Kovalchuk Khrystyna. New risk factors in progression of age-related macular degeneration. Journal of Education, Health and Sport. 2020;10(10):90-101. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2020.10.10.009
https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2020.10.10.009
https://zenodo.org/record/4156249

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 12.10.2020. Revised: 16.10.2020. Accepted: 29.10.2020.

New risk factors in progression of age-related macular degeneration

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Abstract

Background. Allot of studies investigate the etiology and pathogenesis of age-related macular degeneration (AMD). The exudative form of AMD is the leading cause of irreversible blindness in the elderly. At the same time neurosensory retina detachment in AMD has high level of prevalence and severity of comlications but little attention has been paid to explaying the risk factors of retina edema and the possibility of their role on the interstitial fluid accumulation. The investigation of patterns for predicting the risk of retinal detachment is still cosiderable, as this is important for patients selection with high risk of developing retinal detachment of AMD and effective preventive treatment.

Purpose: To investigate new risk factors for the progression of age-related macular degeneration.

Materials and methods. The prospective study included 37 patients (67 eyes) with the exudative form of AMD (Category 4 according to the AREDS). 21 (56.8%) patients had exudative detachment of neurosensory retina. The ophthalmologic examination included anamnesis, visometry, tonometry, biomicroscopy, ophthalmoscopy and optical coherence

tomography. ATP, ADP, adenosine, platelet-activating factor (PAF), adrenaline and isadrin were used to study the functional activity of platelet receptors. The evaluation of platelet aggregation was carried out by a turbidimetric method. Methods of pair correlation analysis and multiple linear regression models were used to analyse the relationship between the values of individual agonists the retinal detachment.

Results. After analysis of nine items, three of which were found to be significantly associated with serous detachment of neurosensory retina, including the activity of PAF receptors, adenosine A2A receptors and α2-adrenoreceptors. The prognosis of the cases of this complication in the general cohort of patients with exudative AMD turned out to be positive in 87.5%.

Conclusions. Correlation between the development of exudative detachment of neurosensory retina and the functional state of Pl can be used in the selection for preventive treatment of patients with retinal detachment in AMD.

Key words: age-related macular degeneration; platelet's receptors; model of prediction of complications.

Allot of studies investigate the etiology and pathogenesis of age-related macular degeneration (AMD) [1, 2, 3]. The exudative form of AMD is the leading cause of irreversible blindness in the elderly [4]. At the same time neurosensory retina detachment in AMD has high level of prevalence and severity of comlications but little attention has been paid to explaying the risk factors of retina edema and the possibility of their role on the interstitial fluid accumulation. The investigation of patterns for predicting the risk of retinal detachment is still cosiderable, as this is important for patients selection with high risk of developing retinal detachment of AMD and effective preventive treatment. Second important point for the diagnosis of serous retinal detachment is the search for methods to analyse the mechanisms of regulation and involment of retinal pigment epithelium (RPE) and Müller cells in the retinal interstitial fluid reabsorption, and the blood-ocular barrier permeability increasing [5, 6]. Promising method for such a diagnosis is an evaluation of platelet receptors activity in vitro. It shows the involvement of pathogenetic factors, including inflammatory mediators (cytokines, chemokines, PAF), purines and catecholamines in the regulation of the platelets function and vascular endothelium, glial cells and RPE [7, 8, 9, 10]. It seems reasonable to discover whether there is a relationship between the retinal detachment of AMD and the activity of the receptors of platelets. If a connection is found, then the pathogenetic risk factors, which can be selected as predictors of retinal detachment need to be founded out.

Purpose: To investigate new risk factors for the progression of age-related macular degeneration.

Materials and methods: The prospective study included 37 patients (67 eyes) with the exudative form of AMD (Category 4 according to the AREDS) [13]. 21 (56.8%) patients had exudative detachment of neurosensory retina. The ophthalmologic examination included anamnesis, visometry, tonometry, biomicroscopy, ophthalmoscopy and optical coherence tomography. Fluorescent angiography was performed according to the indications in case of suspicion of latent neovascularization.

Platelets were isolated from citrate peripheral blood of patients by centrifugation. Platelets were used for evaluation of the receptors functional activity. We studied receptor agonists involved in the AMD pathogenesis, including ATP, ADP and adenosine (correspondingly, ligands of purine P2X-, P2Y- and A2A-receptors), platelet-activatng factor (PAF), adrenaline and isadrine (α2 ligands - and β2-adrenoreceptors, respectively). The adenosine A2A receptors and β2-adrenergic receptors activity was investigated with the combined incubation of ADP with adenosine, or ADP with isadrine, and was calculated as the difference between the ADP-induced platelet aggregation (PlA) and the final platelet aggregation recorded in the combined incubation of ADP with adenosine or ADP with isadrin. Agonists (Sigma, USA) were used in an concentration (EC_{50}) to activate the platelet aggregation (50 ± 5%) in ten healthy volunteers (control group) whose fundus was normal. Spectrophotometric method was used for the aggregation of platelets analysis. All patients gave their informed consent to participate in the study.

