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DETERMINATION OF BUDDING GRADES IN SQUAMOUS CELL CARCINOMAS OF THE OROPHARYNX

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Abstract. Determination of budding grades in squamous cell carcinomas of the oropharynx. Poslavska O.V., Didovets D.Yu., Rakhmanov V.V., Chekan S.M., Alekseenko O.A. *The World Health Organization report on the prevalence of oropharyngeal cancer (OC) places it in sixth place among men and tenth among women worldwide. A pressing problem is the increase in OC cases in recent decades among young patients, which researchers associate infection with high-risk human papillomavirus (HPV). Among all OC, many histological forms are squamous cell carcinomas (SCC). It should be noted that HPV-associated carcinomas have a better prognosis, namely up to 20% of patients do not show recurrence after treatment, which is significantly different from the course observed in patients with HPV-unassociated OC. In the context of cancer prognosis, another important prognostic indicator is interesting - tumor budding (TB), which actively develops on the side of the invasive front of the tumor and is often described in literature as an independent factor of poor prognosis, early vascular invasion and metastasis. There are very few studies comparing the intensity of TB and HPV association for the prognosis of SCC and they mostly concern tumors of other areas of the head and neck, which determines the relevance of this work.The aim of the work is to investigate the distribution of tumor budding gradations according to clinical and morphological characteristics of oropharyngeal squamous cell carcinomas important for prognosis, in particular, to assess the relationship of budding with HPV association (p16 status) and proliferation index (Ki-67 expression).For the study, biopsy and surgical material samples of SCC of the oropharynx of 69 patients (21 women and 48 men) who were in the oncology department of the Dnipropetrovsk Regional Clinical Hospital named after I.I. Mechnikov, Dnipro from 2019 to 2022 and received appropriate treatment were selected. The age of the patients ranged from 45 to 86 years; the average age was 64.09±8.95 years. IHC was performed according to the TermoScientific (TS) protocols with primary antibodies p16 (sp1, RTU, LabVision, USA) and Ki-67 (sp6, RTU, LabVision, USA), and the Lab Vision Quanto (TS, USA) imaging system.The distribution of TB grades according to the three-level system (from 0 to 4 cells - 1 (low), from 5 to 9 clusters – 2 (moderate), from 10 and more – 3 (high) according to clinical and morphological indicators showed a statistically significant difference only by the sex of the patients, namely the gradation of high budding levels among men was significantly higher than among women (10 out of 11 (90.91%) compared to 1 out of 11 (9.09%)), ($p<0.05$), this is probably due to the prevalence of such bad habits among men as smoking and alcohol abuse, which are frequent etiological causes of head and neck cancer development, as an alternative path to the development of OC without HPV infection.Distribution of TB grades in HPV-positive samples (p16 – positive) and HPV-negative (p16-negative) OC demonstrated the largest number of HPV-negative carcinomas in the subgroup with 3 budding grades, namely with grade 1 HPV-negative (p16-negative) were 59.38% of samples (19 out of 32), with grade 2 – 57.69% of samples (15 out of 26), and with grade 3 – already 90.91% of samples (10 out of 11), ($p<0.05$), which confirms the more unfavorable prognosis of HPV-unassociated OC.The proliferation index by Ki-67 did not show a statistically significant relationship with the distribution of TB grades, which makes the latter an independent factor in the prognosis in SCC of the oropharynx.*

