19) (20) (21). They have been found to have the same properties as cryopreserved AM, with a high concentration of trophic factors. There are no studies in the scientific literature comparing AMEED and serum drops. To date, there is no standardized protocol for their production. The antimicrobial effect of photoactivation of riboflavin has been investigated for potential use in infectious diseases in ophthalmology (22). The use of PACK-CXL may have exceptional benefits in the treatment of patients with infectious keratitis, even without the need for AB administration (23) (24). As resistance to AB has increased in recent years, new treatment methods will be needed and in this regard PACK-CXL is a reliable alternative for patients in the future.

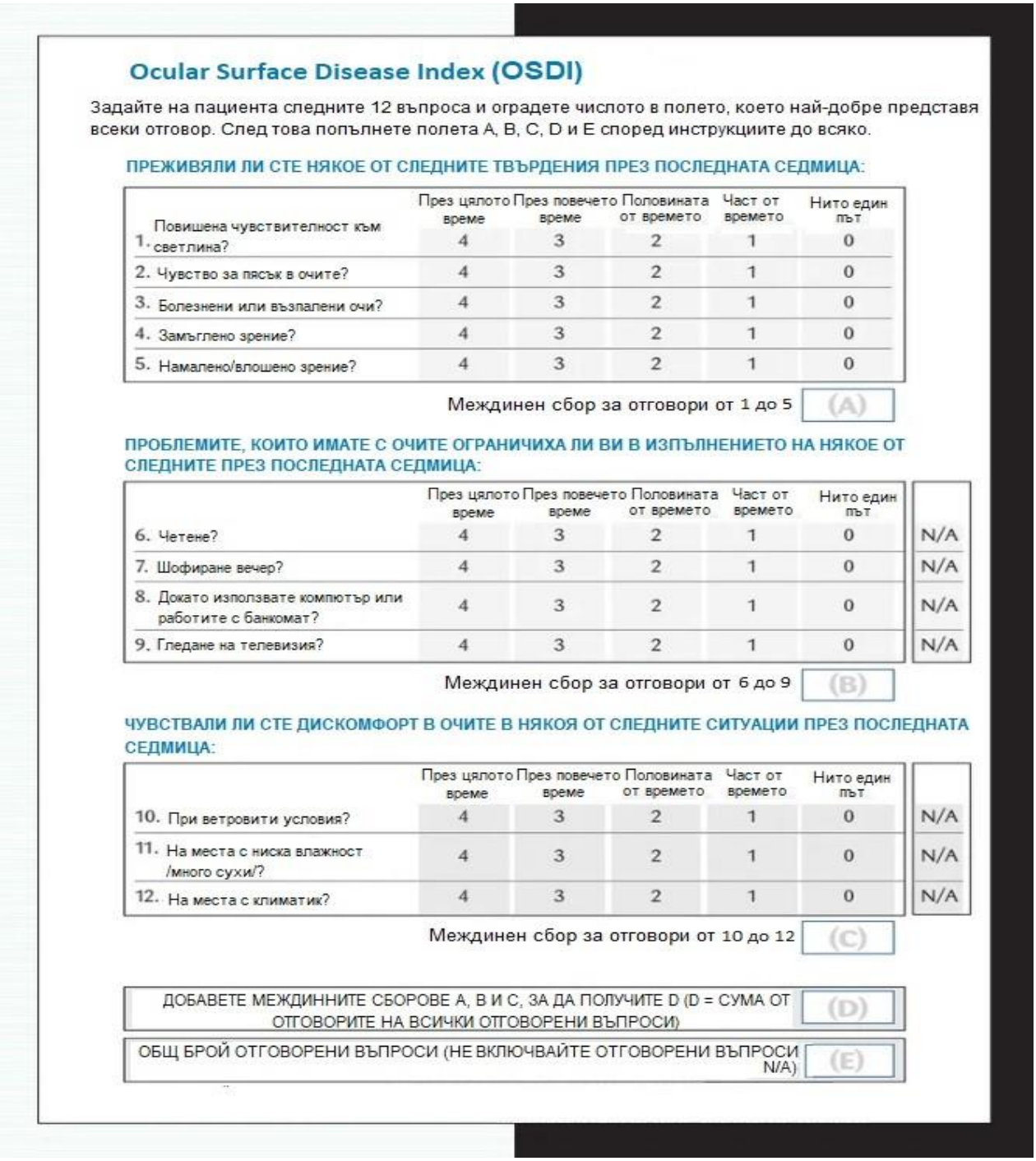
Literature data displays differences in the etiology of PED, possibly due to geographical features, access to health care, gender and countries in the developed or developing world. In terms of the underlying etiology, the leading cause of PED among patients who underwent treatment in USBOBAL-Varna are trauma and burns, followed by inflammatory diseases of the cornea and foreign body. The proportion of patients with bullous keratopathy, corneal dystrophy and postherpetic neuropathy is smaller. We found that in males the leading cause of PED is the presence of a foreign body in the cornea and keratitis, followed by trauma and burns. In females the leading etiological factor is trauma and burns, followed by corneal dystrophies. In our sample, 58.8% of the patients underwent conservative treatment, and the remaining 41.2% underwent surgical intervention. The therapeutic approach in all patients was to eliminate the risk factors that led to the development of PED, reduce the mechanical stress caused by blinking movements by placing TCL and optimize the tear film with the help of preservative-free moisturizing drops. In the group undergoing invasive treatment, AM transplantation was performed in 24.5% of patients, after which TCL was placed and ASD therapy was prescribed and PACK-CXL was initially performed in 16.7% of patients. Referring to the data from the scientific literature in the present clinical study, we decided to use 20% ASD distributed in monodoses of 1ml in 3 out of 4 groups of patients observed by us. Of the patients, 14.7% had PED with a size between 3-5 mm before

