Systemic immune reactivity in patients with ocular demodicosis

Velychko L. M., Bogdanova O. V., Khramenko N. I., Makarova M. B., Linchevska O. G.; Poliakova S. I., Tsukanova I. V.

SI «The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine» Odesa (Ukraine)

Імунологічна реактивність організму хворих на офтальмодемодекоз

Величко Л. М., д-р мед. наук; **Богданова О. В.,** канд. біол. наук; **Храменко Н. І.** канд. мед. наук; **Макарова М. Б.,** канд. мед. наук; **Лінчевська О. Г.,** лікар; **Полякова С. І.,** д-р мед. наук; **Цуканова І. В.** канд. мед. наук

ДУ «Інститут очних хвороб та тканинної терапії ім. В.П.Філатова НАМН України», Одеса (Україна)

Abstract

Purpose: *To assess the status of the immune system in patients with ocular demodicosis.*

Methods: *This study was conducted at the Immunology Laboratory and Clinical Diagnostics Laboratory of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". We examined 47 patients with chronic ocular demodicosis (mean age, 29.7 ± 17.4 years) who were treated at the Consultative Polyclinic of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the NAMS of Ukraine". The control group included 20 healthy donors (mean age, 32.8 ± 15.2 years). Monoclonal antibodies (McABs) and fluorescence microscopy were used in immunohistochemistry studies. Spreadsheets and IBM SPSS Statistics (Demo) software were employed for statistical analysis.*

Results: *Patients with ocular demodicosis exhibited changes in immune system function, reductions in neutrophil phagocytic activity and the absolute count and percentage of CD3 and increases in the neutrophil-to-lymphocyte ratio and percentage of CD16. Treatment for ocular demodicosis should include not only anti-parasitic therapy, but also anti-inflammatory therapy and immune correction.*

Keywords: *Demodex, blepharitis, inflammation, immunology, CD3, CD16*

Резюме

Метою *дослідження було вивчення стану імунної системи організму у пацієнтів із офтальмодемодекозом.*

Методи. *Дослідження проведено в лабораторії імунології та лабораторії клінічної діагностики ДУ «Інститут очних хвороб та тканинної терапії ім. В. П. Філатова НАМН України». Було обстежено 47 пацієнтів із офтальмодемодекозом (29,7±17,4 року), які проходили лікування в консультативній поліклініці ДУ «Інститут очних хвороб та тканинної терапії ім. В. П. Філатова НАМН України» та контрольна група з 20 здорових донорів (32,8±15,2 року). Дослідження проводили методом імуноцитохімії з використанням моноклональних антитіл (МКАТ) та флуоресцентної мікроскопії. Статистичний аналіз здійснювали в електронних таблицях із використанням програми IBM SPSS Statistics (Demo).*

Результати. *У пацієнтів із офтальмодемодекозом спостерігалися зміни у функціюванні імунної системи: зниження абсолютної та відносної кількості CD3 та фагоцитарної активності нейтрофілів, підвищення рів-*

DOI: https://doi.org/10.31288/Ukrj.ophthalmol.202617176

UDC: 617.7

Corresponding Author: Bogdanova O.V., Cand Sc (Biol) and Researcher, Immunology Laboratory, SI «The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine». Email: aleximmun53@gmail.com

Received 2025-07-24
Accepted 2025-10-24

Cite this article as: Velychko LM, Bogdanova OV, Khramenko NI, Makarova MB, Linchevska OG, Poliakova SI, Tsukanova IV. Systemic immune reactivity in patients with ocular demodicosis. Ukrainian Journal of Ophthalmology . 2026;1:71-76.

ня CD16 клітин-кілерів, збільшення показника співвідношення нейтрофіли/лімфоцити. Лікування офтальмодемодекозу має включати, поряд із протипаразитарною терапією, також протизапальну терапію та імунологічну корекцію.