Реферат. Визначення градацій брунькування в плоскоклітинних раках ротоглотки. Пославська О.В., Дідовець Д.Ю., Рахманов В.В., Чекан С.М., Алєксєєнко О.А. *Звіт ВООЗ про поширеність раку ротоглотки (РР) ставить його на шосту позицію серед чоловіків і десяту серед жінок у всьому світі. Актуальною проблемою є зростання кількості випадків РР за останні десятиліття серед молодих пацієнтів, що дослідники пов'язують з інфікуванням вірусом папіломи людини (ВПЛ) високого онкогенного ризику. Серед усіх РР більшість гістологічних форм припадає на плоскоклітинні раки (ПР). Треба зазначити, що асоційовані з ВПЛ-інфекцією карциноми мають кращий прогноз, а саме до 20% пацієнтів не демонструють рецидивів після лікування, що значно відрізняється від перебігу, який спостерігається в пацієнтів з ВПЛ-неасоційованими РР. У контексті прогнозу онкозахворювань цікавим є ще один важливий прогностичний показник – пухлинне брунькування (ПБ), що активно розвивається на боці інвазивного фронту пухлини й часто описане в літературі як незалежний фактор поганого прогнозу, ранньої судинної інвазії та метастазування. Досліджень зіставлення інтенсивності ПБ та ВПЛ-асоціації для прогнозу перебігу ПР вкрай мало і вони здебільшого стосуються пухлин інших ділянок голови та шиї, що й обумовлює актуальність цієї роботи. Мета роботи – дослідити розподіл градацій пухлинного брунькування за важливими для прогнозу клінічними та морфологічними характеристиками плоскоклітинних раків ротоглотки, зокрема оцінити зв'язок брунькування з ВПЛ-асоціацією (p16-статусом) та індексом проліферації (експресії Ki-67). Для дослідження були вибрані зразки біопсійного та операційного матеріалу ПР ротоглотки 69 пацієнтів (21 жінки і 48 чоловіків), що перебували в ЛОР-онкологічному відділенні КП «Дніпропетровська обласна клінічна лікарня ім. І.І. Мечникова» ДОР м. Дніпра в період з 2019 до 2022 р. та отримали відповідне лікування. Вік пацієнтів коливався в діапазоні 45-86 років, середній вік становив 64,09±8,95 року. ІГХ проводили за протоколами TermoScientific (TS) з первинними антитілами p16 (sp1, RTU, LabVision, США) та Ki-67 (sp6, RTU, LabVision, США), й системою візуалізації Lab Vision Quanto (TS, США). Розподіл градацій ПБ за трирівневою системою (від 0 до 4 клітин – 1 (низька), від 5 до 9 кластерів – 2 (помірна), від 10 і більше – 3 (висока) за клініко-морфологічними показниками показав статистично достовірну різницю тільки за статтю пацієнтів, а саме: градація високого рівня ПБ серед чоловіків виявилась значно більшою, ніж серед жінок (10 з 11 (90,91%) порівняно з 1 з 11 (9,09%)), ($p<0,05$), вірогідно це пов'язано з поширенням серед чоловіків таких поганих звичок, як паління та зловживання алкоголем, що є частими етіологічними причинами розвитку раків голови й шиї, як альтернативний шлях розвитку РР без ВПЛ-інфекції. Розподіл градацій ПБ у зразках ВПЛ-позитивних (p16 – позитивних) та ВПЛ-негативних (p16 – негативних) РР продемонстрував найбільшу кількість ВПЛ-негативних карцином у підгрупі з 3 градацією брунькування, а саме: з градацією 1 ВПЛ-негативними (p16-негативними) були 59,38% зразків (19 з 32), з градацією 2 – 57,69% зразків (15 з 26), і з 3 градацією – уже 90,91% зразків (10 з 11), ($p<0,05$), що підтверджує більш несприятливий прогноз ВПЛ-неасоційованих РР. Індекс проліферації за Ki-67 не показав статистично достовірного зв'язку з розподілом градацій ПБ, що робить його незалежним фактором прогнозу ПР ротоглотки.*

Before the emergence of COVID-19, malignant tumors ranked second among the causes of death worldwide [20]. In total, in 2022, almost 20 million cases of cancer were detected in the world, with almost half of them being fatal. About 100 thousand of the recorded cases were localized in the oropharyngeal area [3]. In 2024 in Ukraine more than a million cancer patients were registered, almost 10 thousand with pharyngeal lesions [7]. Statistical data make it possible to understand that cancer, including oropharyngeal squamous cell carcinomas (SCC), is a significant problem that leads to many deaths annually and requires further research.

