ხარვეზებს და პასუხობს კვლევის მთავარ შეკითხვას, რომ განვითარებად ქვეყნებში ვაქცინაციის პროცესი ეფექტურად არ მიმდინარეობს, რაც გამოწვეულია ორი მთავარი ფაქტორით: 1) ვაქცინების მარაგი, 2) საზოგადოების მზაობა და ვაქცინაციის სურვილი. საქართველო, როგორც განვითარებადი ქვეყანა, ანალოგიური გამოწვევის წინაშეა, ვაქცინაციასთან დაკავშირებით საზოგადოების ცნობიერების ამაღლების აქტიური კამპანიის გარეშე ეფექტური ვაქცინაციის პროცესი და სასურველი შედეგის მიღწევა რთული იქნება.

MICROENVIRONMENT ALTERATIONS IN CONJUNCTIVAL NEOPLASTIC LEOSIONS WITH DIFFERENT PROLIFERATION-APOPTOTIC CHARACTERISTICS

[1]Nikolaishvili N., [1]Chichua G., [2]Muzashvili T., [2]Burkadze G.

[1]The New Vision University; [2]Tbilisi State Medical University, Georgia

Recent research shows the important role of tumor immune microenvironment in the formation and progression of different types of cancers [1]. Tumor immune microenvironment is mainly composed of different types of infiltrating T lymphocytes, including CD8+ cytotoxic T cells. In addition, there is the substantial number of Foxp3+ regulatory T cells in the tumor microenvironment [2]. Recently, it has been noted that not only tumor infiltrating lymphocytes (TILs) [3], but also tumor associated neutrophils (TANs) [4], may play an important role in the progression of different malignant tumors. Some studies also indicate that the distribution of TILs and TANs might be associated with the molecular characteristics of different tumors [5]. Many studies have also shown that not only the presence or the absence of TILs and neutrophils in immune tumor microenvironment affect the development and prognosis of solid tumors, but also their specific distribution in the tumor, including for example tumor bead, tumor margin or tumor associated stroma is also important [6]. International immune-oncology working group recommended the evaluation of TILs in standard haematoxylin and eosin (H&E) stained sections in different cancer types [3]. However, many investigators also employ and immunohistochemical evaluation of the different subsets of T cells, by specific markers, including CD3, CD8 and Foxp3.

Pathological assessment of TILs by human eye is considered as a gold standard in diagnostic pathology [3]. However, the human eye based assessment is subjective and characterised with high interobserver variability [7]. Recently, the development of digital pathology applications opened the new window for the detailed and objective quantification of cells in immune tumor microenvironment [7]. One of the widely used application in digital pathology, amongst others is the freely available software QuPath [8]. The software allows the investigator the specific cell quantification and analysis in different tumor areas in both H&E and IHC stained slides, producing the robust and reliable data for further statistical analysis [8].

The role of tumor infiltrating lymphocytes as well as the role of tumor associated neutrophils has not been investigated in conjunctival intraepithelial lesions. The aim of our study was to investigate the distribution patterns of TILs and TANs in different types of conjunctival lesions with different proliferation and apoptotic characteristics.

Material and methods. Study included formalin-fixed and paraffin-embedded (FFPE) tissue sections of 10 normal conjunctivas, 12 actinic keratosis, 25 pterigeas, 14 CoIN1, 12 CoIN2, 8 CoIN3 and 7 squamous cell carcinoma, altogether 88 cases. FFPE tissue blocks were retrieved from the teaching, research and diagnostic laboratory of Tbilisi State Medical University. H&E stained sections were revised and diagnosed by two independent pathologists (T.M., G.B.).

Digital analysis of tumor associated neutrophils (TANs) and tumor infiltrating lymphocytes (TILs). The analysis of TANs and TILs was performed using freely available digital pathology analysis software QuPath (V 0.2.1) as following: 10 randomly selected high power fields of H&E stained sections were captured from each case using the digital camera of Leica 3000 microscope. Then, the images were included in the digital pathology software QuPath. Relevant areas such as the lesion, normal tissue, subepithelial and intraepithelial areas were manually annotated and staining vectors were corrected. The number of TILs was evaluated using QuPath's automatic cell detection system, whilst the number of TANs were counted manually. All cell detections were converted into numbers and finally the average number of TANs and TILs were recorded for each case. The digital analysis algorithm is given in Fig. 1.

Immunohistochemistry. Tissue sections were stained by standard immunohistochemical procedure, using antibodies against: Ki67, Bcl2, p53, CD3, CD8, Foxp3. Similar digital analysis algorithm was used for the counting of CD3, CD8 and Foxp3 in two major areas of the lesions: the subepithelial compartment and in intraepithelial compartment. The average of the detected T cells was recorded. In addition, the Ki67 and Bcl2 labelling index was evaluated by two independent pathologists (G.B. and T.M.) as the percentage of Ki67 and Bcl2 positive cells in the lesion. The Ki67 and Bcl2 labelling index was divided into low (≤10%) and high (>10%) labelling index. The presence of p53 mutations was evaluated as following: the cases with either strong expression of p53 or complete loss of p53 staining were considered as p53 mutant. The cases with the average expression of p53 were considered as wild type (WT).