Ключові слова: *Demodex, блефарит, запалення, імунологія, CD3, CD16.*

Introduction

Demodex blepharitis is a common disease of the eyelid, affecting approximately 25 million Americans [1]. It has long been accepted that the prevalence of Demodex increases with age, affecting more than 80% of those older than 60 years and 100% of those older than 70 years [2]. Demodex mites are present in all races and ethnicities; they contribute to blepharitis in several ways: direct mechanical damage, as a vector for bacteria, and by inducing hypersensitivity and inflammation. Demodex folliculorum can cause anterior blepharitis associated with disorders of eyelashes, and D. brevis can cause posterior blepharitis with meibomian gland dysfunction and keratoconjunctivitis [3]. There was a pronounced increase in the prevalence of D. brevis with increasing age, whereas the prevalence of D. folliculorum tended to remain more constant. Risk factors for Demodex blepharitis include not only chronic disorders (e.g., diabetes) [1], but also environmental changes and eyelid anatomy. Meibum and cosmetic deposits may be accumulated between the eyelashes, creating conditions for bacterial growth and propagation of Demodex species that cause and aggravate blepharitis. Among observational studies investigating the etiology of ocular Demodex in individuals with blepharitis, the reported prevalence ranged from 29% to 90% in symptomatic patients in a hospital setting [4]. Most authors in 2017-2021 reported that Demodex blepharitis accounts for more than 60% of patients with blepharitis [5].

Blepharitis is the most common symptom of ocular demodicosis. The affected follicle usually contains 2 to 8 mites, but the number may be substantially higher. Ocular demodicosis is believed to be parasitological and allergic disease, with host sensibilization with Demodex mite byproducts being a key factor of the pathogenesis. Kin and colleagues [6] reported that, in patients with ocular demodicosis, tear concentrations of cytokines (interleukin (IL)-1β, IL-5, IL-7, IL-12, IL-13, and IL-17), granulocyte colony-stimulating factor and macrophage inflammatory protein-1β were substantially increased. They believe that believe that Demodex plays an aggravating role in inflammatory ocular surface disor¬ders because tear concentrations of IL- 1β and IL-17 significantly decreased after the eradication of Demodex in the patients. Infestation of Demodex mites induces change of tear cytokine levels, IL-17 especially, which cause inflammation of the lid margin and ocular surface [7].

Pyzia and colleagues [8] isolated Staphylococcus aureus, Acinetobacter baumannii, Streptococcus pneumoniae, Klebsiella oxytoca, and Bacillus spp. from the conjunctival sac only in patients infested with D. folliculorum. This indicates an increased probability of colonization by pathogenic bacteria in patients with demodicosis [8]. The composition of the bacterial community in the eyelashes of Demodex blepharitis patients differed from that in eyelashes of healthy volunteers, revealing a decrease in bacterial diversity in infested eyelashes [9].

Therefore, Demodex blepharitis is commonly misdiagnosed or underdiagnosed due to (1) overlap in symptoms with other ocular surface diseases and (2) a sluggish clinical course. This may cause inadequate and ineffective symptomatic treatment.

The purpose of this study was to assess the status of the immune system in patients with ocular demodicosis.

Methods

This study was conducted at the Immunology Laboratory and Clinical Diagnostics Laboratory of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". We examined 47 patients (age, 29.7 ± 17.4 years) with chronic ocular demodicosis who were treated at the Consultative Polyclinic of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". Other inclusion criteria were the absence of comorbidity or addiction. Blood samples were collected after the patient was found to be unresponsive to first-line treatment. No steroids, non-steroidal antiinflammatory drugs, hypotensive mediators or immune suppressors were used in the first stage of treatment.

This study was approved by the Bioethics Committee (Approval number: 2; Approval Date: July 29, 2025), and followed ethical standards as outlined in the Declaration of Helsinki of the World Medical Association and the European Convention on Human Rights and Biomedicine, and relevant laws of Ukraine.

The control group included 20 healthy individuals of a similar age (32.8 ± 15.2 years) and was examined for immunological parameters.