SCC, or epidermoid carcinoma, is one of several types of malignant tumors that originate from squamous epithelial cells. SCC of the head and neck includes the oral cavity, oropharynx, and larynx, and is the most common malignancy arising in this area. The main risk factors are smoking, alcohol consumption, and high-risk human papillomavirus (HPV) [6, 8, 15]. The WHO classification of head and neck tumors, fifth revision, makes a clear distinction between HPV-associated and HPV-non-associated SCCs. The existence of separate terminology for these two tumor types emphasizes the difference in their carcinogenesis based on the potential for involvement of transcriptionally active high-risk HPVs (HPV16, HPV18, etc.) [13]. HPV E7 protein inactivation of pRb leads to over-expression of $p16^{INK4a}$, making it a surrogate marker for HPV. Similarly, HPV E6 protein can inactivate p53 and a lower frequency of p53 mutations is observed in HPV-positive tumors. The p16 marker detects expression of the protein 16^{INK4a}, which is a cyclin-dependent kinase inhibitor and inhibits cell cycle progression at the G1 checkpoint without entering S. Loss of p16 expression due to HPV exposure (deletion, mutation or hypermethylation) is a pathway for the development of SCC and can be used for diagnosis if polymerase chain reaction is too expensive or not available [30].

In the context of cancer prognosis, another important prognostic indicator is interesting – TB, which actively develops on the side of the invasive front of the tumor and is often described in the literature as an independent factor of poor prognosis, early vascular invasion and metastasis. The proliferation index is a very important indicator for determining the activity of the expansion of the

invasive front of the tumor. Tumor budding is most actively manifested precisely at the invasive front, so these indicators should be interconnected. The proliferation index can be obtained by counting the nuclei stained with the Ki-67 marker by immunohistochemical method. This indicator has long become the gold standard in the study of many malignant tumors.

There are very few studies comparing the intensity of TB and HPV association for the prognosis of SCC and they mostly concern tumors of other areas of the head and neck, which determines the relevance of this work.

Aim of the work. To study the distribution of tumor budding gradations according to clinical and morphological characteristics of oropharyngeal squamous cell carcinomas important for prognosis, in particular, to assess the relationship of budding with HPV association (p16 status) and proliferation index (Ki-67 expression).

MATERIALS AND METHODS OF RESEARCH

For the study, biopsy and surgical samples of SCC of the oropharynx of 69 patients (21 women and 48 men) who were in the oncology department of the Dnipropetrovsk Regional Clinical Hospital named after I.I. Mechnikov, Dnipro, Ukraine, in the period from 2019 to 2022 and received appropriate treatment were selected. The age of the patients ranged from 45-86 years; the average age was 64.09±8.95 years. All selected cases were represented by squamous cell carcinomas with keratinization 32 (46.38%) and without keratinization 37 (53.62%). Clinical data on patients with SCC of the oropharynx also included information on the presence of metastases and relapses (the distribution of patients according to clinical data is given in Table 1).

All patients were included in the study after providing their informed written consent. The study was conducted in accordance with the written consent of the participants and in accordance with the principles of bioethics set out in the Declaration of Helsinki "Ethical Principles of Medical Research Involving Humans" [28], the "Universal Declaration on Bioethics and Human Rights (UNESCO)" and national guidelines on bioethical principles of scientific research (excerpt from the protocol of the Biomedical Ethics Committee of Dnipro State Medical University, No. 9 dated October 26, 2021).

Table 1

Distribution of the number of patients with squamous cell carcinoma of the oropharynx according to clinical data, n (%)

Indicator	Clinical data SCCO	Number, n (%)
Sex of patients	**Men**	**48 (69.57)**
	Women	**21 (30.43)**
Age of patients (years)	**40-49**	**5 (7.25)**
	50-59	**17 (24.63)**
	60-69	**26 (37.68)**
	70-79	**18 (26.09)**
	80-89	**3 (4.35)**
Histological form of SCCO	**With keratinization**	**32 (46.38)**
	Without keratinization	**37 (53.62)**
Lymph node involvement in the neck	**N (-)**	**34 (49.28)**
	N (+)	**35 (50.72)**
Presence of recurrences for the period from 2019 to 2022	**Without recurrence**	**64 (92.75)**
	With recurrence	**5 (7.25)**
Total:		**69 (100%)**

Note. SCCO – squamous cell carcinoma of the oropharynx.

Ready-made histological preparations (paraffin sections 4-5 μm thick on glass slides, stained with hematoxylin and eosin) and blocks (fixed in formalin and paraffin-embedded tissues) of selected cases of SCCO for a thorough histological study were taken from the archive of the Dnipro Regional Pathological Anatomical Bureau, Dnipro, Ukraine. When assessing changes in the oropharyngeal mucosa, the internal control (conditional norm) was taken as a multilayered squamous epithelium that fell into the section next to the squamous cell carcinoma at the edges of the resection. Light microscopy in transmitted light was performed with a ZEISS "Primo Star" microscope with a Zeiss Primo Star – Axiocam ERC 5s camera (licensed ZEN 2 blue edition software for obtaining microphotographs) [27].