The number of marker positive cells has been recorded and analysed with the following statistical methods: correlations were assessed using Spearman's rank test and comparisons between

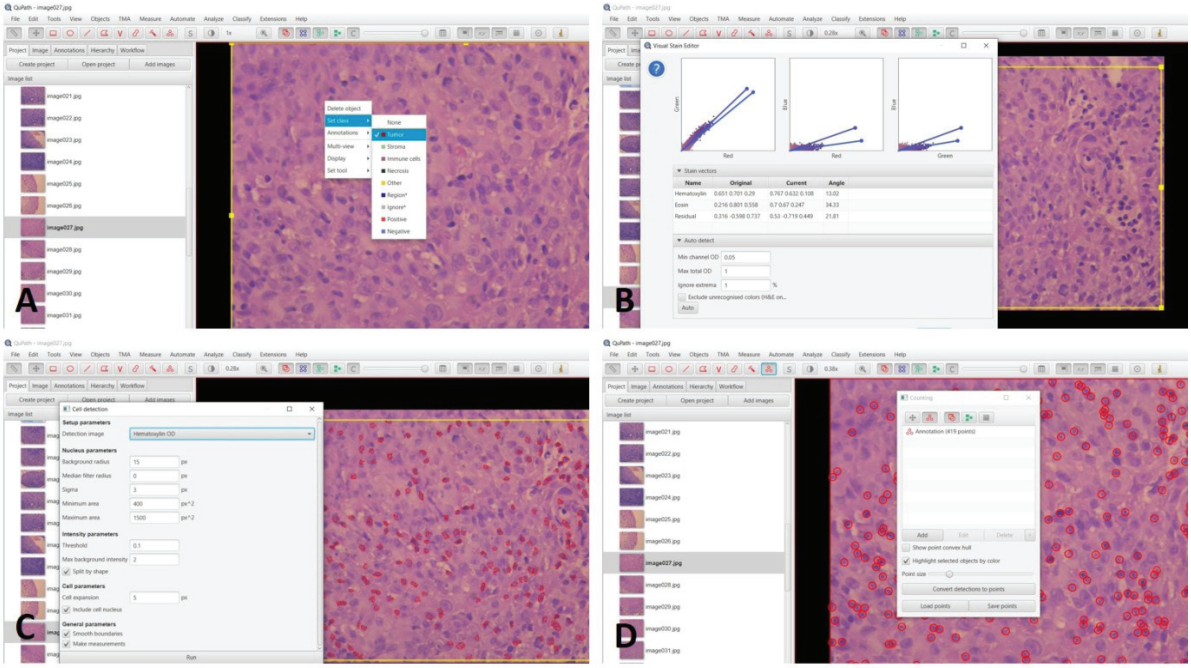


Fig. 1.Tils digital analysis algorithm: A. annotation of the areas of interest, B. estimating the staining vectors, C. TILs detection, D. converting the detection into points

Table 1. Distribution of neutrophils, lymphocytes and neutrophil/lymphocyte ratio in conjunctival lesions

	TANs		TILs		NLR	
	Intraepithelial	Subepithelial	Intraepithelial	Subepithelial	Intraepithelial	Subepithelial
Normal Conjunctiva	0	4	3	23.5	na	0.17
Actinic Keratosis	0	15	7	46.6	na	0.32
Pterygea	0	4	0	21	na	0.19
CoIN1	3	10	9	115	0.33	0.09
CoIN2	4	12	10	122	0.40	0.10
CoIN3	9	22	16	155	0.56	0.14
CSCC	23	35	33	201	0.70	0.17

Red squares mark the highest number and blue squares mark to lowest number, TANs, tumor associated neutrophils, TILs, tumor infiltrating lymphocytes, NLR, neutrophil/lymphocyte ratio, CoIN, conjunctival intraepithelial neoplasia, CSCC, conjunctival squamous cell carcinoma

groups were evaluated using Mann-Whitney and Kruskal-Wallis test. The sensitivity and specificity of the test was assessed using 95% confidence interval. P value <0.05 was considered as statistically significant. All statistical tests were performed using SPSS statistical software V20.00.

Results and discussion. Neutrophil infiltration was not detected in the intraepithelial compartments of normal conjunctiva, actinic keratosis and pterigea. Mean neutrophil count was 4±1.2 in subepithelial component of normal conjunctiva, 15±4.3 in actinic keratosis and 4±2.3 in pterigea. In CoIN1 the mean intraepithelial neutrophil count was 3±1.1 and subephitelial neutrophil count was 10±2.3. In CoIN2 mean intraepithelial neutrophil count was 4±2.2 and subepithelial neutrophil count was 12±4.3. In CoIN3 mean intraepithelial neutrophil count was 9±3.3 and mean subepithelial neutrophil count was 35±7.8. In squamous cell carcinoma mean intraepithelial neutrophil count was 23±4.8 and mean subepithelial lymphocyte count was 35±7.2.

Mean intraepithelial lymphocyte count was 3±0.9 and mean subepithelial lymphocyte count was 23.5±6.7 in normal conjunctiva. In actinic keratosis mean intraepithelial lymphocyte count was 7±2.3 and subepithelial lymphocyte count was 46.6±6.9. In pterigea intraepithelial lymphocytes were not detected, whilst mean subepithelial lymphocyte count was 21±3.8. In CoIN1 mean intraepithelial lymphocyte count was 9±3.5 and subephitelial lymphocyte count was 115±15.7. In CoIN2 mean intraepithelial lymphocyte count was 10±3.7 and subepithelial lymphocyte count was 122±15.4. In CoIN3 mean intraepithelial lymphocyte count was 16±4.4 and mean subepithelial lymphocyte count was 155±23.8. In squamous cell carcinoma mean intraepithelial lymphocyte count was 33±5.7 and mean subepithelial lymphocyte count was 201±30.8.

The neutrophil/lymphocyte ratio (NLR) was not possible to count in intraepithelial compartment of normal conjunctiva, actinic keratosis and pterigea, as there were no neutrophils detected in these lesions. The mean NLR in subepithelial compartment of normal conjunctiva was 0.17, in actinic keratosis it was 0.32 and in pterigea it was 0.19. In CoIN1 the mean NLR in intraepithelial compartment was 0.33 and insubepithelial compartment

was 0.09. In CoIN2 mean NLR in intraepithelial compartment was 0.4 and in subepithelial compartment was 0.1. In CoIN3 mean NLR in intraepithelial compartment was 0.56 and in subepithelial compartment was 0.14 and in CSCC mean NLR in intraepithelial compartment was 0.7 and in subepithelial compartment was 0.17.