starting therapy. At week 1, in 8.8% of them, we observed complete recovery of the epithelial defect, and at month 1, the results are even more impressive - in 14.7%, the absence of an epithelial defect was found. Already at week 1 after starting treatment, 61.80% of all four groups of patients had no epithelial defect. The results were even more impressive at month 1, as in 84.30% we observed complete recovery of corneal integrity. The obtained results support the data from the conducted studies available in the literature. One of the objective indicators that we monitored in our sample of patients with PED is the degree of injection. Before starting treatment, patients with ciliary injection in 2-3 quadrants (51.0%) predominated, followed by those with injection along the entire circumference (33.3%). We found a significant improvement in this objective indicator already at week 1, as in 36.3% of patients, injection was absent. The increase in the number of patients with conjunctival injection is impressive - 35.3%, which is due to the presence of a conjunctival suture in those in whom AM was placed. Thirty days after the prescribed therapy, 81.4% of our patients lacked an injection. Visual acuity is another objective indicator that we studied and monitored in this dissertation. The results show a significant improvement in its values in all groups of patients. The best results were reported in patients with a foreign body in the cornea, with inflammatory diseases (keratitis), including those with postherpetic keratopathy. We observed a weaker response in patients with GVHD and those with bullous keratopathy. We also reported good results in patients with trauma and burns. Most of the patients included in this dissertation had moderate degree of burn, which explains the good results observed in this group. The results clearly demonstrate the positive effect of the applied treatment (conservative and operative/invasive) on the proper recovery of the AOS, as well as that the personalized approach and the individual therapeutic plan play a key role in the successful response to both subjective symptoms and objective signs.

A large part of patients with AOS diseases have a deteriorated quality of life. Pain, discomfort, the sensation of a foreign body and sand are symptoms that limit daily life and reduce the working capacity of patients with PED, which leads to significant socio-economic losses (25). Sometimes the symptoms are strongly expressed, do not respond to the applied conventional therapy and significantly impair the quality of life of patients (26). There is a lot of data in the scientific literature proving the positive effect of ASD on the epithelialization process, but unfortunately

there is an absence of data on the way in which the application of ASD affects subjective symptoms in patients with PED. In this dissertation, we monitored and evaluated the way in which subjective symptoms (pain, redness and foreign body sensation) are affected in patients with PED, undergoing different treatment regimens. In 100% of the patients included in this dissertation, a TCL was placed, which led to a significant reduction in pain syndrome and improved comfort. Regarding pain symptoms, in our sample of patients, we found a significant reduction in pain already in the first week of the prescribed treatment, with 63.70% of the observed patients having no pain, and 17.60% having mild pain. In the first month after starting therapy, the results are even more impressive, with 88.0% of patients experiencing no pain, only 3.0% experiencing severe symptoms, and only 1.0% experiencing severe symptoms. Of the patients we observed, 92.2% experienced pain before starting the respective therapy. The placement of TCL significantly reduces pain and improves ocular comfort. There is an abundance of data in the scientific literature supporting our results. No complications related to wearing CL were found in the patients we observed. Redness is another subjective indicator that, from a cosmetic point of view, worries patients and their relatives. Before starting therapy, 42.20% of the patients we observed had pronounced redness and 17.60% had severe redness. We found significant improvement already in the first week, with 50.0% of the patients having none and 35.30% having mild redness. Only in 2.0% of the observed patients did we find severe redness. The results are even better 1 month after the therapy, with 83.30% of the patients having no redness and mild redness found in 6.90%. Despite the applied therapy, in 8.80% of the patients we reported moderate redness and in 1.0% - severe. Foreign body sensation is the third subjective symptom that we followed among our patients with PED. Before starting treatment, we found a notable foreign body sensation in 43.1% of them, and severe - in 27.5%. This subjective symptom significantly disrupts ocular comfort, stimulates the patient to rub their eyelids, which in turn causes additional mechanical stress (trauma) on the compromised AOS. This leads to a deepening of the problem and deterioration of the processes of epithelial defect repair. We found a significant reduction in foreign body sensation during the 1st week, with 65.70% of patients experiencing no foreign body sensation and 18.0% experiencing mild symptoms. Only 1.0% of the patients we observed had persistent severe foreign body sensation.

