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Neutrophil activation marker CD15+ as a prognostic factor of ocular surface damage in type 2 diabetics

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Purpose: *To evaluate the prognostic value of the neutrophil activation marker CD15+ in ocular surface damage in type 2 diabetics.*

Material and Methods: *This study was conducted from January 2021 to January 2021 at the Pirogov Vinnytsia Regional Clinical Hospital, the clinical site of the Medical University. A total of 60 type 2 diabetics (120 eyes) were enrolled, with an average age of 62.1 ± 5.24 years.*

Results: *The prognostic value of the percentage expression of CD15+ in ocular surface damage in type 2 diabetics was for the first time evaluated. A rather high sensitivity of the percentage expression of CD15 (88.3%) allows using it as a parameter for screening for the presence of ocular surface damage in type 2 diabetics. Type 2 diabetics with a percentage of >27% of CD15+ cells had 10.52 times increased odds of ocular surface damage (95%CI, 4.34–25.50; p < 0.0001), and this value of the percentage of CD15+ cells can be used for the purpose of screening for ocular surface damage with a sensitivity of 88.3% (95% CI, 77.8-94.2) and specificity of 63.3% (95% CI, 50.7-74.4).*

Introduction

The mechanism of ocular surface damage in diabetes mellitus (DM) is still poorly understood due to the complex pathophysiology of the disease [1]. Chronic hyperglycemia contributes to defective wound healing in the outer cornea, abnormalities in nerve fibers in the subbasal plexus, and loss of corneal endothelial pump [1]. In DM, pathological changes can be observed in meibomian gland (MG) function. MG dysfunction results in increased meibum viscosity, stasis of the meibum in the duct, and gradual MG loss, leading to quantitative and qualitative changes in the tear film lipid layer. Destabilization of the lipid layer can lead to excessive tear evaporation and increased tear osmolarity [2]. All these factors contribute to structural changes in the eyelids, tear apparatus and cornea.

The ocular surface milieu is special with regard to immune properties. It not only performs a barrier function, but also protects its inherent immune cells from potential damage associated with their excessive activity in response to infectious, mechanical or chemical agents. Neutrophil granulocytes (CD45, CD15) are common innate immune cells mostly due to their phagocytic properties, direct migration to inflammation foci and infiltration in adjacent tissues [3]. The neutrophil marker CD15 is known to contribute to cell adhesion to the epithelium and play a key role in eliciting and maintaining an internal inflammatory response [3]. The CD15 carbohydrate epitope is expressed in mature human neutrophils, monocytes, and promyelocytes [4]. This cell surface glycan is involved in cell-to-cell interaction, phagocytosis, and stimulation of degranulation and oxidative burst [4, 5].

Studies reported on the involvement of CD15 in various disorders. Because elevated CD15 levels have been reported in malignant solid tumors affecting lungs, ovaries, thyroid gland, colorectal tract, etc., CD15 is mostly used as a marker of a relapse-free course and outcome [6].

A recent study by O'Rourke and colleagues [7] found increased concentrations of dendritic cells (CD11c+ HLA-DR+), neutrophils (CD15+ CD11c+) and T-cells (CD4+, CD8+) in the aqueous humor of patients with the acute flare of anterior uveitis versus healthy controls. They concluded that the acute uveitis microinvironment contributes to immune cell response and intraocular inflammation.

Mun and colleagues [8] hypothesized that ageing neutrophils play a role of the development of dry eye disease (DED) in elders by inducing chronic inflammation of vessels and affecting lacrimal glands and ocular surfaces [8]. In addition, neutrophils are involved in the system of acquired immunity due to their interaction with T-cells and B-cells [9, 10, 11, 12].

Given the range of activity and the sources of expression of CD15+, the percentage expression of CD15+ may be a prognostic factor of the severity of ocular surface immune inflammation [8, 9].

The purpose of the study was to evaluate the prognostic value of the neutrophil activation marker CD15+ in ocular surface damage in type 2 diabetics.

Material and Methods

This study was conducted from January 2021 to January 2021 at the Pirogov Vinnytsia Regional Clinical Hospital, the clinical site of the Pirogov Vinnytsia National Medical University. A total of 60 type 2 diabetics (120 eyes) were enrolled, with an average age of 62.1 ± 5.24 years. Two groups were formed for evaluating the prognostic value of neutrophil CD15 expression. Group 1 comprised 60 eyes with conjunctival impression cytology evidence of ocular surface damage corresponding to Nelson's grade 2 or 3 squamous metaplasia. Group 2 comprised 60 eyes with conjunctival impression cytology evidence of ocular surface damage corresponding to Nelson's grade 0 or 1 squamous metaplasia [13].