Mean intraepithelial CD3+lymphocyte count was 4±2.2 in normal konjunctiva, 5.6±1.2 in actinic keratosis, 0 in pterigea, 7.2±2.2 in CoIN1, 8±3.6 in CoIN2, 12.8±4.1 in CoIN3 and 111±15.7 in CSCC. Mean subepithelial CD3+ lymphocyte count was 18.8±4.1 in normal conjunctiva, 37.3±4.8 in actinic keratosis, 16.8±2.6 in pterigea, 92±10.7 in CoIN1, 97.6±7.8 in CoIN2, 124±14.8 in CoIN3 and 257.6±25.9 in CSCC.

Mean intraepithelial CD8+ lymphocyte count was 3±2.1 in normal konjunctiva, 3.92±1.9 in actinic keratosis, 0 inpterigea, 5.04±2.2 in CoIN1, 5.06±1.3 in CoIN2, 8.96±2.2 in CoIN3 and 77.7±15.7 in CSCC. Mean subepithelial CD8+ lymphocyte count was 13.2±4.1 in normal conjunctiva, 26.11±2.2 in actinic keratosis, 11.76±2.9 in pterigea, 64.4±10.9 in CoIN1, 68.3±6.1 in CoIN2, 86.8±12.3 in CoIN3 and 180.3±15.9 in CSCC.

Mean intraepithelial Foxp3+ lymphocyte count was 1±0.2 in normal konjunctiva, 4.1±1.6 in actinic keratosis, 0 inpterigea, 2.1±1.2 in CoIN1, 2.9±1.3 in CoIN2, 6.2±2.8 in CoIN3 and 58.7±12.4 in CSCC. Mean subepithelialFoxp3+ lymphocyte count was 3.2±1.1 in normal conjunctiva, 7.8±2.9 in actinic keratosis, 4.1±2.3 in pterigea, 20.1±5.9 in CoIN1, 23.9±4.3 in CoIN2, 41.7±7.3 in CoIN3 and 78.7±14.3 in CSCC.

Mean intraepithelial Foxp3+/CD8+ lymphocyte ratio was 0.33 in normal konjunctiva, 1.05 in actinic keratosis, 0.42 in CoIN1, 0.57 in CoIN2, 0.69 in CoIN3 and 0.76 in CSCC. Mean subepithelial Foxp3+/CD8+ lymphocyte ratio was 0.24 in normal conjunctiva, 0.3 in actinic keratosis, 0.35 in pterigea, 0.31 in CoIN1, 0.35 in CoIN2, 0.48 in CoIN3 and 0.44 in CSCC.

Table 2. Distribution of CD3, CD8 and Foxp3+ lymphocytes, as well as Foxp3+/CD8 lymphocyte ratio in conjunctival intraepithelial lesions

	CD3		CD8		Foxp3		Foxp3/CD8 ratio	
	Intraepithelial	Subepithelial	Intraepithelial	Subepithelial	Intraepithelial	Subepithelial	Intraepithelial	Subepithelial
Normal Conjunctiva	4	18.8	3	13.2	1	3.2	0.33	0.24
Actinic Keratosis	5.6	37.3	3.92	26.11	4.1	7.8	1.05	0.30
Pterigea	0	16.8	0	11.76	0	4.1	Na	0.35
CoIN1	7.2	92	5.04	64.4	2.1	20.1	0.42	0.31
CoIN2	8	97.6	5.06	68.3	2.9	23.9	0.57	0.35
CoIN3	12.8	124	8.96	86.8	6.2	41.7	0.69	0.48
CSCC	111	257.6	77.7	180.3	58.7	78.7	0.76	0.44

Red squares mark the highest number and blue squares mark to lowest number, CoIN, conjunctival intraepithelial neoplasia, CSCC, conjunctival squamous cell carcinoma

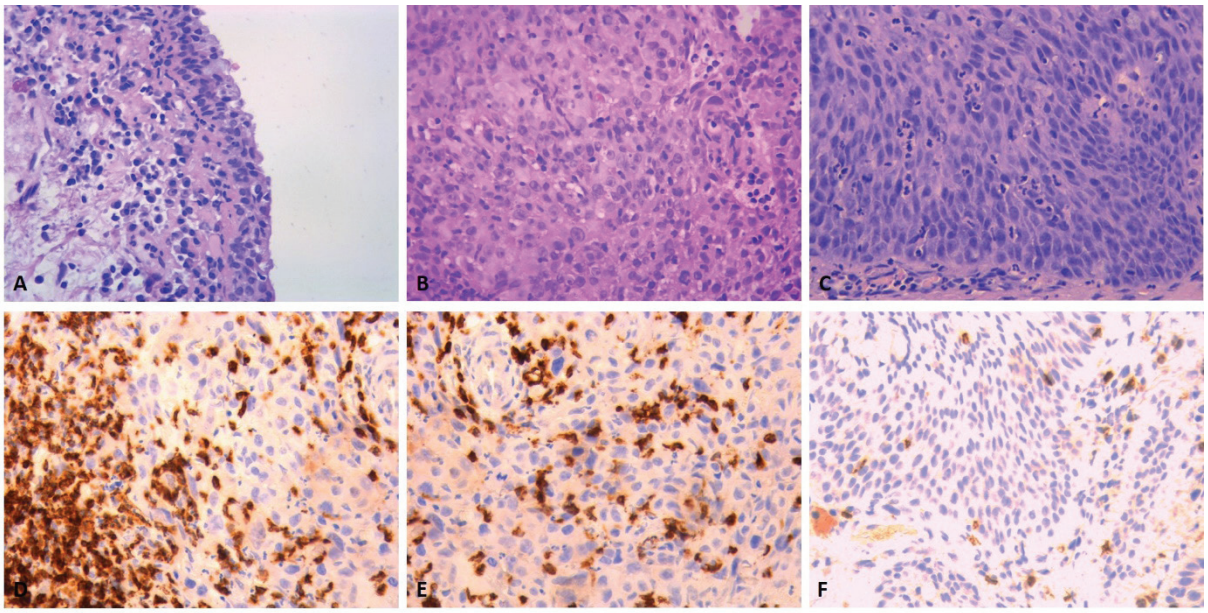


Fig. 2. A. intraepithelial and subepithelial lymphocytes in normal conjunctiva, B. intraepithelial lymphocytes in CSCC, C. intraepithelial neutrophils in CSCC, D. CD3+ T cells in CSCC, E. CD8 + T cells in CSCC, F. Foxp3+ T regulatory cells in CSCC, IHC, x200