To obtain new sections of SCCO, adhesive Super Frost Plus slides were used for IHC. Then, deparaffinization, rehydration, temperature unmasking of antigens, inhibition of endogenous peroxidase activity, and incubation of sections with primary antibodies in humid chambers at a temperature of 23-25°C for 30 minutes were performed [17]. Primary monoclonal antibodies to the p16 oncoprotein (sp1, RTU, Lab Vision, USA) were used to detect HPV in SCCO samples, and the Ki-67 marker (sp6, RTU, Lab Vision, USA) was used to calculate the proliferation index. The Ultra Vision Quanto imaging system and the DAB chromogen (brown) were selected according to the manufacturer's protocols [17].

Evaluation of immunohistochemical staining. According to the recommendations of the authors de Ferreira C. et al. (2021) [5], the reaction with the p16 marker in SCCO tumor cells was considered positive for HPV if it demonstrated strong diffuse nuclear-cytoplasmic staining in more than 75% of the cells (Fig. 1).

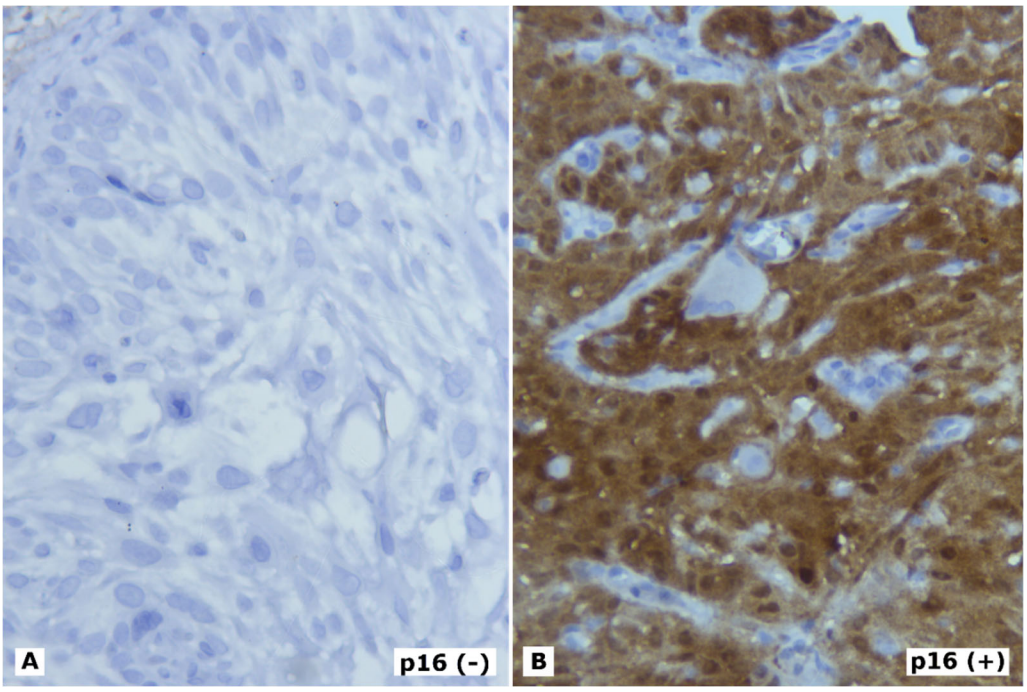

Fig. 1. A. Negative expression of p16 in the SCCO sample (HPV (-) status), IHC method, (×400). B. Positive expression of p16, diffuse nuclear-cytoplasmic staining of more than 75% of SCCO cells (HPV (+) status), IHC method, (×400)

According to the recommendations of the authors Yadav P. et al. (2019) [30], the calculation of the proliferation index in SCCO samples was carried out by the expression of the Ki-67 marker as the percentage of stained cells per 1000 tumor cells in the ×400 microscope field: according to the gradations 0 (<10% of stained cells), 1 (10%-50%) and 2 (>50%). Gradations 1 and 2 were considered a positive reaction, 0 – a negative reaction (Fig. 2).