To analysis the link between the exudative detachment of the neurosensory retina and the values of the individual agonists, we used the methods of pair correlation analysis (Spirman's rank correlation coefficient was calculated) and multivariable linear regression models (generalized regression models); the adequacy of the model was evaluated by corrected determination parameter ($R^2_{adjusted}$). For the estimation of the degree of influence of each of the factor characteristics in the multivariable model, partial correlation coefficients (r_{part}) were calculated. The statistical analysis was performed with EZR v. package. 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical interface to R (The R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

Nine characteristics as an age, duration of disease, sex, functional activity of P2X and P2Y purine receptors, A2A- adenosine and FAT- receptor, α2 and β2 adrenergic receptors were used. For the selection of significant features, the method of stepwise exclusion /

inclusion was applied (a critical threshold for the exclusion of the sign - $p_1 > 0.1$ and a critical threshold of inclusion - $p_2 < 0.05$). In the course of the analysis, three signs were found to be strongly associated with this complication, namely the activity of the receptor A2A, PAF-receptor and α2-adrenergic receptor. The logistic regression model based on the distinguished features was adequate ($\chi^2 = 102.7$, three degrees of freedom, $p < 0.001$). The area under the curve (AUC) of the model was 0.99 (95% VI 0.96 - 1.00), which is indicative of a strong correlation between the risk of retinal detachment and the activity of the A2A receptor, the PAF-receptor, and the α2-adrenergic receptor. *Table* 1 shows the coefficients of the model. The analysis showed that the risk of retinal detachment increased ($p = 0.004$) with an increase in A2A receptor activity, VS = 1.2 (95% VI 1.1-2.2) per each unit of increase in this index (standardized by the values of PAF - receptor and α2-adrenergic receptor activity). Besides, the rise in ($p = 0.004$) of the risk of retinal detachment was detected with an increase in the activity of the PAF- receptor, VS = 1.6 (95% VI 1.1 - 2.2) per each unit of an increase in this index (standardized by the values of A2A- receptor and α2-adrenergic receptor activity). There was a decrease ($p = 0,01$) in the risk of retinal detachment with an increase in the activity of the α2-adrenergic receptor, VS = 0.6 (95% VI 0.4 - 0.9) per each unit of an increase in this index (standardized by the values of A2A receptor and FAT receptor activity). When selecting the optimal threshold, the sensitivity of the model was 100% (95% VI 83.9% - 100%); the specificity of the test was 88.0% (95% VI 81.0% - 93.1%); the predictive value of the positive result was 87.5 % (95% CA 69.6% - 95.5%), %), and the predictive value of the negative outcome was 100%.

Table 1

The coefficients of the three-factor logistic regression model for predicting the risk of retinal detachment

Factor sign	The value of the coefficient of the model, b ± m	The level of significance of the difference, p	The ratio of odds, VS (95% VI)
A2A-receptor activity	0.19±0.07	0.004	1.2 (1.1 – 2.4)
PAF-receptor activity	0.45±0.16	0.005	1.6 (1.1 – 2.2)
α2-adrenergic receptor activity	–0.48±0.18	0.01	0.6 (0.4 – 0.9)

A three-factor model of retinal detachment risk (Y) with A2A receptor activity, (X1), PAF-receptor (X2) and α2-adrenergic receptor (X3) was obtained. It should be noted that, basically, the risk of retinal detachment is determined by the level of PAF-receptor activity. The area under the curve of operating characteristics (AUC) for this index was 0.93 (95% VI 0.87 - 0.97), which is the evidence of excellent consistency of the prediction model, and a very strong correlation between the level of the PAF-receptor activity and the risk of retinal detachment. When choosing the optimal threshold for the activity of the PAF-receptor > 62%, the patient is predicted to be in the risk of retinal detachment. When selecting this threshold, the sensitivity of the test is 95.2% (95% VI 76.2% - 99.9%), the specificity of the test is 79.2% (95% VI 71.0% - 85.9%), prognosticity of the positive the result is 43.5% (95% VI 35.0% - 52.3%), the predictive value of the negative result is 99.0% (95% IU 93.6% - 99.9%).

Noteworthy is the low predictive value of the positive result (43.5%) as opposed to the value received while using the 3-factor logistic regression model for prediction of the risk of detachment (87.5%). It is logical that an increase in the number of indices reflecting the pathogenesis of retinal detachment led to an increased probability of this pathology verification. Interestingly, in eight (50%) out of 16 patients with exudative AMD, who had no detachment of the neurosensory retina, the rate of PAF-induced platelet aggregation exceeded 62%, that is, false-positive results are predicted. Further analysis of those patients could enable to determine the causes of errors in predicting the risk of detachment of the neuronsensative retina.

