ABSTRACT

Introduction: The incidence of head and neck squamous cell carcinoma (HNSCC) is on a continuous increasing trend worldwide, despite known risk factors and primary prevention of the disease. Secondary prevention is ineffective due to the lack of screening programmes. Tertiary prevention is therefore a research and clinical area of interest. In this context, the search for new prognostic factors that would improve outcomes and meet the goals of tertiary prevention is desirable. Therefore, research in immuno-oncology and the study of immune biomarkers in the tumor microenvironment (TME) offers new perspectives in this regard. In the era of personalized medicine, the use of biomarkers to estimate disease prognosis is a promising strategy for optimizing therapeutic approaches to improve overall treatment outcomes with a positive impact on tertiary prevention of disease.

Material and methods: To evaluate the prognostic potential of immune biomarkers in TME of HNSCC patients, a prospective monocentric cohort study "ONKOL-01-Head and Neck" was initiated at the Department of Oncology, Ostrava University Hospital, from June 2020. The study enrolled adult patients aged 18 to 90 years with histologically veryfied HNSCC in the oral cavity, nasal cavity, oropharynx, larynx and hypopharynx; clinical stage I-IVb, indicated for radical radiotherapy and/or radiochemotherapy. Patients with histologic types other than HNSCC, as well as patients with distant metastases, synchronous or multiple malignancies, recurrent tumors, or patients after prior radiation or the use of chemotherapy were excluded from the study. Availability of biopsy samples of tumour tissue in paraffin blocks was reqiured. Immunohistochemical analysis was used to evaluate the presence of immune biomarkers in tumor tissue. The primary endpoint of the study assessed the association between the expression of ligand of programmed death receptor 1 (PD-L1) and tumor infiltrating lymphocytes (TILs) and overall survival (OS). Secondary endpoints focused on other cancer specific survival rates. Associations between biomarkers and survival rates were assessed by crude and adjusted hazard ratios (cHR, aHR, respectively) obtained from Cox proportional hazards regression.

Results: Among a total of 55 patients enrolled in the study between June 1, 2020 and August 9, 2022 within a median follow-up of 19,7 months, there were 21 (38,2 %) all-cause deaths and 15 (27,3 %) cancer-related deaths. An overall survival (OS) rate of 61,8 % and a disease-specific survival (DSS) rate of 72,7 % were recorded. A significant association between survival rates and a ≥ 10% difference in PD-L1 expression on immune versus tumor cells (high PD-$L1_{IC}$ expression) was documented regardless of the type of analysis (univariate or multivariate). In addition, a stronger association was confirmed for OS and the composite biomarker high PD-$L1_{IC}$ expression along with either median-higher CD8+ TIL count or increased TIL density ≥30%, as indicated by an aHR of 0,08 (95% CI, 0,01 to 0,52) and 0,07 (95% CI, 0,01 to 0,46), respectively. Similar results were demonstrated for other specific survival rates.

Conclusion: The early results of this study demonstrated the strong prognostic potential of immune biomarkers (high PD-$L1_{IC}$ in association with increased TIL density) in patients with advanced HNSCC undergoing definitive treatment with radiotherapy and/or radiochemotherapy. The methodology used for biomarker assessment and definition of high PD-$L1_{IC}$ expression and increased TIL density appears to be accurate and reliable and could therefore be implemented in clinical practice for future studies. The prognostic potential of the combination of biomarkers could be used to stratify patients into risk groups according to survival prognosis, which would allow to optimize follow-up protocols and tailor treatment strategies.

Trial registration: The study is registered with *Clinicaltrials.gov.* – NCT05941676

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