Statistical analysis of data was performed in the R software environment version 3.4.1 (2017-06-30) – "Single Candle" Copyright (C) 2017; The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit), which is freely distributed under the GNU General Public License. The significance of the difference in the study groups according to the quantitative distribution of samples was determined by Fisher's exact test due to small samples. The difference was considered significant at $p<0.05$ [24].

RESULTS AND DISCUSSION

All observations of SCCO were divided into three groups according to the gradations of TB, according to the general recommendations used for SCC of the head and neck, namely: if there are from 0 to 4 single cells or clusters of up to 5 cells, the TB gradation was considered 1 (low), from 5 to 9 clusters – 2 (moderate), from 10 and more – 3 (high gradation) (Fig. 3). To identify the relationship between clinical and morphological indicators of PRRG and the calculated grades of tumor budding, the number of cases was listed in Table 2.

It should be noted that both among men and women, the distribution between low and moderate gradations of TB was almost equal (20 out of 48, and 18 out of 48 for men, and 12 out of 21, and 8 out of 21 for women), but the gradation of high budding level among male patients significantly prevailed over that of female patients (10 out of 11 (90.91%) compared to 1 out of 11 (9.09%), which according to Fisher's exact test showed a statistically significant difference in distribution ($p<0.05$).

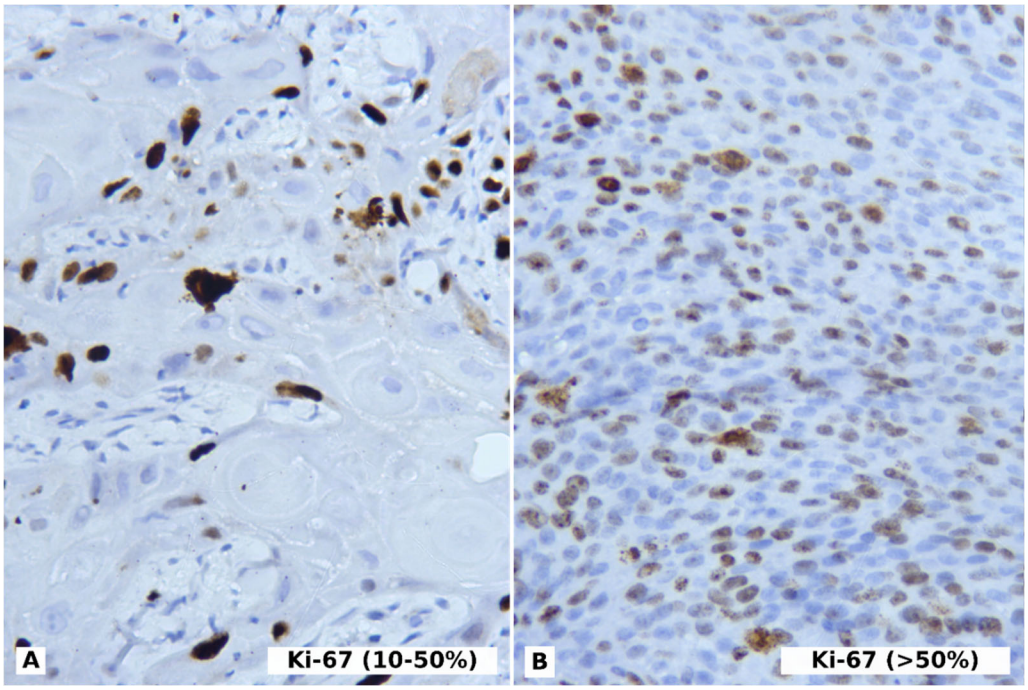

Fig. 2. A. Gradation 1 of Ki-67 marker expression (from 10% to 50% of stained nuclei of SCCO cells), IHC method, (×400). B. Gradation 2 of Ki-67 marker expression (>50% of stained nuclei of SCCO cells), IHC method, (×400)

Low level of TB was most often found in the age groups from 50 to 59 years and from 60 to 69 years (12 out of 32, 37.50% and 10 out of 32, 31.25%, respectively). Moderate level in the age group from 60 to 69 years (13 out of 26, 50.00%), and high in the age group from 70 to 79 years (6 out of 11, 54.55%), but no statistically significant difference was found (p>0.05).