Demodex infestation was diagnosed by identification of the parasite at the root of the lashes with light microscopy. Lash sampling was performed by epilating the lashes (at least 4 lashes from each eyelid). The collected lash was placed on a microscopic slide, 1-2 drops of distilled water were added, and the slide was covered with a cover slip. The epilated lashes were examined under a microscope. The number of Demodex mites observed and the number of lashes epilated were recorded and mite density was calculated as the number of mites per lash. Patients with confirmed presence of Demodex had comprehensive immunological investigations.

A 4-5-ml sample of fasting heparinized blood was obtained from the cubital vein with a disposable vacuum system, and twice diluted with 0.9% NaCl. Monoclonal antibodies (McABs) and fluorescence microscopy were used for immunological investigations [10].

Fluorescein isothioocyanate (FITC)-conjugated McABs manufactured by the Kavetsky Institute of Experimental pathology, Oncology and Radiobiology (Kyiv, Ukraine) were used. Major immune system parameters, absolute counts and percentages of CD3 (T cells), CD4 (T helper/inducer cells), CD8 (T suppressor/cytotoxic cells), CD16 (Natural Killer cells) and CD19 (B lymphocytes) were examined.

A summary of the method procedure is as follows. First, peripheral blood mononuclear cells from whole blood are separated through density gradient centrifugation using Ficoll separating solution (Simesta, Odesa, Ukraine) with density of 1.076 g/cm3 and washed twice by centrifugation to obtain lymphocyte suspension. Second, smears are prepared and fixed with formalin vapor, a drop of the specific McAB is placed on the smear, and smears are incubated with specific FITC-conjugated McABs for three hours. Finally, smears are washed and examined under a fluorescence microscope (iSCOPE, Euromex, Arnhem, The Netherlands).

Additionally, granulocyte phagocytic activity was assessed. A summary of the method procedure is as follows. First, the granulocyte suspension is prepared using a Ficoll density gradient centrifugation technique (Ficoll separating solution with density of 1.119 g/cm3 from Simesta), and cells are washed twice by centrifugation. Second, washed neutrophils are resuspended and diluted with physiological saline to a concentration of 2-4*106 cell/μL. Third, 0.05 mL of neureophil suspension and 0.05 mL of 0.1% suspension of latex beads (Simko Ltd, Lviv, Ukraine) is added to a plate well, centrifuged at 200 g for 5 min and incubated at +4oC for 30 min. Fourth, smears are prepared and fixed with ethanol. Finally, the percentage of cells that engulfed at least one latex bead is determined using a fluorescence microscope (iSCOPE, Euromex, Arnhem, The Netherlands) at an objective lens magnification of x80 and eyepiece magnification of x15.

The neutrophil to lymphocyte ratio (NLR) reflects the activity of systemic inflammation and immune response and was calculated as a ratio of neutrophil cell count percentage and lymphocyte cell count percentage.

Spreadsheets and IBM SPSS Statistics software were employed for statistical analysis. The Shapiro-Wilks test was used to check for normality. Mean and standard deviation (SD) were calculated for quantitative data. Student t-test was used to assess differences in normally distributed continuous variables between groups. P values ≤ 0.05 were considered significant.

Results

Absolute cell count and percentage for the CD3 T cell subset in the peripheral blood of patients with ocular demodicosis were 734.2 ± 33.4 cell/μL and 53.3 ± 4.8%, respectively (Fig. 1), and were statistically significantly lower compared to healthy controls (1167 ± 86.1 cell/μL and 62.5 ± 3.2%, respectively; $p < 0.01$).

Absolute cell count and percentage for granulocyte phagocytic activity in the peripheral blood of patients with ocular demodicosis were 685.7 ± 61.4 cell/μL and 42.8 ± 4.1%, respectively (Fig. 2), and were statistically significantly lower compared to healthy controls (1732.2 ± 125.3 cell/μL and 65.4 ± 6.3%, respectively; $p < 0.01$).

The percentage for the CD16 natural killer (NK) subset in the peripheral blood of patients with ocular demodicosis was 22.3 ± 1.8% (Fig. 3), and was statistically significantly higher compared to healthy controls (14.3 ± 1.5%, respectively; $p < 0.01$).