Percentage expression of CD15 in the peripheral blood was determined with the method we reported previously [14] and impression cytology of the bulbar conjunctiva was performed using our methodology published elsewhere [15, 16].

The study was approved by the Bioethics Committee and the principles of the Declaration of Helsinki were followed throughout. Written informed consent was obtained from all study participants. This study is a part of the research project A Comprehensive Approach to the Pathognetic Components and Improving the Diagnosis, Prevention, Treatment and Rehabilitation of Patients with Anterior Segment Disease (Reg. no. 0123U103539) by the Department of Ophthalmology at the Pirogov Vinnytsia National Medical University.

Statistical analyses were conducted using Statistica 10.0 (StatSoft, Tulsa, OK, USA) software and the Epitools Epidemiologic Calculator [17]. Data are presented as mean plus or minus standard deviation (SD) or percentages. Binary logistic regression was used to examine the association between the percentage expression of CD15 and the presence of ocular surface damage. Determination of the area under Receiver Operating Characteristic (ROC) curve was performed to calculate the sensitivity, specificity and the overall model correctness. The model was considered adequate if the AUC was significantly different from 0.5. The level of significance $p \leq 0.05$ was assumed. Youden's index was used to determine the optimal cut-off value for the percentage expression of CD15 [17]. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to determine the association of the percentage expression of CD15 with the presence of ocular surface damage. A risk factor was considered significant if the adjusted OR was greater than one.

Results

The mean relative level of CD15 expression was significantly higher in the group of patients with signs of Nelson's grade 2 or 3 squamous metaplasia ($p < 0.00001$). Therefore, an increase in the percentage of CD15-positive cells in this subgroup may indicate activation of neutrophil-mediated processes in the presence of ocular surface damage (Table 1).

Figure 1 compares graphically the percentage of CD15+ cells for both groups.

A ROC-curve analysis found a significant association ($p = 0.0001$) between the percentage of CD15+ cells and the presence of ocular surface damage (AUC = 0.84; 95%CI = 0.773-0.908). An AUC of 0.84 indicates that the developed logistic model is adequate and the diagnostic test under investigation has a high prognostic value, which allows using the percentage of CD15+ cells as a biochemical risk marker of ocular surface damage in type 2 diabetics. In addition, a small confidence interval value indicates a high accuracy of the calculated AUC value (Fig. 2). A percentage of 20% or 24% of CD15+ cells (with a sensitivity of 99.9% or 90%, respectively) may be reasonable for use in screening settings, but the prognostic value of these percentages of CD15+ cells is limited by low specificity values (33.3% or 51.7%, respectively).

For the purpose of confirmation of the diagnosis, one should be guided by the specificity of a test. Particularly, 99.9% of patients without ocular surface damage will have a percentage of CD15+ cells <42%. A variant with a specificity of 90%, a sensitivity of 51.7% and a cut-off point of 37% seems to be a more balanced one. A percentage of 27% of CD15+ cells was an optimal cut-off point (Table 2).

The results of the analysis of association power indicate that patients with a percentage of >27% of CD15+ cells had 10.52 times increased odds of ocular surface damage ($p< 0.0001$) (Table 3).

Table 1. Percentage of CD15+ cells (M±SD) in study group type 2 diabetics with different Nelson grades of squamous metaplasia

Characteristic	Group 1 (n=60)		Group 2 (n=60)		p
Nelson grades of squamous metaplasia	Nelson grade 2	Nelson grade	Nelson grade 0	Nelson grade 1	n/a
n	45	15	0	60	n/a
$CD15^+$ (%)	36.7±9.0		23.4±8.9		p<0.00001*

Note: n, number of eyes; p, significance of difference between groups; n/a, not applicable