Low (≤10) Ki67 labelling index was detected in all 10/10 (100%) cases of normal konjunctiva, in 7/12 (58.3%) cases of actinic keratosis, in 23/25 (92%) cases of pterigea, in 9/14 (64.3%) cases of CoIN1, in 8/12 cases of CoIN2 (66.7%), in 2/8 (25%) cases in CoIN3 and 0/7 (0%) cases in conjunctival squamous cell carcinoma. High (>10) Ki67 labelling index was not detected in normal konjunctival epithelium, it was detected in 5/12 (41.7%) cases of actinic keratosis, in 2/25 (8%) cases of pterygeum, in 5/14 (35.7%) cases of CoIN1, in 4/12 (33.3%) cases of CoIN2, in 6/8 (75%) cases of CoIN3 and in all 7/7 (100%) cases of CSCC.

Low (≤10) Bcl2 labelling index was not detected in normal konjunctiva, it was detected in 4/12 (33.3%) cases of actinic keratosis,

in3/25 (12%) cases of pterigea, in 4/14 (28.6%) cases of CoIN1, in 5/12 (46.7%) cases of CoIN2, in 6/8 (75%) cases in CoIN3 and 7/7 (100%) cases in conjunctival squamous cell carcinoma. High (>10) Bcl2 labelling index wasdetected in all 10/10 (100%) cases of normal konjunctival epithelium, in 8/12 (66.7%) cases of actinic keratosis, in 22/25 (88%) cases of pterygeum, in 10/14 (71.4%) cases of CoIN1, in 7/12 (58.3%) cases of CoIN2, in 2/8 (25%) cases of CoIN3 and none of the cases in CSCC (0%).

Mutated p53 was not detected in normal konjunctival epithelium, it was detected in 3/12 (25%) cases of actinic keratosis, 8/25 (32%) cases of pterygeum, 4/14 (28.6%) cases in CoIN1, 5/12 (41.7%) cases in CoIN2, 6/8 (75%) cases in CoIN3 and all 7/7 (100%) cases in CSCC.

All 10/10 cases of normal conjunctiva were grouped as Ki67 low/Bcl2 high.In addition, Ki67 low/Bcl2 high group included 7/12 (58.3%) actinic keratosis, 22/25 (88%)pterigea, 9/14 (64.2%) CoIN1, 7/12 (58.3%) CoIN2, 2/8 (25%) CoIN3 and 0/7 (0%)CSCC.Ki67 high/Bcl2 low group included 5/12 (41.7%) actinic keratosis, 3/25 (12%)pterigea, 5/14 (35.7%) CoIN1, 5/12 (41.7%) CoIN2, 6/8 (75%) CoIN3 and 7/7 (100%)CSCC. Ki67 high/Bcl2 low group did not include normal conjunctival samples.

The distribution of neutrophils, lymphocytes, CD3+ T cells, CD8+ T cells and Foxp3+ T regulatory cells in different proliferation apoptotic groups as well as in groups with different p53 mutation status is given in tables 1 and 2.

Table 3. Distribution of Ki67, Bcl2 and mutant P53 in conjunctival lesions. mut., mutant

	N	Ki67		Bcl2		mut. P53	
		≤10	>10	≤10	>10	No	Yes
Normal Conjunctiva	10	10	0	0	10	10	0
Actinic Keratosis	12	7	5	4	8	9	3
Pterigea	25	23	2	3	22	17	8
CoIN1	14	9	5	4	10	10	4
CoIN2	12	8	4	5	7	7	5
CoIN3	8	2	6	6	2	2	6
CSCC	7	0	7	7	0	0	7

Table 4. Distribution of neutrophils and lymphocytes in different proliferation/apoptotic groups and p53 status cases of conjunctival lesions

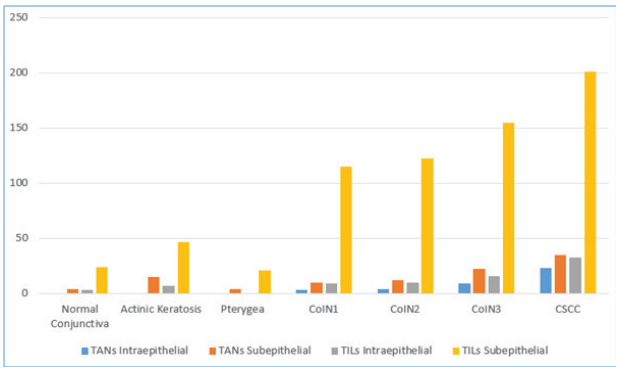
		TANs		TILs	
		Intraepithelial	Subepithelial	Intraepithelial	Subepithelial
Normal Conjunctiva	Ki67 low/Bcl2 high, WT P53	0	4	3	23.5
Actinic Keratosis	Ki67 low/Bcl2 high	0	7	2	32.3
	Ki67 High/Bcl2 Low	0	18	5	52.7
	mut. P53	0	29	11	59.8
	WT P53	0	14	4	28.1
Pterigea	Ki67 low/Bcl2 high	0	2	0	16
	Ki67 High/Bcl2 Low	0	4	0	21
	mut. P53	0	6	0	27
	WT P53	0	0	0	19
CoIN1	Ki67 low/Bcl2 high	2	5	4	59
	Ki67 High/Bcl2 Low	5	12	6	103
	mut. P53	6	14	18	142
	WT P53	0	4	8	90
CoIN2	Ki67 low/Bcl2 high	2	3	5	81
	Ki67 High/Bcl2 Low	4	4	8	144
	mut. P53	8	10	21	151
	WT P53	3	2	9	93
CoIN3	Ki67 low/Bcl2 high	3	12	10	74
	Ki67 High/Bcl2 Low	5	20	26	112
	mut. P53	11	26	29	182
	WT P53	7	10	14	120
CSCC	Ki67 high/Bcl2 Low mut. P53	23	35	33	201