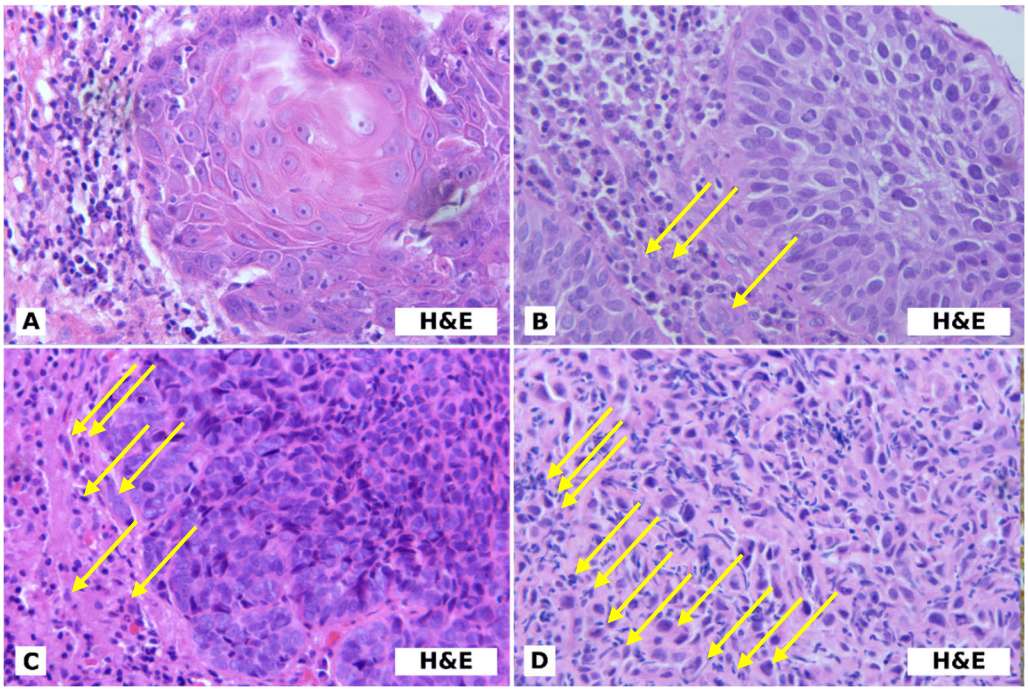

Fig. 3. A. Absence of budding in SCCO samples (gradation 1), H-E staining, (×400). B. TB grade 1 - low (from 0 to 4 single cells or clusters up to 5 cells, yellow arrows), H-E staining, (×400). C. TB grade 2 (from 5 to 9 clusters, yellow arrows), H-E staining, (×400). D. TB grade 3 (from 10 or more single cells or clusters up to 5 cells, yellow arrows), H-E staining, (×400)

High and moderate levels of TB were divided almost equally between forms with and without keratinization (moderate level – 13 out of 26, 50% with keratinization and 13 out of 26, 50% without keratinization; high level – 6 out of 11, 54.55% with keratinization and 5 out of 11, 45.45% without keratinization). For low level there was some difference between forms with and without keratinization (13 out of 32, 40.63% for samples with keratinization and 19 out of 32, 59.37% for samples without it without it), (p>0.05).

Table 2

Distribution of tumor budding grades of squamous cell carcinomas of oropharynx by clinical and morphological parameters, n, %

