SUMMARY

Head and neck cancer encompasses a group of malignant tumors situated in the head and neck region, excluding the brain. Squamous cell carcinoma is the most prevalent histological type, accounting for approximately 92-95% of cases. Despite the relative ease of diagnosis, 60% of patients in this group die within 5 years. This epidemiological situation can be attributed to persistent exposure to risk factors, primarily the high prevalence of smoking, often coupled with alcohol consumption. Additionally, the low efficacy of early detection and treatment programs for head and neck cancer contributes to the poor outcomes. Despite various approaches to surgical and adjuvant therapies, there has been limited progress in improving overall survival or recurrence-free survival rates in recent years. Deepening the understanding of the factors involved in the development of oropharyngeal squamous cell carcinoma (SCC) would expand our current knowledge of this cancer, potentially leading to improved treatment strategies and perhaps prevention of this disease.

The focus of my research, presented in this dissertation, revolves around evaluating the prevalence of human papillomavirus (HPV) and Epstein-Barr virus (EBV) in individuals diagnosed with oropharyngeal squamous cell carcinoma (OSCC) in North-eastern Poland. In addition, the assessment of the expression of proliferation proteins (Ki-67, p53 protein), receptors of inflammatory cells of the tumor microenvironment (CD3, CD20, CD138), and CTLA-4 and PD-L1 particles in these lesions and the correlation of the results with histoclinical data (cancer differentiation grade, lesions, and risk factors for developing SCC).

The study includes patients diagnosed with oropharyngeal squamous cell carcinoma and who underwent treatment at the Department of Maxillofacial and Plastic Surgery of the University Clinical Hospital in Bialystok between the years 2010 and 2020.

The analyzed material in this study comprises archival tissue samples in the form of paraffin blocks containing specimen samples collected from 105 patients in total. The material was assessed according to protocol, taking into account the degree of differentiation (G) and the pTNM stage of the examined lesions according to AJCC. The study evaluated protein expression Ki-67, p53, PD-L1, CTLA-4, as well as inflammation markers CD3, CD4, CD8, CD20, CD138. The evaluation of the preparations was conducted using a light microscope, specifically examining 10 representative fields of view at a magnification of 100x. To determine HPV infection, an indirect method, that is immunohistochemistry was employed to evaluate the expression of the p16 protein, while the viral genome was assessed using PCR techniques. The

presence of EBV infection was detected through immunohistochemistry, specifically by evaluating the expression of the LMP-1 protein. The study also considered various clinical parameters, including age, sex, location of the lesion, disease severity, and the overall 5-year survival rate. Pathomorphological data were then correlated with the clinical information obtained during the observation period, which involved regular check-ups of the patients.

The collected results underwent statistical analysis, with a statistical significance level set at $p<0.05$. The findings of the study revealed a predominance of older patients (around 70 years of age) in advanced stages (III and IV) of the disease, smoking cigarettes and abusing alcohol. The expression of the p53 protein was observed in all examined patients with SCC, similar to the expression of the Ki-67 protein. The intensity of expression varied based on the degree of lesion differentiation and advancement. The expression of the p16 protein served as an indirect indicator of HPV infection, with its reaction correlating with the presence of the HPV virus determined using the PCR method. The expression of the p16 protein and the occurrence of HPV infection were contingent upon the degree of SCC differentiation and severity. Notably, the presence of inflammatory cells within the tumor microenvironment of the analyzed lesions, particularly CD138-expressing plasma cells, held significance. Their presence correlated with the level of SCC differentiation. EBV infection was infrequent in the examined lesions and was found in 23.8% of cases. The expression of CTLA-4 protein in the analyzed lesions did not demonstrate a correlation with the degree of differentiation and advancement of SCC. Conversely, PD-L1 protein was expressed in both invasive squamous cell carcinoma cells and inflammatory cells within the tumor microenvironment. PD-L1 expression was predominantly observed in lesions of patients with stage III and IV disease.

The findings obtained in this study emphasize the importance of further investigating the interplay between HPV, EBV, CD138, PD-L1, CTLA-4, p16, and p53 in HNSCC. Inconsistencies found in the available literature highlight the need for standardization and unification of diagnostic methods. Additionally, conducting additional research is recommended to identify predictive and prognostic risk factors. This could pave the way for the development and implementation of personalized diagnostic and therapeutic approaches for each case of squamous cell carcinoma of the head and neck, ultimately aiming to achieve optimal therapeutic outcomes.