Based on the study, the results show that despite the existence of different therapeutic approaches, treatment requires addressing both the causal primary condition and the underlying subjective symptoms. The complex cycle of a thorough examination, etiological diagnosis, treatment, and follow-up involves a number of medical structures, offices, equipment, units, and personnel. In order to optimize the therapeutic approach, we created a scorecard for assessing the subjective and objective signs of patients with persistent epithelial defects.



Ocular Surface Disease Index (OSDI)

Задайте на пациента следните 12 въпроса и оградете числото в полето, което най-добре представя всеки отговор. След това попълнете полета A, B, C, D и E според инструкциите до всяко.

ПРЕЖИВЯЛИ ЛИ СТЕ НЯКОЕ ОТ СЛЕДНИТЕ ТВЪРДЕНИЯ ПРЕЗ ПОСЛЕДНАТА СЕДМИЦА:

	През цялото време	През повечето време	Половината от времето	Част от времето	Нито един път
1. Повишена чувствителност към светлина?	4	3	2	1	0
2. Чувство за пясък в очите?	4	3	2	1	0
3. Болезнени или възпалени очи?	4	3	2	1	0
4. Замъглено зрение?	4	3	2	1	0
5. Намалено/влошено зрение?	4	3	2	1	0

Междинен сбор за отговори от 1 до 5 (A)

ПРОБЛЕМИТЕ, КОИТО ИМАТЕ С ОЧИТЕ ОГРАНИЧИХА ЛИ ВИ В ИЗПЪЛНЕНИЕТО НА НЯКОЕ ОТ СЛЕДНИТЕ ПРЕЗ ПОСЛЕДНАТА СЕДМИЦА:

	През цялото време	През повечето време	Половината от времето	Част от времето	Нито един път	
6. Четене?	4	3	2	1	0	N/A
7. Шофиране вечер?	4	3	2	1	0	N/A
8. Докато използвате компютър или работите с банкомат?	4	3	2	1	0	N/A
9. Гледане на телевизия?	4	3	2	1	0	N/A

Междинен сбор за отговори от 6 до 9 (B)

ЧУВСТВАЛИ ЛИ СТЕ ДИСКОМФОРТ В ОЧИТЕ В НЯКОЯ ОТ СЛЕДНИТЕ СИТУАЦИИ ПРЕЗ ПОСЛЕДНАТА СЕДМИЦА:

	През цялото време	През повечето време	Половината от времето	Част от времето	Нито един път	
10. При ветровити условия?	4	3	2	1	0	N/A
11. На места с ниска влажност /много сухи/?	4	3	2	1	0	N/A
12. На места с климатик?	4	3	2	1	0	N/A

Междинен сбор за отговори от 10 до 12 (C)

ДОБАВЕТЕ МЕЖДИННИТЕ СБОРОВЕ A, B И C, ЗА ДА ПОЛУЧИТЕ D (D = СУМА ОТ ОТГОВОРИТЕ НА ВСИЧКИ ОТГОВОРЕНИ ВЪПРОСИ) (D)

ОБЩ БРОЙ ОТГОВОРЕНИ ВЪПРОСИ (НЕ ВКЛЮЧВАЙТЕ ОТГОВОРЕНИ ВЪПРОСИ N/A) (E)

КАРТА НА ПАЦИЕНТА

1. Име, презиме, фамилия		
2. Пол		
3. Местоживеене		
4. Възраст		
5. Кратка анамнеза		
6. Диагноза		
7. Проведено лечение		
8. Зрителна острота		
- Преди започване на лечение	VOD=	VOS=
- 1 седмица след започване на лечението	VOD=	VOS=
- 2 седмици след начало на лечението	VOD=	VOS=
- 30 дни след начало на лечението	VOD=	VOS=

9. Болка		Липсва	Лека	Умерена	Тежка
- Преди започване на лечение	ДО- ЛО-				
- 1 седмица след започване на лечението	ДО- ЛО-				
- 2 седмици след начало на лечението	ДО- ЛО-				
- 30 дни след начало на лечението	ДО- ЛО-				

10. Зачервяване		Липсва	Леко	Умерено	Тежко
- Преди започване на лечение	ДО- ЛО-				
- 1 седмица след започване на лечението	ДО- ЛО-				
- 2 седмици след начало на лечението	ДО- ЛО-				
- 30 дни след начало на лечението	ДО- ЛО-				