Red squares mark the highest number and blue squares mark to lowest number, TANs, tumor associated neutrophils, TILs, tumor infiltrating lymphocytes, WT, wild type, CoIN, conjunctival intraepithelial neoplasia, CSCC, conjunctival squamous cell carcinoma

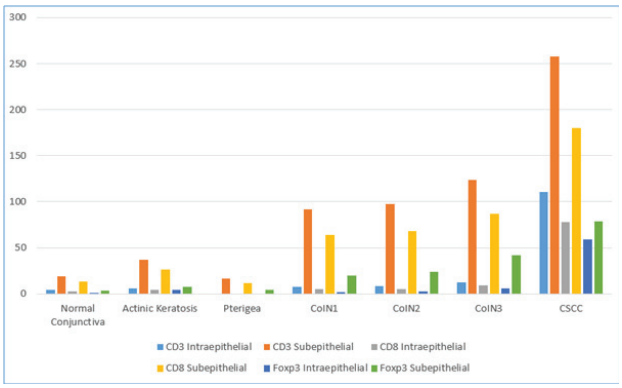
Table 5. Distribution of CD3, CD8 and Foxp3 in different proliferation/apoptotic groups and p53 status cases of conjunctival lesions

		CD3		CD8		Foxp3	
		Intraepithelial	Subepithelial	Intraepithelial	Subepithelial	Intraepithelial	Subepithelial
Normal Conjunctiva	Ki67 low/Bcl2 high, WT P53	2	16	1	12	0	7
Actinic Keratosis	Ki67 low/Bcl2 high	2	27	1	19	0	10
	Ki67 High/Bcl2 Low	4	49	2	36	1	16
	mut. P53	8	42	4	29	2	23
	WT P53	3	24	2	15	0	9
Pterigea	Ki67 low/Bcl2 high	0	11	0	6	0	3
	Ki67 High/Bcl2 Low	0	16	0	12	0	4
	mut. P53	0	19	0	14	0	12
	WT P53	0	12	0	7	0	3
CoIN1	Ki67 low/Bcl2 high	3	42	2	32	1	17
	Ki67 High/Bcl2 Low	4	79	3	61	2	41
	mut. P53	14	123	9	97	6	56
	WT P53	6	81	4	68	2	33
CoIN2	Ki67 low/Bcl2 high	4	56	3	39	1	20
	Ki67 High/Bcl2 Low	6	112	4	99	2	59
	mut. P53	17	130	12	114	9	71
	WT P53	5	71	4	43	3	23
CoIN3	Ki67 low/Bcl2 high	8	59	7	56	4	48
	Ki67 High/Bcl2 Low	21	96	16	80	12	55
	mut. P53	23	142	17	112	14	73
	WT P53	10	101	8	79	4	52
CSCC	Ki67 high/Bcl2 Low mut. P53	111	257.6	77.7	180.3	58.7	78.7

Red squares mark the highest number and blue squares mark to lowest number, TANs, tumor associated neutrophils, TILs, tumor infiltrating lymphocytes, WT, wild type, CoIN, conjunctival intraepithelial neoplasia, CSCC, conjunctival squamous cell carcinoma



Graph 1. Distribution of neutrophils and lymphocytes in different conjunctival lesions. TANs, tumor associated neutrophils, TILs, tumor infiltrating lymphocytes

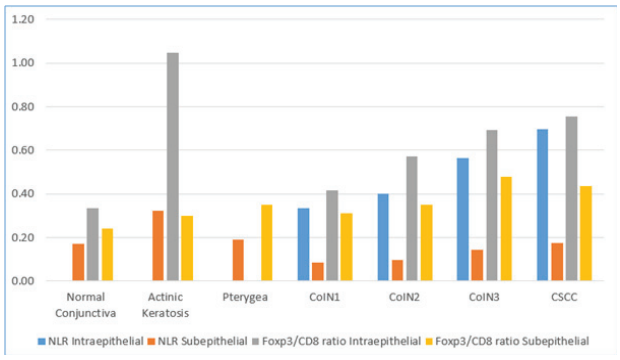


Graph 2. Distribution of CD3, CD8 and Foxp3 in different conjunctival intraepithelial lesions

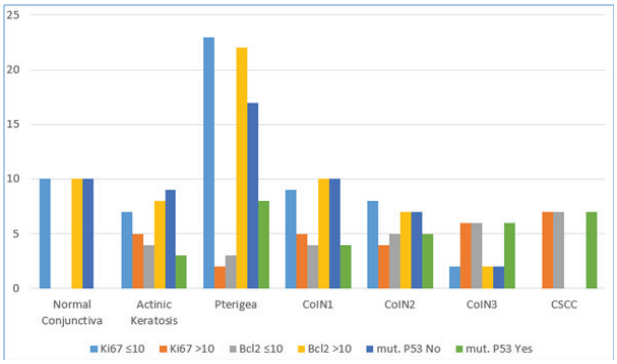
The distribution analysis of neutrophils and lymphocytes in different conjunctival lesions, as well as in normal conjunctiva indicated that the number of subepithelial neutrophils and lymphocytes are always higher in all cases compared to intraepithelial neutrophils and lymphocytes. The number of subepithelial, as well as intraepithelial lymphocytes are significantly increased during the progression of conjunctival intraepithelial lesions, showing highest infiltration in CoIN3 and CSCC. In addition, the infiltration with lymphocytes is significantly higher compared to the infiltration with neutrophils. With regards to pterigea, intraepithelial lymphocytes and neutrophils were not detected in this lesion.

The analysis of the distribution of CD3+, CD8+ and Foxp3+ lymphocytes in different conjunctival lesion showed that there is the significant correlation between these three markers. The distribution of mentioned markers is somewhat similar in CoIN1 and CoIN2, which is significantly higher compared to normal konjunctiva, actinic keratosis and pterigea and significantly lower compared to CoIN3 and CSCC. The highest infiltration with CD3, CD8 and Foxp3 was detected in CSCC. Similar to the H&E based analysis of neutrophils and lymphocytes, the number of CD3+, CD8+ and Foxp3+ lymphocytes are significantly higher in subepithelial compartment, compared to intraepithelial compartment. These markers, were not detected in intraepithelial compartment of pterigea.