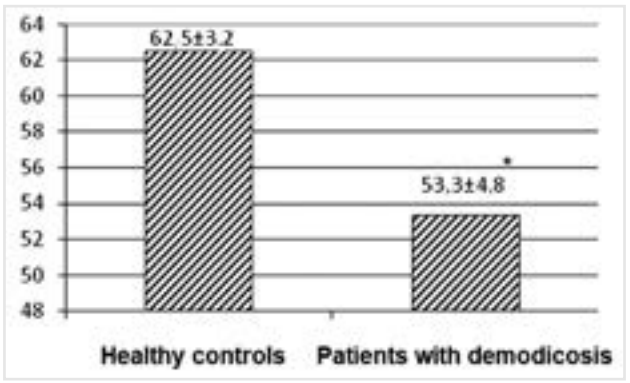

Fig. 1. Mean ± standard deviation values for the percentage of CD3 (T-cells) in healthy controls (n = 20) and patients with ocular demodicosis (n = 47). Note: * - p<0.01.

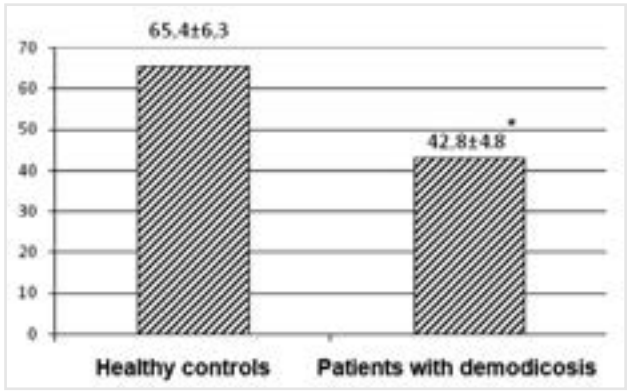

Fig. 2. Mean ± standard deviation values for the percentage of neutrophil phafocytic activity in healthy controls (n = 20) and patients with ocular demodicosis (n = 47). Note: * - p<0.01.

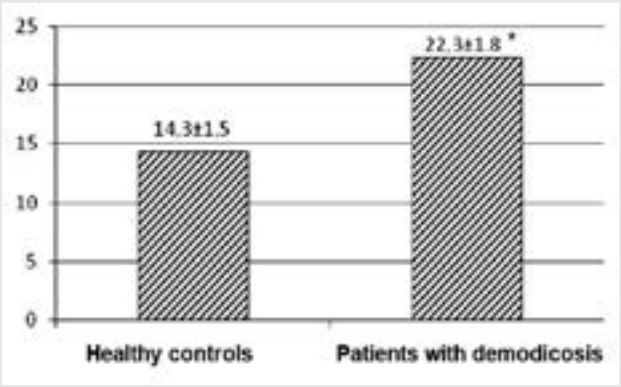

Fig. 3. Mean ± standard deviation values for the percentage of CD16 (natural killer cells) in healthy controls (n = 20) and patients with ocular demodicosis (n = 47). Note: * - p<0.01.

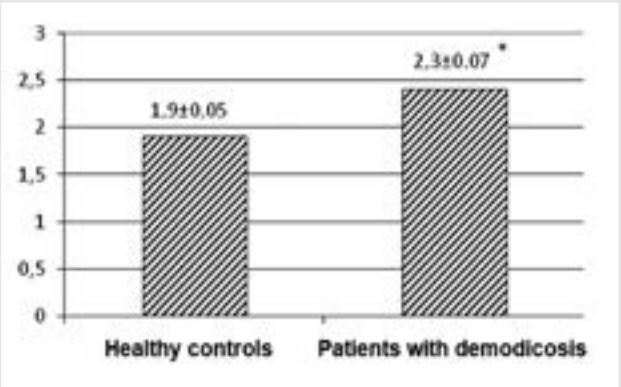

Fig. 4. Mean ± standard deviation values for neutrophil-to-lymphocyte ratio in healthy controls (n = 20) and patients with ocular demodicosis (n = 47). Note: * - p<0.01.