11. Усещане за чуждо тяло		Липсва	Леко	Умерено	Тежко
- Преди започване на лечение	ДО- ЛО-				
- 1 седмица след започване на лечението	ДО- ЛО-				
- 2 седмици след начало на лечението	ДО- ЛО-				
- 30 дни след начало на лечението	ДО- ЛО-				

БЕЛЕЖКИ

12. Инекция		Липсва	Лека	Умерена	Тежка
- Преди започване на лечение	ДО- ЛО-				
- 1 седмица след започване на лечението	ДО- ЛО-				
- 2 седмици след начало на лечението	ДО- ЛО-				
- 30 дни след начало на лечението	ДО- ЛО-				
13. Хиперемия		Липсва	Лека	Умерена	Тежка
- Преди започване на лечение	ДО- ЛО-				
- 1 седмица след започване на лечението	ДО- ЛО-				
- 2 седмици след начало на лечението	ДО- ЛО-				
- 30 дни след начало на лечението	ДО- ЛО-				
14. Размер на епителния дефект		Липсва дефект	0.5-2.00мм	2.00-3.00мм	3.00-5.00мм
- Преди започване на лечение	ДО- ЛО-				
- 1 седмица след започване на лечението	ДО- ЛО-				
- 2 седмици след начало на лечението	ДО- ЛО-				
- 30 дни след начало на лечението	ДО- ЛО-				
15. Биомикроскопия					
- Преди започване на лечение					
- 1 седмица след започване на лечението					
- 2 седмици след начало на лечението					
- 30 дни след начало на лечението					
16. Предно-сегментна оптична кохерентна томография					
- Преди започване на лечение					
- 1 седмица след започване на лечението					
- 2 седмици след начало на лечението					
- 30 дни след начало на лечението					
17. Ин виво конфокална биомикроскопия					
- Преди започване на лечение					
- 1 седмица след започване на лечението					
- 2 седмици след начало на лечението					
- 30 дни след начало на лечението					

Among the new directions in therapeutic approaches for the treatment of PED is the transplantation of cultured epithelial cells. The most common technique, called cultured limbal epithelial transplantation (CLET), involves the expansion of epithelial cells from limbal explants onto human amniotic membrane (27). The results after performing CLET are extremely good, with patients showing significant improvement in the condition of their corneas within the next 1–2 years (27). Another method with promising results is cultured oral mucosal epithelial transplantation (COMET) (28). Oral mucosal cells are suitable because they are relatively undifferentiated and do not keratinize rapidly, making them relatively easy to culture (28). In addition, they express differentiation markers such as the protein K3, which is an indicator of corneal epithelial differentiation (28). In clinical studies involving patients with LSCD resulting from chemical burns, COMET has been successful in 75.0% of cases (28). The principles of multipotent stem cell differentiation can be used to treat patients with PED (19). Regenerative therapy involves mesenchymal stem cells (MSCs), often derived from bone marrow. Umbilical cord stem cells are another alternative for the treatment of PED (28). Another therapeutic option to promote repair in severe, refractory PED is an artificial lattice created using tissue engineering techniques that can be placed directly into the defect itself. A group of researchers have created a stromal corneal equivalent using (SMILE)-derived lenticules in combination with fibrin glue. Given that SMILE is becoming increasingly popular and attractive for the treatment of myopia in our time, it is expected that lenticules will become increasingly available. It remains unclear whether this strategy will be effective in recipients with significant corneal inflammation, rather than just mechanical protection of epithelial and stromal defects. ECM proteoglycans play an important role in the process of restoring corneal integrity. In PED and corneal trauma, their level is abnormal and this leads to a delay in re-epithelialization. This has led to the idea of including them in PED therapy, with the aim of improving ECT and promoting cell migration and adhesion. Vitronectin (VN) is one of the main cell adhesion-promoting proteins present in ECT (29). It has been found that the application of VN to mechanically or chemically injured rabbit eyes accelerates epithelialization (29). The scientific database shows encouraging studies and a number of clinical trials working towards optimizing the therapeutic approach and ensuring a harmless, safe and effective healing process for patients.