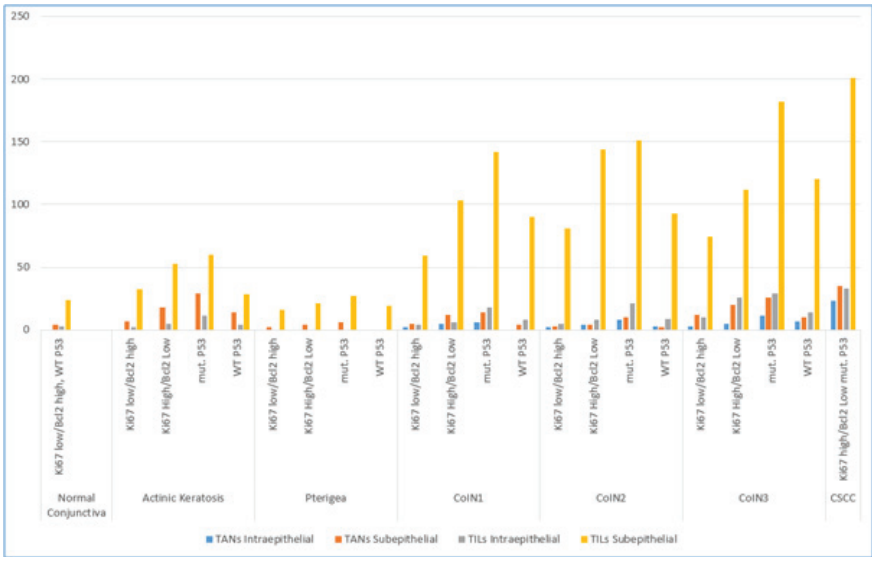
The analysis of neutrophil/lymphocyte ratio also indicated that this ratio is significantly increased during the progression of conjunctival intraepithelial lesions, reaching it's maximum in CSCC. The analysis of CD8/Foxp3 ratio indicated that the highest CD8/Foxp3 ratio is detected in the intraepithelial component of actinic keratosis. However, it is also significantly increased during the progression of conjunctival intraepithelial neoplasia, reaching its maximum in CSCC. With regards to CD8/Foxp3 ratio in subepithelial compartment, also it was increased in the progression of conjunctival intraepithelial neoplasia, this difference did not reach the statistical significance.



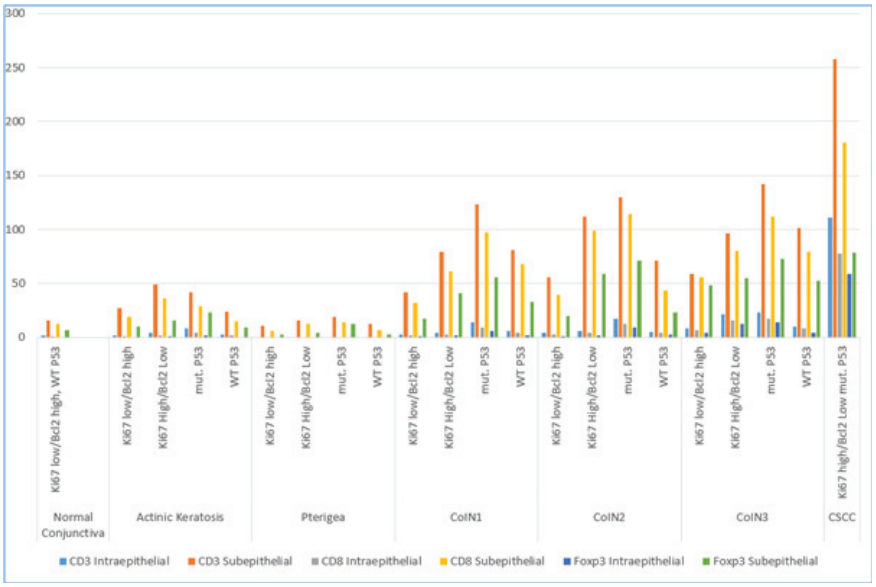
Graph 3. Distribution of neutrophil/lymphocyte ratio (NLR) and Foxp3/CD8 ratio in different conjunctival lesions



Graph 4. Distribution of Ki67, Bcl2 and p53 in different conjunctival lesions



Graph 5. Distribution of neutrophils and lymphocytes in different conjunctival lesions, with different proliferation/apoptotic and p53 status.TANs, tumor associated neutrophils, TILs, tumor associated lymphocytes, mut., mutant, WT, wild type



Graph 6. Distribution of CD3, CD8 and Foxp3 in different conjunctival lesions with different proliferation/apoptotic and p53 status

The analysis of the proliferation and apoptotic markers in conjunctival intraepithelial lesions indicated that high proliferation activity, measured as Ki67 labelling index >10, was not detected in normal conjunctiva, whilst there were no cases with low Ki67 labelling index in CSCC. The proliferation index was also significantly increased during the progression of conjunctival intraepithelial lesions, whilst an apoptotic index based on Bcl2 labelling was significantly decreased. With regards to p53 mutations, it was not detected in normal conjunctiva, whilst it was detected in all cases of CSCC. p53 mutations were also detected in the minority of samples with actinic keratosis and pterigea.

The correlation analysis between the infiltration with intraepithelial neutrophils and Ki67 labelling index showed the positive correlation ($r=30.8$, $p<0.05$) in all conjunctival lesions, whilst there was a negative correlation between infiltration with intraepithelial neutrophils and Bcl2 labelling index ($r=-52.3$, $p<0.05$). In addition, the infiltration with intraepithelial neutrophils was positively correlated with the presence of p53 mutations ($r=42.3$, $p<0.05$). The correlation between subepithelial neutrophils and Ki67, Bcl2 and p53 did not reach the significance. Similar to neutrophils the infiltration with intraepithelial lymphocytes were positively correlated with the Ki67 labelling index ($r=61.3$, $p<0.05$) and negatively correlated with Bcl2 labelling index ($r=-44.8$, $p<0.05$). Intraepithelial lymphocyte infiltration was also significantly correlated with the presence of p53 mutations ($r=35.9$, $p<0.05$). The infiltration with subepithelial lymphocytes were not significantly correlated with the proliferation and apoptotic markers and p53. However, the highest numbers of both subepithelial and intraepithelial lymphocytes was seen in Ki67 high/Bcl2 low and p53 mutated cases in all conjunctival lesions.