NLR was statistically larger in patients with ocular demodicosis than in controls (2.3 ± 0.07 (Fig. 4) versus 1.9 ± 0.05, p < 0.05).

Discussion

Our immunological studies demonstrated marked T-cell immune deficiency (a reduction in the absolute cell count and percentage for the CD3 T cell subset) in patients with ocular demodicosis. It is likely that T-cell immune deficiency is a factor of susceptibility to mite infestation. Additionally, in patients with ocular demodicosis, we observed a reduction in the percentage of granulocyte phagocytic activity and an increase in the percentage of CD16 NK cells that may infiltrate the palpebral conjunctival epithelium.

Functional cooperation among lymphocytes and neutrophils can substantially impact the status of the immune system and systemic immune reactivity. Recently, there has been an increase in studies on NLR, a new hematological parameter of systemic in-flammation and stress which reflects the activity of systemic non-specific inflamma-tion and immune response and may be an important prognostic factor for the dis-ease. An increase in neutrophil count with a decrease in lymphocyte count is believed to be a sign of infection progression [11]. To the best of our knowledge, no study has reported on the NLR in patients with ocular demodicosis. Of note, in our patients, the percentage of neutrophil granulocytes in the peripheral blood was within the normal range, but the phagocytic activity of these cells was low. Therefore, decreased phagocytic activity of neutrophil granulocytes is an unfavorable prognostic sign in Demodex infestation, with the reduction in the capacity of neutrophils for rapidly in-creasing secretion of cytotoxic components leading the activation of these cells in the focus of inflammation. In order to compensate for cytotoxic product deficiency, the immune system of a patient with demodicosis increases the percentage of CD16 NK cells that also have cytotoxic properties. This is confirmed by the fact that the per-centage of CD16 NK cells in patients with chronic Demodex blepharitis was statisti-cally significantly higher than in healthy controls (Fig. 3). Clinical research confirmed the sensitivity of NLR for diagnosis/stratification of systemic infection, sepsis, bacte-remia as well as its robust predictive and prognostic value [12]. NLR reflects online dynamic relationship between innate (neutrophils) and adaptive cellular immune re-sponse (lymphocytes) during illness and various pathological states. NLR should be investigated daily, and follow-up its absolute values and dynamic course in acute dis-ease or critical illness. Altunisik and colleagues [13] aimed to evaluate complete blood count parameters and NLR in rosaceous patients with and without demodex mite and to compare with healthy controls. The lower red cell distribution width (RDW) levels in the demodex (+) group suggested that demodex mite did not have an increasing effect on inflammation and was effective in the disease through alternative pathways.

Local immunity of the conjunctiva plays an important role in ocular inflammatory disorders. Additionally, in ocular inflammation, local antibody synthesis takes place in the lacrimal gland, and a certain degree of transudation of serum antibodies to tears is also often encountered, especially in severely inflamed eyes [15]. Puchkovska and colleagues [16] investigated local immunity in ocular inflammation and found an increase in antibody titers, a positive relationship of tear and serum antibody levels, increased immunoglobulin (Ig)M and decreased IgA levels. Tear levels of IL-7, IL-12, and IL-17 were increased significantly in the Demodex blepharitis group compared to the Demodex-free blepharitis group [7].

It should be noted that the tear levels of local and cellular immunity parameters in patients with Demodex blepharoconjunctivitis reflect an active inflammatory process. Others reported that patients with Demodex blepharitis showed increased tear levels of IgG, IgM, IL-17, IL-7, and IL-12, and decreased tear levels of IgA. Our patients, however, showed reduced levels of cellular immunity and neutrophil phagocytic activity.