Firstly, it was necessary to establish whether the rate of PAF-induced platelet aggregation really differed in 16 patients with out retinal detachment, if 62% was taken as the cut-off value of the quantitative sign of receptor functional activity. The findings of the analysis showed that this methodical approach allowed dividing the study group into two subgroups with different status of activity of the PAF-receptor. In subgroup A, there was revealed the hyperreactivity of the receptor (by 23.1% higher, $p < 0.001$); subgroup B was found to have the upper limit of the normreactivity.

Secondly, we had to identify the receptors whose activity differed in subgroups A and B, and therefore could affect the predictive value of the positive result. The solution to this problem allowed specifying the mechanisms of interaction of the PAF receptor with other receptors, which may result in its increased reactivity These were: an increased activity of P2Y receptors (by 11.5%, $p < 0.05$), and a decrease in the reactivity of α2-adrenergic receptors (by 11.0%, $p < 0.01$).

Furthermore, after the comparison of the activity of the receptors in subgroup A and the general group of patients having exudative detachment of the neurosensory retina, we found it possible to identify specific mechanisms of platelet receptors interaction inherent in cases of retinal detachment (development of complication), and general (non-specific) mechanisms of such interaction when platelets were involved in the development of the exudative AMD. The revealed increase in the activity of the P2X-receptor and the PAF receptor and the decrease in the reactivity of α2-adrenoreceptors of platelets (Table 2) can be attributed to specific mechanisms associated with retinal detachment.

Table 2

Differences in induced PlA (%) between the patients with wet AMD with no exudative detachment of the neurosensory retina and those having this complication

Inductor	No exudative retinal detachment in subgroup A patients (n = 8)		Exudative retinal detachment (n = 21)	
PlA	$\overline{X}$ ± SD	Min–Max	$\overline{X}$ ± SD	Min –Max
Adenosine (1.0 μM)	83.6±1.0	80.0-88.0	74.9±2.7	56.0-92.0
ADF (5,0 μM)	82.5±1.4	76.0-87.0	73.4±1.3	62.0-84.0
ATF (500 μM)	69.4±1.1	66.0-76,0	76.9±0.7***	70.0-83.0
PAF (150.0 μM)	66.6±1.5	64.0-77.0	71.9±1.8*	60.0-89.0
Adrenalin (5.0 μM)	74.7±2.1	62.0-80.0	61.1±0.9***	55.0-72.0
Isadrin (10.0 μM)	43.0±2.3	35.0-52.0	48.6±2.0	36.0-65.0

Notes: * - the probability of PlA differences between patients with and without exudative retinal detachment with $p < 0.05$; *** with $p < 0.001$.

The activity of β2-adrenergic receptors was within the normal range. Hyperreactivity of purine P2Y- and A2A-receptors of platelets, which does not differ ($p > 0.05$) in patients with and without retinal detachment, suggests that the interaction of these receptors occurs irrespective of the development of this complication, and therefore can be attributed to non-specific mechanisms of the the exudative AMD pathogenesis. In particular, the presence of hyperreactivity of P2Y-receptors reflects the pro-aggregant condition of platelets, which may result in the increased hydrostatic pressure in the capillaries, the enhanced fluid transport

through the vessels of the microcirculation bed and the accumulation of interstitial fluid (swelling in the tissue) [10].

Moreover, the predictive value of the positive result in patients with an exudative AMD without retinal detachment may be affected by other complications if they are associated with hyperreactivity of the PAF receptors. Thus, out of 8 patients of subgroup A, CNV was detected in 7 (87.5%) cases, geographical atrophy of pigment epithelium - in 4 (50%) individuals, the association of CNV and geographical atrophy occurred in 3 (37.5%) patients.

Available methods of visualization of retinal structures are highly informative when there are pronounced morphological changes. However, often the efficacy of the treatment is determined by the duration of the period from the beginning of the development of the pathological process in the retina, i.e. the principle of early diagnosis should be the “gold standard”. In this regard, the issue of developing the models to determine the risk of AMD complications occurrence becomes relevant, as they would be beneficial for preventive treatment. It should be noted that studies in the field of AMD complications prediction are based on the assessment of demographic and environmental factors, genetic markers and morphological parameters of the macula. Schmidt-Erfurth [2018] offers to use the artificial intelligence in order to enable the personalized prediction of AMD progression. The efficacy developed model in the prediction of the progression of choroidal neovascularization and geographic atrophy was 0.68 and 0.80, respectively. The most important quantitative features of the progression of retinal pathology were the thickness of the outer part of the retina, hyperreflexive lesions and drusen. It is interesting to compare the possibilities of predicting the complications of AMD with artificial intelligence. Peng Y. et al. [10] developed a neural network system (DeepSeeNet) to classify patients' retinal pathology automatically on the AREDS Simplified Severity Scale using bilateral ophthalmoscopy. The system is ahead of retinal specialists in the detection of large drusen (accuracy of 0.742 against 0.696) and pigment deviations (accuracy of 0.890 versus 0.813;). Thus, the accuracy of predicting complications of AMD based on analysis of platelet receptor activity coincides with those reported in the literature when standard risk factors were used. However, the Pl study in vitro has a significant advantage because specific pathogenetic factors of serous retinal detachment in AMD are identified, and therefore an individual assessment of the risk of this complication in the patient is carried out. The study of the functional activity of Pl receptors allows, to some extent, analyzing the influence of factors of pathogenesis of AMD on other cells that have receptors to these factors.