The correlation analysis of the distribution of CD3+ intraepithelial lymphocytes showed the positive correlation between CD3+ T cells and Ki67 labelling index ($r=38.9$, $p<0.05$) and negative correlation between CD3+ T cells and Bcl2 labelling index ($r=-36.3$, $p<0.05$). Similar significant association was not seen between the distribution of CD3+ subepithelial T cells and proliferation and apoptotic index. Even though, both intraepithelial and subepithelial CD3+ lymphocytes were significantly higher in Ki67 high/Bcl2 low cases. Similar pattern was seen with regards to correlation of CD3+ intraepithelial T cell infiltration and p53 mutations ($r=59.3$, $p<0.05$). The correlation analysis of CD8+ intraepithelial T cells also showed the significant associations with Ki67 labelling index ($r=54.2$, $p<0.05$) and Bcl2 labelling index ($r=-51.3$, $p<0.05$). Similar results were found with the correlation analysis of Foxp3+ intraepithelial T regulatory cells and Ki67 labelling index ($r=39.1$, $p<0.05$) and Bcl2 labelling index ($r=-36.3$, $p<0.05$).

To the best of our knowledge we are first who analysed the distribution of TILs and TANs in different types of conjunctival lesions with various proliferation and apoptotic features and p53 status. However, previous studies in other tumors, such as cervical carcinoma for example, also indicate that the number of TILs is increased in the progression of cervical intraepithelial neoplasia and it also correlates with the clinical outcome and response to immunotherapy in cervical cancer [9]. Therefore, we could speculate that the increased TILs in conjunctival intraepithelial lesions, might also guide the treatment decision with modern immunotherapeutic drugs which offers the new treatment opportunities for patients suffering from CoIN disease or CSCC. In addition to TILs, similar to our study the neutrophil/lymphocyte ration was also studied in cervical cancer patients. The study results, indicated that NLR, could also serve as not only prognostic but as well as predictive factor [10]. There are not also many studies investigating the relationship between proliferation and apoptotic features or p53 status in different cancers. However, one recent study from Lee at al., indicated that increased p53 expression is associated with higher numbers of TILs [11]. This is in line with our findings as we have also seen high TILs and TANs in p53 mutated cases, including those with higher expression of p53.

Conclusions. The number of intraepithelial TILs as well as the number of intraepithelial TANs are significantly increased during the progression of conjunctival intraepithelial lesions. TILs as well as TANs are significantly associated with the higher proliferation rate, lower apoptotic rate and p53 mutation status in conjunctival intraepithelial lesions. The highest numbers of TILs and TANs were seen in Ki67 high/Bcl2 low and p53 mutated groups.

REFERENCES

1. Hinshaw D. C.. Shevde L. A. The tumor microenvironment innately modulates cancer progression // Cancer Res., 2019. - vol. 79, № 18. - pp. 4557–4567.
2. Petitprez F, Sun CM, Lacroix L, Sautès-Fridman C, de Reyniès A, Fridman WH. Quantitative Analyses of the Tumor Microenvironment Composition and Orientation in the Era of Precision Medicine. // Front Oncol. 2018 Sep 25;8:390. doi: 10.3389/fonc.2018.00390. PMID: 30319963; PMCID: PMC6167550.
3. Hendry S. et al. Assessing Tumor-Infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in Melanoma, Gastrointestinal Tract Carcinom // Adv. Anat. Pathol.- 2017.- vol. 24, №. 6. - pp. 311–335.
4. Jaillon S, Ponzetta A, Di Mitri D, Santoni A, Bonecchi R, Mantovani A. Neutrophil diversity and plasticity in tumour progression and therapy. Nat Rev Cancer. 2020 Sep;20(9):485-503. doi: 10.1038/s41568-020-0281-y. Epub 2020 Jul 21. PMID: 32694624.
5. Fujimoto Y. et al. Prognostic significance of tumor-infiltrating lymphocytes may differ depending on Ki67 expression levels in estrogen receptor-positive/HER2-negative operated breast cancers. // Breast Cancer, 2019. - vol. 26, № 6. - pp. 738–747.
6. Fridman W. Pagès H., F., Sautès-Fridman C. Galon J. The immune contexture in human tumours: impact on clinical outcome // Nat. Rev. Cancer, 2012. - vol. 12, №. 4. - pp. 298–306.
7. Saltz J, Gupta R, Hou L, et al. Cancer Genome Atlas Research Network, Shmulevich I, Rao AUK, Lazar AJ, Sharma A, Thorsson V. Spatial Organization and Molecular Correlation of Tumor-Infiltrating Lymphocytes Using Deep Learning on Pathology Images. // Cell Rep. 2018 Apr 3;23(1):181-193.e7. doi: 10.1016/j.celrep.2018.03.086. PMID: 29617659; PMCID: PMC5943714.
8. Bankhead P.et al. QuPath: Open source software for digital pathology image analysis. // Sci. Rep. - 2017. - vol. 7, № 1, p. 16878.
9. Otter S. J., Chatterjee J., Stewart A. J., Michael A. The Role of Biomarkers for the Prediction of Response to Checkpoint Immunotherapy and the Rationale for the Use of Checkpoint Immunotherapy in Cervical Cancer // Clin. Oncol. - 2019. - vol. 31, №. 12, pp. 834–843.
10. Huang Q.-T. et al. Prognostic significance of neutrophil-to-lymphocyte ratio in cervical cancer: A systematic review and meta-analysis of observational studies // Oncotarget. - 2017.- vol. 8, №. 10. - pp. 16755–16764.