Therefore, immunological abnormalities in patients with ocular demodicosis contribute to disease progression, and local treatment fails to prevent complications. Elimination of parasites is practically impossible after parasitic infestation of deep epidermal and dermal layers in complicated disease. Demodex mite fragments were found by histomorphological examination of biopsy specimens from patients suspected for eyelid tumors. Normally, almost complete mite destruction occurs within 2-3 hours after the death of a mite. If this process is disrupted, a proliferative process is triggered [17]. Studies at the Pathomorphology Laboratory of the institute found Demodex species in several types of eyelid and conjunctival tumors and tumor-like processes; this expands our understanding of the role of Demodex infection in blepharitis. Taken together, the above allows us to consider post-demodecosis pathomorphosis as a stand-alone problem of ocular pathology [17, 18]. The chemical mediators secreted by Demodex spread over the cornea as the eyelids rub them during blinking and cause inflammation in either the superior or inferior part of the cornea [19], which is very difficult to treat. Although Demodex blepharitis is one of the commonest eye disorders, it is still a problem due to prolonged treatment course (30-45 days), insufficient efficacy of therapy and frequent relapses [20, 21]. Adjunctive immunotherapy may substantially improve the efficacy of treatment for demodicosis.

In conclusion, it should be noted that patients with ocular demodicosis exhibit some changes in immune system function, reductions in the absolute count and percentage of CD3 and neutrophil phagocytic activity, and increases in the NLR and the percentage of CD16. Treatment for ocular demodicosis should include not only anti-parasitic therapy, but also anti-inflammatory therapy and immune correction.

Author Contributions

LMV: Conceptualization, Project Administration, Data Analysis and Interpretation; OVB: Data Curation, Analysis and Interpretation, Writing – Original Draft Preparation, Writing – Review and Editing; NIK: Data Curation and Investigation; MBM: Data Curation and Investigation; OGL: Data Curation and Investigation; SIP: Writing – Original Draft Preparation, Writing – Review and Editing; IVT: Data Curation and Investigation. All the authors have read and approved the final manuscript.

Ethical Statement

This study involved human subjects, was approved by the Bioethics Committee (Approval number: 2; Approval Date: July 29, 2025), and followed ethical standards as outlined in the Declaration of Helsinki. Informed consent was obtained from all subjects. This study did not include animal experiments.

Statements and Declarations

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Sources of support

None.No sponsor or funding organization was involved in the development of this work and conducting this study.

Disclaimer

This manuscript reflects the views of the authors and may not reflect the views of their institution.

Conflict of interest

The authors state that they have no conflict of interest that could influence their view on the subject matter or material described and discussed in this manuscript.

Data Availability Declaration

All the data obtained or examined during this study has been incorporated into this published article.

Abbreviations

Ig, immunoglobulin; IL, interleukin; McABs, monoclonal antibodies

References

1. Rhee MK, Yeu E, Barnett M, Rapuano CJ, Dhaliwal D, Nichols K K, et al. Demodex Blepharitis: A Comprehensive Review of the Disease, Current Management, and Emerging Therapies. Eye Contact Lens. 2023 Aug 1;49(8):311-318. doi: 10.1097/ICL.0000000000001003.
2. Cheng AMS, Sheha H, Tseng SCG. Recent advances on ocular Demodex infestation. Curr Opin Ophthalmol. 2015;26:295–300 DOI: 10.1097/ICU.0000000000000168
3. Pathogenic role of Demodex mites in blepharitis. Curr Opin Allergy Clin Immunol. 2010 Oct;10(5):505-10. DOI: 10.1097/ACI.0b013e32833df9f4
4. Zhang AC, Muntz A, Wang M, Craig JP, Downie LE. Ocular demodex: A systematic review of the clinical literature. Ophthalmic Physiol Opt. 2020;40:389–432. DOI:10.1111/opo.12691
5. Biernat MM, Rusiecka-Ziółkowska J, Piątkowska E, Helemejko I, Biernat P, Gościniak G. Occurrence of demodex species in patients with blepharitis and in healthy individuals: A 10-year observational study. Jpn J Ophthalmol. 2018;62:628–633 10.1007/s10384-018-0624-3
6. Kim JH, Chun YS, Kim JC. Clinical and immunological responses in ocular demodecosis. J Korean Med Sci. 2011 Sep; 26(9):1231-7. doi:10.3346/jkms.2011.26.9.1231
7. Kim JT, Lee SH, Chun YS, Kim JC. Tear cytokines and chemokines in patients with Demodex blepharitis. Cytokine. 2011 Jan;53(1):94-9. doi: 10.1016/j.cyto.2010.08.009.
8. Pyzia J, Mankowska K, Czepita M, Kot K, Lanocha-Arendarczyk N, Czepita D. Demodex Species and Culturable Microorganism Co-Infestations in Patients with Blepharitis. Life (Basel). 2023 Aug 29;13(9):1827. DOI:10.3390/life13091827.
9. Zou D, Lu X, Song F, Zhong X, Chen H, Zhang J et al. Characteristics of bacterial community in eyelashes of patients with Demodex blepharitis. Parasit Vectors. 2024 Feb 14;17(1):64. doi: 10.1186/s13071-024-06122-x.
10. Gluzman DF, Skliarenko LM, Nadgornaia VA, Kriachok IA. [Immunocytochemistry in tumor diagnosis]. Kyiv: Morion; 2003. Russian.
11. Forget P, Khalifa C, Defour J, Latinne D, Van Pel M, De Kock M What is the normal value of the neutrophil-to-lym-