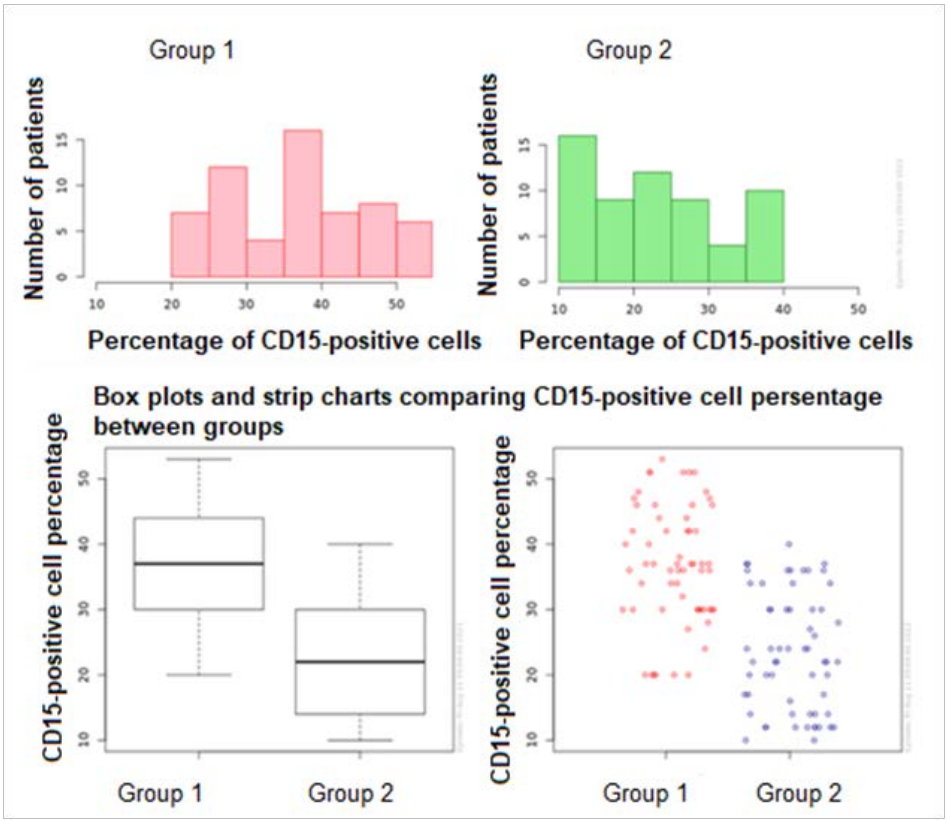

Fig. 1. Comparing graphically the percentage of CD15+ cells for both groups

Discussion

In recent years, increased attention has been given to the role of the immune system in developing ocular surface damage and DED [10, 18].

A human blood neutrophil half-life is less than 1 day normally, but can significantly increase in the setting of inflammation [9]. Autophagy is a mechanism of regulation of half-life of granulocytes positive for CD15, CD66b, CD63 and CD11b; consequently, it is a mechanism of regulation of NETosis-mediated inflammation. Impaired autophagy is accompanied by the accumulation of aging neutrophils, abnormal expression of cell surface molecules and increased proportion of neutrophil extracellular traps (NETs) [10].