Clinical and morphological data of SCCO	Quantity n=69 (100 %)	Gradations of tumor budding, n (%)		
		1 (low) n=32 (46.38%)	2 (moderate) n=26 (37.68 %)	3 (high) n=11 (15.94 %)
Men	48 (69.57)	20 (62.50)	18 (69.23)	10 (90.91)
Women	21 (30.43)	12 (37.50)	8 (30.77)	1 (9.09)
P			p<0.05	
Age (years)				
average age	64.09±8.95	61.69±8.83	66.00±9.05	66.55±7.95
40-49	5 (7.25)	3 (9.38)	1 (3.85)	1 (9.09)
50-59	17 (24.63)	12 (37.50)	4 (15.38)	1 (9.09)
60-69	26 (37.68)	10 (31.25)	13 (50.00)	3 (27.27)
70-79	18 (26.09)	7 (21.87)	5 (19.23)	6 (54.55)
80-89	3 (4.35)	0 (0)	3 (11.54)	0 (0)
P			p>0.05	
Keratinization (+)	32 (46.38)	13 (40.63)	13 (50.00)	6 (54.55)
Keratinization (-)	37 (53.62)	19 (59.37)	13 (50.00)	5 (45.45)
P			p>0,05	
Metastases (-)	34 (49.28)	17 (53.13)	12 (46.15)	5 (45.45)
Metastases (+)	35 (50.72)	15 (46.87)	14 (53.85)	6 (54.55)
P			p>0.05	
Recurrence (-)	64 (92.75)	29 (90.62)	25 (96.15)	10 (90.91)
Recurrence (+)	5 (7.25)	3 (9.38)	1 (3.85)	1 (9.09)
P			p>0.05	

Notes: SCCO – squamous cell carcinoma of the oropharynx, p – difference was considered significant at p<0.05, the difference between groups was assessed by Fisher's exact test.

Almost equally there were divided the grades of TB in samples with and without metastases (low level – 17 out of 32, 53.13% without metastases and 15 out of 32, 46.87% with metastases; moderate level – 12 out of 26, 46.15% without metastases and 14 out of 26, 53.85% with metastases; high level – 5 out of 11, 45.45% without metastases and 6 out of 11, 54.55% without metastases), no statistically significant difference was found (p>0.05).

Among all three gradations of TB, cases without relapses occurred equally more often than cases with them (29 out of 32, 90.62% without relapses for low level, 25 out of 26, 96.15% for moderate and 10 out of 11, 90.91% for high), therefore, no statistically significant difference was found (p>0.05).

The next stage of our study was the calculation of the proliferation index in the areas of the invasive front and the division into gradations. The calculation of the proliferation index is most conveniently performed by IHC using nuclear expression of the Ki-67 marker, which is the gold standard for diagnosing cell division for tumors of different localizations. To identify the relationship between HPV status and proliferative activity of SCCO and calculated budding grades, the number of cases with different expression of p16 and Ki-67 markers was listed in Table 3.

Distribution of TB grades was shifted towards HPV-negative cases (for low-level HPV-negative cases were 19 out of 32 samples, 59.38%, for moderate TB – 15 out of 26, 57.69%, for high TB – 10 out of 11, 90.91%, respectively), which was reflected in a statistically significant difference between these subgroups (p<0.05). Unlike p16 expression, the Ki-67 marker demonstrated a more even distribution: among all three gradations of TB, cases with grade 1 Ki-67 expression occurred equally more often than

with grade 2 (25 out of 32, 78.13% for low TB, 18 out of 26, 69.23% for moderate TB, and 9 out of 11, 81.82% for high TB) and therefore no statistically significant difference was found between these subgroups (p>0.05).

TB was first described in 1954 by the Japanese scientist Imai T., but it began to be used as a prognostic criterion only at the beginning of the 21st century. TB is a histological indicator characterized by the presence of single tumor cells or small clusters (up to 5 cells) scattered in the stroma at various distances from the invasive growth front of a malignant tumor [2; 14; 22]. TB is an important marker of an aggressive course of a malignant tumor with loss of intercellular contacts and a high probability of vascular invasion, which should be used to assess the prognosis of cancer. In many studies, the level of TB was associated with a worse prognosis for patients [4, 10, 12, 18, 26]. This is especially relevant for colorectal carcinomas, where this indicator is included in the WHO recommendations as mandatory when assessing the aggressiveness of cancers of this localization after numerous studies have confirmed the importance of TB in large cohorts of patients [13]. Studies of the TB process in head and neck tumors are less common, and mostly concern the oral cavity, but they also show the significance of this indicator for prognosis [1, 9, 11, 16, 19, 21-23, 25]. For example, in the study of Narayanan A. V. et al. (2025) it was determined that the level of TB is an important indicator, along with the degree of tumor differentiation and metastasis to the lymph nodes of the head and neck, which can be used to predict the development of SCC of the oral cavity [14].