The advancement of technology and the development of AI systems are also observed in the fields of ophthalmology, in particular, we consider their application in the field of pathological conditions characterized by persistent epithelial defects. Compared with images obtained from fundus cameras and optical coherence tomography, which have been popular choices in artificial intelligence (AI) research over the past decade, the less unified modality of biomicroscope images limits its application in AI models (30). In a study by Weinjia and colleagues, EyeHealer was created as a large-scale dataset of ocular lesion segmentation (31). The study identified the need for further development in the field of neural networks, the application of blue-filter image training, and a narrower field of view of the biomicroscope, but it also opens up the possibility of developing mobile applications for diagnostics, recommendations, and treatment suggestions (31). According to the diverse applications of AI in diagnosing Ophthalmic Ophthalmic Diseases, AI has shown significant advantages through data and image analysis (31). However, many studies on the application of AI to the diagnosis of ocular surface diseases and their satisfactory results still have many limitations and challenges (31). AI can classify images into different types according to the characteristics of the disease, such as classification and stage. In addition, AI can also detect and segment the anatomical structure in the image, such as the shape of the lesion, to realize the automatic quantization of image biomarkers and perform auxiliary diagnosis. Therefore, based on these advantages, the application of AI technology in clinical diagnosis and treatment offers endless potential and significant prospects. With the continuous progress of science and technology, the ongoing improvements in AI and the establishment and improvement of relevant legal systems, AI will be better applied to clinical diagnosis and treatment in ophthalmology, especially in economically disadvantaged areas and those lacking medical resources in the near future. In addition, if the clinical diagnosis and treatment course can be fully established through AI, the work stress of clinical medical staff will be greatly reduced and their work efficiency will be improved, allowing them to perform the most adequate and complete diagnosis and propose the best treatment plan for patients. The full implementation of AI will bring fundamental changes to clinical ophthalmic diagnosis and treatment.

CONCLUSIONS

1. Eye diseases leading to persistent epithelial defects require precise diagnosis and selection of an appropriate, individual therapeutic approach. Modern medicine offers various alternatives in this area.

2. In the current literature, there is limited data on studies investigating visual acuity in patients with persistent epithelial defects and studies assessing subjective symptoms in patients with PED are lacking.

3. The literature analysis performed shows a lack of uniformity in protocols for the preparation and use of autologous serum drops. Before the definitive role of serum drops in the treatment of anterior ocular surface pathologies can be established in a large randomized controlled trial, this must be evaluated in more detail.

4. Worldwide, there is no data on a uniform scorecard for taking anamnesis of subjective and objective symptoms of patients suffering from diseases associated with persistent epithelial defects.

5. In the conducted study and the assessment of the etiology of diseases, characterized by persistent epithelial defects, we found that the highest frequency is in patients with eye pathologies such as conditions after mechanical or chemical trauma, followed by those with foreign bodies and keratitis.

6. The analysis of corneal structures, through highly specialized examinations, proved that the results of our study do not differ significantly from those of other authors, as the findings when performing anterior segment optical coherence tomography and in vivo confocal microscopy before starting therapy are identical.

7. The analysis of the therapeutic approaches performed showed that the individual approach to the treatment of patients with PED, with prominent subjective and clinical symptoms, leads to a significantly faster recovery time and positive clinical results. The correct treatment method shows a lack of relapses and complete epithelialization in our patients.

8. The assessment of subjective symptoms in patients with persistent epithelial defects showed that the greatest impact on visual function and quality of life is exerted by pain, followed by the sensation of a foreign body, which after the treatment with an individual approach, significantly decreased, with the symptoms remaining in only 1% of the participants.

9. The results of the study display a significant improvement in visual acuity in all groups of patients, with the best results being reported in patients with a foreign body in the cornea. A large part of the patients included in this dissertation had a moderate degree of burn, which also explains the good results observed in this group

10. The data from the treatment in terms of etiology confirm the efficacy of crosslinking in patients with corneal infiltrates due to its bactericidal effect.

SUMMARY

Anterior ocular surface disorders can negatively affect the quality of life by causing pain, irritation, redness and reduced vision. Several conditions are common causes of persistent epithelial defects, including dry eye syndrome, trauma and burns, Sjögren's syndrome, keratitis and keratopathies, corneal degenerations and dystrophies, graft-versus-host disease and limbal stem cell deficiency. The type of ocular surface disorder, as well as its severity, determine the treatment regimen. PEDs are diagnosed by their type and the history taken - the inability of epithelial adhesion is responsible for the characteristic findings, including superficial ulceration, non-adherent epithelial border, with or without involvement of the stroma and underlying structures. The findings characteristic of PED can be established by observation under a biomicroscope or highly specialized test – IVCM for detailed cellular study of morphological changes and AS-OCT for useful, fast and non-contact visualization of corneal defects and monitoring of the response to treatment. The rapid development of artificial intelligence, concepts for deep machine learning, portable devices for quantitative analysis leads to new promising methods for implementation in personalized ophthalmology. Treatment options may include artificial tears, lubricating gels, vitamins, autologous serum drops, therapeutic contact lenses and various surgical methods. The use of autologous serum drops as a method for treating dry eyes and anterior ocular surface disorders is widespread in practice, due to their content of growth factors that promote epithelial healing and their unique composition and antibacterial and anti-inflammatory properties. The duration of use is no more than a few weeks due to logistical reasons and their preparation and dosing require special facilities to maintain sterility and special equipment. They lead to significantly faster improvement of signs and symptoms and do not contain preservatives. Homologous serum and umbilical cord blood serum are alternative treatment options. Amniotic membrane transplantation can be considered as an alternative method for the treatment of persistent epithelial defects and sterile ulcers that are refractory to conventional treatment, as the thick basement membrane and avascular stromal matrix promote epithelial healing. It has been proven to be a safe and effective method for the treatment of PED. In conclusion, the prospects of personalized ophthalmology look promising. Advances in new technologies to improve molecular diagnostics, researches in developing new therapies, fresh