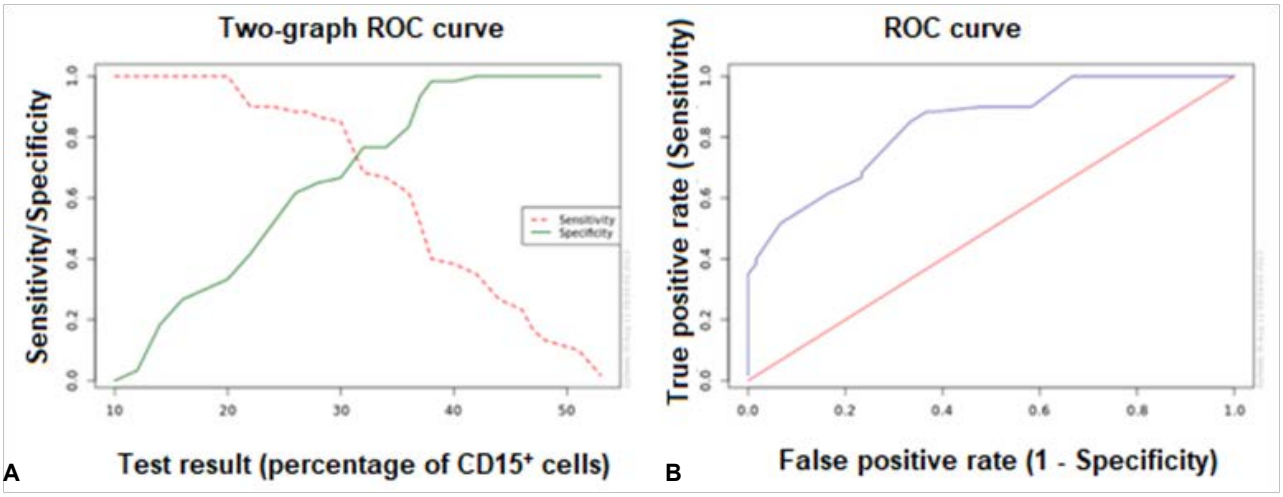

Рис. 2. A – двохграфікова ROC-крива залежностей чутливості та специфічності від показника відносного рівня CD15+. B – стандартна ROC-крива із зазначенням AUC.

Table 2. Optimal sensitivity and specificity for testing the prognostic value of the percentage of CD15+ cells

Youden's index	Sensitivity	Specificity
0.5166	88.3%	63.3%
	95% CI = 77.8 -94.2	95% CI = 50.7 -74.4

Note: CI, confidence interval

Table 3. Association of the risk for developing ocular surface damage with a percentage of CD15+ cells > 27%

Group 1 n=60		Group 2 n=60		OR	95% CI	Z score	p-value
TN	51	TN	39	10.52	4.34 – 25.50	5.211	p<0.0001
FN	9	FN	21				

Note: CI, confidence interval; FN, false negative; n, number of eyes; OR, odds ratio; P-value, significance of difference between groups; TN, true negative; z-score, normalized data;

An interesting study by Postnikoff and colleagues [19] sought to compare normal and dry eye subjects for daily variation in open eye leukocyte composition. On average, there were about twice as many leukocytes collected from dry eye subjects (1.5 × 106 ± 1.7 × 106) versus normals (7.3 × 105 ± 1.1 × 106) at awakening, however, this was not statistically significant (p = 0.21). The ratio of granulocytes to lymphocytes at awakening was, however, significantly higher in dry eye subjects versus normals (p = 0.05). The latter finding supports the inflammation mechanism as explanation for the occurrence of dry eye, and suggests increased levels of CD15+ cells in the ocular surface milieu.

Some studies described the role of Matrix Metalloproteinase 9 (MMP-9) in the diagnosis and approaches to treatment of DED. Thus, Sambursky and colleagues [20] concluded that the capacity to determine accurately the increased levels of MMP-9 will result in early diagnosis and more adequate treatment of ocular surface disease.

To the best of our knowledge, the current study is the first aiming to diagnose ocular surface damage in type 2 diabetics by assessing the percentage of CD15+ cells.

Previously, we have conducted a study to assess the role of the marker of neutrophil activation in blood of type 2 diabetics. The mean expression of CD15+, a neutrophil activation marker, in the peripheral blood, was 46.7% for patients with T2DM plus DED and punctuate keratopathy, and 28.5% for 32 patients with T2DM plus DED and intact cornea (p=0.0001) [14].

Therefore, in the current study, we consider the cell surface antigen CD15 as a potential marker of ocular surface damage in type 2 diabetics.

A limitation of this study is that our study sample included only patients older than 60 years of age, although T2DM with associated ocular surface changes can be found also in younger patients. Another limitation was a tendency of elder patients to the subclinical degenerative disease and the development of neoplasms which could increase the percentage of CD15-positive cells in the peripheral blood.

Further studies will be conducted to determine the percentage of CD15-positive cells among bulbar conjunctival cells (e.g., conjunctival epithelial cells) in type 2 diabetics of different age groups to avoid the above potential effects.

Conclusion

First, the prognostic value of the neutrophil activation marker CD15+ in ocular surface damage in type 2 diabetics was for the first time evaluated.

Second, a rather high sensitivity of this biomarker (88.3%) allows using it as a parameter for screening for the presence of ocular surface damage in type 2 diabetics.

Finally, type 2 diabetics with a percentage of >27% of CD15+ cells had 10.52 times increased odds of ocular surface damage (95% CI, 4.34–25.50; p < 0.0001), and this value of the percentage of CD15+ cells can be used for the purpose of screening for ocular surface damage with a sensitivity of 88.3% (95% CI = 77.8-94.2) and specificity of 63.3% (95% CI = 50.7-74.4).

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Disclosures

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Abbreviations: *DED, dry eye disease; DM, diabetes mellitus; MG, meibomian glands; MMP-9, Matrix Metalloproteinase 9*