Table 3

Distribution of budding grades of squamous cell carcinomas of oropharynx by HPV status and Ki-67 expression, n, %

IHC data of SCCO	Quantity n=69 (100 %)	Gradations of tumor budding, n (%)		
		1 (low) n=32 (46.38 %)	2 (moderate) n=26 (37.68 %)	3 (high) n=11 (15.94 %)
HPV (+), (p16+)	25 (36.23)	13 (40.62)	11 (42.31)	1 (9.09)
HPV (-), (p16-)	44 (63.77)	19 (59.38)	15 (57.69)	10 (90.91)
P			p<0.05	
Gradations Ki-67				
G 1 (10%-50%)	52 (75.36)	25 (78.13)	18 (69.23)	9 (81.82)
G 2 (>50%)	17 (24.64)	7 (21.87)	8 (30.77)	2 (18,18)
P			p>0.05	

Notes: SCCO – squamous cell carcinoma of the oropharynx, p – difference was considered significant at p<0.05, the difference between groups was assessed by Fisher's exact test.

However, there are very few studies of TB grades for SCCO in general, and none in Ukraine at all, despite the large number of cases of morbidity and mortality from SCCO. In addition, there is no precise instruction for the use of TB grades for SCCO in the practice of pathologists, because the fact of using TB as a histological criterion for the prognosis of the course of SCCO in this location is poorly studied.

Information on the TB grades for head and neck cancers is found in individual publications as subjective recommendations of individual scientists. For example, Claudio Cacchi et al. (2024) suggest using the TB grades as follows: if there are 0 to 4 single cells or clusters of up to 5 cells, the TB level is 1 (low), from 5 to 9 clusters the level is 2 (moderate), 10 or more clusters the level is 3 (high) [2]. At the same time, Shreshtha Ghosh and Priyadarshini Guha (2023) propose a TB grade with a division only into high and low levels, where low is less than 5 buds, and high is 5 or more cells [21].

For oral cancers, the TB grades are mostly similar to the option from the study by Claudio Cacchi et al. (2024), namely in the studies of Anand Vijaya Narayanan et al. (2025), or Nan Xie et al. (2016) used the budding gradations as follows: if there are from 0 to 4 single cells or clusters of up to 5 cells, the level of TB is 1 (low), from 5 to 9 clusters the level is 2 (moderate), 10 or more clusters the level is 3 (high) [2; 14; 26]. But for SCCO such information was not found.

In this work, for the first time for our region, the distribution of TB gradations of SCCO was analyzed with regard to sex, age, histological form, regional lymph node involvement and relapses after treatment. For the first time, an analysis of the relationship

between HPV status and proliferative activity of SCCO according to TB grades was performed to determine the significance of the latter in the prognosis of tumors this localization.

CONCLUSION

1. The distribution of tumor budding grades according to the three-level system (from 0 to 4 cells – 1 (low), from 5 to 9 clusters – 2 (moderate), from 10 and more – 3 (high) according to clinical and morphological indicators showed a statistically significant difference only by the sex of the patients, namely the gradation of high budding levels among men was significantly higher than among women (10 out of 11 (90.91%) compared to 1 out of 11 (9.09%), ($p<0.05$), this is probably due to the prevalence of such bad habits among men as smoking and alcohol abuse, which are frequent etiological causes of head and neck cancer development, as an alternative path to the development of oropharyngeal cancer without human papillomavirus infection.

2. The distribution of tumor budding grades in human papillomavirus-positive samples (p16-positive) and human papillomavirus-negative (p16-negative) oropharyngeal cancer demonstrated the largest number of human papillomavirus-negative carcinomas in the subgroup with 3 budding grades, namely with grade 1 human papillomavirus-negative (p16-negative) were 59.38% of samples (19 out of 32), with grade 2 – 57.69% of samples (15 out of 26), and with grade 3 – already 90.91% of samples (10 out of 11), ($p<0.05$), which confirms the more unfavorable prognosis of HPV-unassociated oropharyngeal cancer.

3. The Ki-67 proliferation index did not show a statistically significant relationship with the distribution oftumor budding grades, which makes the latter an independent factor in the prognosis ofs quamous cell carcinoma of the oropharynx.

Prospects for further research. Continuation of studying tumor budding process in squamous cell carcinoma of other locations is promising and has not yet been studied enough to identify additional prognostic criteria.

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