approaches to already known pathologies, AI software for telemedicine and screening and data sharing systems give personalized ophthalmology the potential to revolutionize this medicinal branch as we know it.

CONTRIBUTIONS

Contributions of a cognitive nature

1. A detailed review of the scientific literature on anatomy, etiology, mechanisms of occurrence, diagnostic and treatment methods and the opportunities that modern medicine provides and develops for patients with persistent epithelial defects has been made.

2. A comprehensive analysis of scientific data on artificial intelligence systems, progress in new technologies, AI software for telemedicine and screening and data sharing systems has been done, which provide numerous opportunities for diagnostic, informative and therapeutic purposes.

Contributions of a scientific and applied nature

1. An analysis of the subjective and objective signs of patients with persistent epithelial defects and the response to the prescribed therapy, who underwent treatment at USBOBAL-Varna, has been made.

2. An analysis of the etiology, subjective symptoms and visual acuity, as well as a microstructural analysis of corneal changes using modern diagnostic methods before and after the treatment, has been performed.

3. Based on the in-depth analysis of the therapeutic approaches carried out, it was found that the individual approach to the treatment of patients with persistent epithelial defects is essential for the restoration of their visual function and improvement of their quality of life.

4. A scorecard was prepared for the assessment of subjective and objective signs of patients with persistent epithelial defects. Its application in the conducted study shortened the time for data collection and provided an opportunity to assess several clinical factors.

Practical contributions

1. For the first time in our country, a detailed analysis and assessment of the etiology, methods of treatment and diagnosis of patients with persistent epithelial defects was made.

2. The advantages of treatment through biological therapy and an individual approach to the patient as a more reliable method for the treatment of patients with persistent epithelial defects than conventional moisturizers were established.

3. In surgical treatment of refractory cases, amniotic membrane transplantation is a cost-effective and readily available alternative to modern ophthalmology for the treatment of patients with persistent epithelial defects.

PUBLICATIONS RELATED TO THE DISSERTATION

1. Nikolova, N., & Grupcheva, C. (2022). Serum drops—the last resort for topical treatment after refractive surgery and laser in situ keratomileusis-induced neuropathic epitheliopathy (LINE). Scripta Scientifica Medica, 54(2), 25-29.

2. Nikolova-Petkova, N., & Hristova, E. (2021). Modern approaches in the biological therapy of the anterior ocular surface. Journal of the Union of Scientists-Varna. Medicine and Ecology Series, 26(1), 10-14.

ABSTRACT

Aim

The aim of this dissertation is to perform a detailed analysis of a wide range of eye diseases characterized by persistent epithelial defects and to assess the clinical effectiveness of the applied therapeutic approaches.

Materials and methods

The present study was conducted at the Department of Eye Diseases and Visual Sciences of the Medical University - Varna on the territory of the University Specialized Hospital for Eye Diseases for Active Treatment USBOBAL - Varna for a period of 4 years - from 01.12.2017 to 01.12.2021. A total of 102 patients treated in hospital and prehospital care were included in the study. The selection of patients included in the study was based on the following defined criteria - patients with chronic recurrent diseases of the anterior ocular surface, aged over 18 years and from Northeastern Bulgaria. Exclusion criteria from the study were patients under 18 years of age, having coagulation status disorders and systemic diseases or in case they did not sign an informed consent form. The study methodology included a documentary method, clinical methods – biomicroscopy (Carl Zeiss, Meditec AG), anterior segment optical coherence tomography (Cirrus HD-OCT 5000, Carl Zeiss Meditec, Inc.) and in vivo confocal biomicroscopy (Heidelberg Retina Tomograph II – Rostock Cornea Module, Heidelberg Engineering GmbH, Germany), determination of best-corrected visual acuity, taking anamnesis and assessing subjective signs of irritation, pain, redness and foreign body sensation. Based on the data from the conducted studies, the etiology and size, depth and localization of the epithelial defect of each of the patients included in the study were determined. Depending on the etiology, size and depth of the epithelial defect, the presence/absence of infiltrate, the patients were divided into four randomized groups. In some of the patients, autologous serum drops were prepared according to a standard operating protocol described by Geerling and associates. The patients were informed in detail about the method of storage and administration of the drops. Surgical treatment was performed in two of the patient groups. This was a transplantation of cryopreserved amniotic membrane fixed on nitrocellulose paper, supplied by the "Center for Translational Medicine and Cell Therapy" at the Medical