phocyte ratio? BMC Res Notes. 2017 Jan 3;10(1):12. DOI: 10.1186/s13104-016-2335-5
12. Zahorec R Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy. 2021;122(7):474-488. doi: 10.4149/BLL2021078.
13. Altunisik N, Turkmen D, Sener S. Investigation of the relationship between inflammatory blood parameters and rosacea and demodex infestation. J Cosmet Dermatol. 2020 Aug;19(8):2105-2108. doi: 10.1111/jocd.13254.
14. Certificate of copyright registration for work № 119857 dated 19.06.2023. Authors: Khramenko NI, Drozhzhyna GI, Velychko LM, Bogdanova OV. [Method for diagnosing recurrence in patients with recurrent stromal herpetic keratitis based on the ratio of neutrophils to lymphocytes in peripheral blood].
15. Friedman MG. Antibodies in human tears during and after infection. Surv Ophthalmol. 1990 Sep-Oct;35(2):151-7. doi: 10.1016/0039-6257(90)90070-c.
16. Puchkovskaya NA, Shulgina KS, Minev MG, Ignatov RK. [Immunology of Eye Pathology]. Moscow: Meditsina; 1983. Russian.
17. Artemov O, Lytvynenko M, Neskoromna N, Chebotarova S, Prus R, Oluwafemi A, et al. Pathomorphosis of eyelid tumor pathology in demodectic infection. Inter Collegas. 2024;11(1): 5-10. https://doi.org/10.35339/ic.11.1.aln
18. Artemov OV, Lytvynenko MV, Chumachenko IV, Bondarenko AV, Dotsenko NV Ostapchuk KV, et al. The influence of the demodex mite on the morphological picture of eyelid papilloma. Georgian Med News. 2024 Jul-Aug:(352-353):50-54.
19. Chatterjee S, Gupta J, Srinivas SP, Rao SK. Demodex and the eye – A review. Indian J Ophthalmol. 2024 Dec 23;73(1):10–18. doi: 10.4103/IJO.IJO_1591_24.
20. Martínez-Pulgarín DF, Ávila MY, Rodríguez-Morales AJ. Interventions for Demodex blepharitis and their effectiveness: A systematic review and meta-analysis. Cont Lens Anterior Eye. 2021 Dec;44(6):101453. doi: 10.1016/j.clae.2021.101453.
21. Navel V, Mulliez A, Benoist d'Azy C, Baker JS, Malecaze J, Chiambaretta Fet al. Efficacy of treatments for Demodex blepharitis: A systematic review and meta-analysis.. Ocul Surf. 2019 Oct;17(4):655-669. doi: 10.1016/j.jtos.2019.06.004.