11. Lee M., Park I. A., Heo S.-H.et al. Association between p53 Expression and Amount of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer// J. Pathol. Transl. Med.- 2019. - vol. 53, №. 3, 180-187.

SUMMARY

MICROENVIRONMENT ALTERATIONS IN CONJUNCTIVAL NEOPLASTIC LEOSIONS WITH DIFFERENT PROLIFERATION-APOPTOTIC CHARACTERISTICS

[1]Nikolaishvili N., [1]Chichua G., [2]Muzashvili T., [2]Burkadze G.

[1]The New Vision University; [2]Tbilisi State Medical University, Georgia

Different studies indicate that tumor infiltrating lymphocytes (TILs) and tumor associated neutrophils (TANs) play an important role during the progression of malignant tumors. We have analysed the distribution of tumor associated neutrophils (TANs) and tumor infiltrating lymphocytes (TILs) in different conjunctival lesions, with different proliferation and apoptotic characteristics. The distribution of TILs and TANs were evaluated in standard haematoxylin and eosin (H&E) stained sections using the digital pathology software QuPathin normal conjunctiva, actinic keratosis, pterigea, conjunctival intraepithelial neoplasias (CoIN1-3) and conjunctival squamous cell carcinoma (CSCC). In addition, the expression of following markers were investigated using standard immunohistochemistry: Ki67, Bcl2, p53, CD3, CD8 and Foxp3. The study results indicated that the number of TILs and TANs are significantly increased during the progression of conjunctival intraepithelial lesions. Also, the number of TILs and TANs significantly correlate with higher proliferation index, lower apoptotic index and the p53 mutation status.

Keywords: malignant tumors, tumor infiltrating lymphocytes, tumor associated neutrophils, conjunctival lesions, conjunctival intraepithelial lesions

РЕЗЮМЕ

ИЗМЕНЕНИЯ МИКРОСРЕДЫ В НЕОПЛАСТИЧЕСКИХ ПОРАЖЕНИЯХ КОНЪЮНКТИВЫ С РАЗНЫМИ ПРОЛИФЕРАТИВНО-АПОПТОЗНЫМИ ХАРАКТЕРИСТИКАМИ

[1]Николаишвили Н.Д., [1]Чичуа Г.А., [2]Музашвили Т.З., [2]Буркадзе Г.М.

[1]Университет New Vision, Тбилиси; [2]Тбилисский государственный медицинский университет, Грузия

Ранее проведенные исследования показали, что инфильтрирующие опухоль лимфоциты, также как и ассоциированные с опухолью нейтрофилы, играют значимую роль в процессе прогрессии опухоли.

Цель исследования - определить особенности распределения инфильтрирующих опухоль лимфоцитов и ассоциированных с опухолью нейтрофилов в поражениях конъюнктивы с различными пролиферативно-апоптозными характеристиками.

В препаратах, окрашенных стандартным гематоксилином и эозином определены особенности распределения лимфоцитов и нейтрофилов в нормальной конъюнктиве, актиновом кератозе, птеригиуме, интраэпителиальных поражениях (CoIN1-3) конъюнктивы и в плоскоклеточной карциноме конъюнктивы с помощью цифровой программы QuPath. Стандартным иммуногистохимическим методом изучены следующие молекулярные маркеры: Ki67, Bcl2, p53, CD3, CD8 и Foxp3.

Результаты исследования показали, что инфильтрация интраэпителиальными лимфоцитами и нейтрофилами значительно возрастает в процессе прогрессии интраэпителиальных поражений конъюнктивы и находится в статистически значимой корреляции с высоким пролиферативным индексом, низким апоптозным индексом и со статусом мутаций p53.

რეზიუმე

მიკროგარემოს ცვლილებები კონიუქტივის ნეოპლაზიურ დაზიანებებში სხვადასხვა პროლიფერაციულ-აპოპტოზური მახასიათებლებით

[1]ნ. ნიკოლაიშვილი, [1]გ. ჩიჩუა, [2]თ. მუზაშვილი, [2]გ. ბურკაძე

[1]უნივერსიტეტი New Vision, თბილისი; [2]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, საქართველო

სხვადასხვა კვლევებით ნაჩვენებია, რომ სიმსივნის მაინფილტრირებელი ლიმფოციტები, ისევე როგორც სიმსივნესთან ასოცირებული ნეიტროფილები, მნიშვნელოვან როლს თამაშობენ რიგი სიმსივნეების პროგრესიის პროცესში.

კვლევის მიზანს წარმოადგენს სიმსივნის მაინფილტრირებელი ლიმფოციტების და სიმსივნესთან ასოცირებული ნეიტროფილების განაწილების თავისებურებების შესწავლა კონიუქტივის დაზიანებებში სხვადასხვა პროლიფერაციული და აპოპტოზური მახასიათებლებით.

სტანდარტული ჰემატოქსილინით და ეოზინით შეღებილ ანათლებში შეფასებული იყო ლიმფოციტების და ნეიტროფილების განაწილება ციფრული პათოლოგიის პროგრამის QuPath-ის გამოყენებით ნორმალურ კონიუქტივაში, აქტინურ კერატოზში, პტერიგეუმში, კონიუქტივის ინტრაეპითელურ ნეოპლაზიებში (CoIN1-3) და კონიუქტივის ბრტყელუჯრედოვან კარცინომაში. სტანდარტული იმუნოჰისტოქიმიური მეთოდის გამოყენებით შეფასებული იყო შემდეგი მარკერები: Ki67, Bcl2, p53, CD3, CD8 და Foxp3.

კვლევის შედეგებმა აჩვენა, რომ ინტრაეპითელური ლიმფოციტებით და ნეიტროფილებით ინფილტრაცია მნიშვნელოვნად იზრდება კონიუქტივის ინტრაეპითელური დაზიანებების პროგრესიის პროცესში და სტატისტიკურად სარწმუნო კორელაციაშია მაღალ პროლიფერაციულ იდექსთან, დაბალ აპოპტოზურ ინდექსთან და p53 მუტაციის სტატუსთან.