University - Varna and corneal cross-linking (PACK-CXL) - a minimally invasive intervention that uses ultraviolet A rays (UVA) and riboflavin (vitamin B2) to slow down or even stop the progression of corneal ectasia and to treat some corneal pathologies. The collected data was processed using the following statistical methods - analysis of variance (ANOVA), variance, correlation and regression analysis. Comparative analysis was used to study cause-and-effect relationships, and the results obtained are presented in graphical and tabular form. In all analyses, an acceptable level of significance of $p<0.05$, $p<0.01$, $p<0.001$ at a confidence interval of 95% was assumed. The data was statistically processed using SPSS v.20, using descriptive indicators for quantitative and qualitative variables and are presented in tabular and graphical form.

Results

Over a period of 4 years (2017-2021), 102 patients with PED were treated at USBOBAL-Varna. The results show that the male gender predominates (62.7%). The average age of patients with PED is 40.5 years. In terms of the underlying etiology, the leading causes of PED among patients are trauma and burns, followed by inflammatory diseases of the cornea and foreign body ingress. When examining patients with PED, the following indicators were used to assess subjective symptoms: pain, redness and foreign body sensation. They were monitored before starting therapy, at the 1st, 2nd week and 30 days after treatment. The results show that 92.2% of patients experience pain before starting treatment. A significant improvement in the pain syndrome is observed already in the first week, with 63.7% of patients having no pain syndrome, and in the first month - in 86.3% of them. A statistically significant difference was found in the perception of pain in patients with various diseases before treatment and in the first week after treatment ($p<0.001$), before treatment and in the first month after treatment ($p<0.001$), as well as in the first week and in the first month after treatment ($p<0.001$). The most prominent pain symptoms (severe and pronounced) before the start of treatment were those of patients with inflammatory diseases (keratitis), trauma, burns and the presence of a foreign body. Before the start of therapy, 96.1% of patients reported a "red eye". The proportion of patients with notable redness prevailed - 42.2%, followed by those with moderate - 30.4%. A significant impact on this subjective indicator was observed already in the first week. In 50% of patients there was no redness, in 35.3% there was mild, in 12.7% - moderate, and only in 2% - pronounced redness. The absence of patients with severe redness already in the

1st week of the start of therapy is impressive. The analysis of the data shows that the most pronounced redness before the start of therapy is observed in patients with keratitis, followed by patients with injuries and burns and the presence of a foreign body. In the first month after the start of treatment, 83.3% have no redness, 8.8% report moderate, and 6.9% have mild redness. Severe redness is observed in patients with keratitis, injuries and burns, as well as in those with bullous keratopathy. It is impressive that in the first month, 80.4% of patients have no redness, while before the start of therapy there were only 4%. The largest percentage of patients - 43.1% reported a pronounced foreign body sensation, in 27.5% the symptoms were severe, and in 22.5% they were moderately pronounced. The rapid response of the patients to the applied treatment is impressive. At the 1st week, only 5.9% had a severe foreign body sensation, in 8.8% the symptoms were moderate, and in 18.6% - mild. In 65.7% of the studied patients, there was no foreign body sensation already at the 1st week after the start of the therapy. This percentage was even higher in the first month, as it was absent in 89.2% of the patients. In terms of the underlying etiology, the most pronounced foreign body sensation (severe) was observed in patients with trauma and burns, followed by patients with inflammatory diseases (keratitis).

The size of the epithelial defect is the first objective sign that we monitored in our sample of patients and is also used as a criterion for the effectiveness of the applied therapy. A significant difference in the size of the epithelial defect was observed between the individual groups before the start of therapy. The highest percentage of epithelial defects with a size between 3-5 mm was observed in the group with injuries and burns - 11.8%, and of epithelial defects with a size < 5 mm - in the group with keratitis. In the first month, 22.5% of patients with injuries and burns observed epithelialization, followed by patients with a foreign body - 16.7% and keratitis - 12.7%. The rapid process of epithelialization, which we established in the 1st week after the inclusion of the respective therapeutic plan, is impressive. In 61.8% of patients, complete restoration of the integrity of the corneal epithelium was observed. In most of the remaining patients, a significant reduction in the size of the PED was found. In 84.3% of patients there was no epithelial defect, in 3.9% we observed persistence of a defect with a size of 2-3 mm, in 2% with a size of 3-5 mm, and in 9.8% the size was 0.5-2.0 mm in the first month after treatment. Injection is another objective indicator that we monitored in our patients with PED. The results for the injection in the period