The key to ensuring the transport of the retinal interstitial fluid is RPE. Transepithelial fluid transport in RPE is controlled by adenosine receptors, with a high dose of adenosine accelerating active transfer through A1 receptors and a low dose slowing through A2 receptors [15]. The problem of receptor interaction in the regulation of RPE cell function was first discussed by Collison D. [16], who established that adenosine could potentiate the effect of ATP, and therefore A1- and A2A-adenosine receptors interact with P2X receptors in the process of increasing the level of intracellular Ca^{2+}. A similar effect was reproduced by α2- and β2-adrenoceptors through cAMP-dependent and independent mechanisms in RPE cells.

It is established that RPE is the most active site of 5'AMP dephosphorylation; moreover, degradation of 5'AMP and adenosine formation is blocked by catecholamines (norepinephrine, adrenalin and phenylephrine) [17]. Thus, adrenaline lowers the level of adenosine and this phenomenon is reflected in our model.

Purines, in particular, ATP and adenosine, act as neuro- and gliotransmitters in the sensory retina, where they participate in the bilateral neuron-glial signaling. A review by Wurm A. [18] summarizes current knowledge about the expression and functional importance of P1 (adenosine) and P2 (nucleotide) receptors in Müller glial cells. Under physiological conditions, Müller cells maintain neural activity and blood-ocular barrier integrity, whereas gliotic changes can contribute to retinal degeneration and edema formation. Reichenbach A. et al. [7] believe that the main function of Müller cells is the absorption of fluid from the retina, which is mediated by transcellular transport of water in combination with electric current through potassium channels. Retinal detachment is characterized by the gliosis of Müller cells and microglial cells, which have shown an increase in Ca2 + levels on the stimulation of purinergic P2X receptors [19]. In a recent report Aires I.D. [20] has shown that blockade of the adenosine A2A receptor prevents the neuroinflammatory response and protects the retinal ganglion cells. In particular, the A2A receptor antagonist (SCH 58261) was shown to reduce the microglial reactivity, expression, and release of proinflammatory cytokines. These results support the assumption that hyperactivity of A2A receptors on Müller cells may be a risk factor for neurodegenerative changes and retinal edema.

The concept of inflammation initiation and its relation to the AMD pathogenesis is discussed by Kauppinen A. [21]. Oxidative stress is thought to reduce intracellular recirculation and degradation of lipid metabolites, resulting in a decrease in heterophagy / autophagy, which induces inflammation. The presence of chronic inflammation in the retina dictates the need to identify informative indicators, which would not only determine the severity of inflammation and predict the risks of complications in dry and exudative forms of

AMD, but also optimize therapy. Pinna A. et al [22] analyzed the six most significant biomarkers of inflammation based on the number of shaped blood cells. The results of the study showed that all of these indicators were unreliable biomarkers of the inflammation in AMD.

From our point of view, a promising indicator of inflammation may be the sensitivity of the PAF-receptors Pl, as this reflects their stimulation by activated leukocytes capable of PAF secreting. The result of the interaction of blood cells is the formation of platelet-leukocyte aggregates that provide the recruitment of leukocytes from the vascular bed to the tissues of the retina [23]. Currently, PAF is seen as: (a) a key pro-inflammatory mediator, (b) an element of immune system suppression, (c) a modulator of local angiogenic and cytokine networks [24, 25, 26] provides the evidence of the PAF comntribution in the AMD pathogenesis.

Conclusions

PAF -receptors, A2A receptors, and α2-adrenergic receptors functional activity are independent factors for the prediction of the development of exudative detachment neurosensory retina in the Pl study. The predictive value of the positive result of this complication in the total cohort of patients with exudative form of AMD was 87.5% (95% CI 69.6% - 95.5%) under the combination of independent prognostic factors. This fact reflects the interaction of PAF -receptors, A2A receptors and α2-adrenoceptors in the pathogenesis of interstitial fluid accumulation in the retina. The revealed relationship between the development of serous neuro retinal detachment and the functional state of Pl may be beneficial for the selection of patients with retinal detachment in AMD for preventive treatment.

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