before the start of therapy show that the proportion of patients with ciliary injection in 2-3 quadrants prevails - 51%, followed by those with injection along the entire circumference - 33.3%. A significant improvement in this indicator was observed already in the 1st week, as in 36.3% of patients there was no injection, while in the period before the start of therapy this percentage was only 2%. The increase in the proportion of patients with conjunctival injection in the 1st week is impressive. - 35.3% (compared to - 2.9% before therapy), which is associated with the invasive/operative therapeutic approach (placement of AM) in some patients. At the 1st month, the proportion of patients lacking an injection reached 81.4%. The proportion of patients with conjunctival injection remained higher – 11.8%. This phenomenon was observed only in the groups of patients undergoing invasive treatment. The last objective sign that we examined and included in our study was visual acuity. The results show a significant improvement in this indicator in all groups of patients. The best results were reported in patients with a foreign body in the cornea, with inflammatory diseases (keratitis), including those with postherpetic retinopathy. We observed a weaker response to this indicator in patients with GVHD and those with bullous keratopathy. We also reported good results in patients with trauma and burns. Most of the patients included in this dissertation had moderate degree of burn, which explains the observed results in this group.

Patients were randomized into four groups based on the assessment of subjective symptoms and objective findings. Patients with superficial epithelial defects without involvement of the corneal stroma were subjected to conservative treatment (58.40%). In patients in whom we found the presence of an ulcer (involvement of the stroma without the presence of infiltrate), we performed AMT and placement of TCL, in combination with 20% ASE after the 7th day. In 16.7% of the patients included in this dissertation, we found the presence of corneal infiltrate. In them, we performed PACK-CXL, then placed AM and TCL, and after 1 week we assigned 20% ASE. Of the subjects in our clinical sample, 58.8% had undergone conservative treatment, and in 42.1% it was necessary to carry out surgical treatment. The results of the conducted study show a positive impact on all indicators after carrying out the mentioned treatment.

The analysis of the corneal structures, through highly specialized tests, proved that the results of our study do not differ significantly from those of other authors, as the findings when performing

anterior segment optical coherence tomography and in vivo confocal microscopy before starting therapy are identical. Results display that a significant difference in the studied quadrants, we observed in traumas, keratitis, keratopathies, entropion and foreign bodies. We measured the smallest thickness of the limbal epithelium in patients with corneal dystrophies and the largest - in patients with dry eye. The results show that the lowest density of long nerves are in patients with bullous keratopathy, keratitis and GVHD. The lowest density of subbasal nerve plexus was observed among patients with foreign body, bullous keratopathy and GVHD. The highest density of both indicators was observed among patients with corneal dystrophies.

Conclusion

Disorders of the anterior ocular surface can negatively affect the quality of life by causing pain, irritation, redness and reduced vision. Several conditions are common causes of persistent epithelial defects, including dry eye syndrome, trauma and burns, Sjögren's syndrome, keratitis and keratopathies, corneal degenerations and dystrophies, graft-versus-host disease and limbal stem cell deficiency. The type of ocular surface disorder and its severity determine the treatment regimen. The findings characteristic of PED can be identified by observation under a biomicroscope or by highly specialized studies – IVCM for detailed cellular examination of morphological changes and AS-OCT for useful, rapid and non-contact visualization of corneal defects and monitoring of the response to treatment. The rapid development of artificial intelligence, concepts for deep machine learning, portable devices for quantitative analysis leads to new promising methods for implementation in personalized ophthalmology. Treatment options may include artificial tears, lubricating gels, vitamins, autologous serum drops, therapeutic contact lenses and various surgical methods. The use of autologous serum drops as a method for treating dry eyes and anterior ocular surface disorders is widespread in practice, due to their content of growth factors that promote epithelial healing and their unique composition and antibacterial and anti-inflammatory properties. They lead to a significantly faster improvement in signs and symptoms and do not contain preservatives. Homologous serum, cord blood serum, and autologous fingerstick serum are alternative treatment options. Amniotic membrane transplantation may be considered as an alternative method for the treatment of persistent epithelial defects and sterile ulcers that are refractory to conventional treatment, as the thick basement membrane and avascular

stromal matrix promote epithelial healing. It has been proven to be a safe and effective method for the treatment of PED. In conclusion, the prospects for personalized ophthalmology appear promising. Advances in new technologies to improve molecular diagnostics, research to develop new therapies, fresh approaches to already known pathologies, AI software for telemedicine and screening, and data sharing systems give personalized ophthalmology the potential to revolutionize the specialty as we know it.

Keywords persistent epithelial defects, epidemiology, artificial intelligence, biological therapy, personalized approach

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