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Original Research

Human papillomavirus as prognostic marker with rising prevalence in neck squamous cell carcinoma of unknown primary: A retrospective multicentre study

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Abstract Patients with neck squamous cell carcinomas of unknown primary tumour (NSCCUP) present with lymph node metastasis without evidence for a primary tumour. Most patients undergo an aggressive multimodal treatment, which induces severe, potentially unnecessary toxicity. Primary tumours of NSCCUP can be hidden in the oropharynx. Human papillomavirus (HPV) is causally involved in a subgroup of oropharyngeal squamous cell carcinomas (OPSCC) associated with early lymph node metastasis and good prognosis. Detection of markers for HPV transformation in NSCCUP could allow focussing on the oropharynx in primary tumour search and could be of value for choice and extent of treatment.

In a retrospective multicentre study (Germany, Italy and Spain), we analysed metastatic lymph nodes from 180 NSCCUP patients for the presence of HPV DNA, HPV E6*I mRNA and cellular $p16^{INK4a}$ overexpression, a surrogate marker for HPV-induced transformation. HPV status, defined as positivity for viral mRNA with at least one additional marker, was correlated with clinical parameters and survival outcome.

A substantial proportion (16%) of NSCCUP were HPV-driven, mainly by HPV16 (89%). HPV prevalence increased with year of diagnosis from 9% during 1998–2004 to 23% during 2005–2014 ($p = 0.007$). HPV-driven NSCCUP had significantly better overall and progression-free survival rates ($p \leq 0.008$).

Based on this survival benefit, it is contended that HPV RNA status should be included in NSCCUP diagnosis and in therapeutic decision-making. Deintensification of radiation in patients with HPV-driven NSCCUP, while concurrently concentrating on the oropharynx appears to be a promising therapeutic strategy, the efficacy of which should be assessed in prospective trials. To our knowledge, this is the largest study on HPV in NSCCUP.

1. Introduction

Among head and neck cancers, neck squamous cell carcinomas from unknown primary tumour (NSCCUP) are probably the most clinically challenging. Patients present with enlarged neck lymph nodes. Biopsies or cytology reveal squamous cell carcinoma metastases, which are most often unilateral at neck level II [1]. Diagnostic workup includes physical examination and imaging, such as computed tomography (CT), magnetic resonance imaging (MRI) and/or positron emission tomography with CT (PET/CT). To localise the primary site, an endoscopy of the upper aerodigestive tract is performed with biopsies of suspicious areas and routinely of the tonsils and base of tongue, which are common sites of hidden primary tumours [2]. In a United States (US) study analysing 236 patients with NSCCUP, 45% of identified primary tumours were in the tonsillar fossa and 44% in the base of tongue [3].

NSCCUP are generally treated with aggressive multimodal therapy similar to locally advanced head and neck cancers. According to the European Society for Medical Oncology (ESMO) guidelines, this may include neck dissection followed by comprehensive irradiation of the bilateral neck and potential head and neck primary sites [4]. In advanced stages, induction chemotherapy or chemoradiation might be applied. This treatment often induces severe side-effects. Focussed treatment would be preferable to reduce toxicity. There is a need for biomarkers guiding the search for the primary site and for prognostic markers.

Oncogenic human papillomavirus (HPV) types are discussed as biomarkers in NSCCUP [5]. HPVs, in particular HPV16, are causally associated with a subset of oropharyngeal squamous cell carcinoma (OPSCC) [6–9]. Expression of viral E6 and E7 oncoproteins leads to inactivation of the cellular proteins p53 and pRb, respectively, resulting in cell cycle stimulation, suppression of apoptosis and transformation [10,11]. HPV prevalence in head and neck tumours is highest in the oropharynx [12], particularly in the palatine tonsils followed by the base of tongue, but low outside the oropharynx [9,13]. Patients with HPV-positive OPSCC have a better survival rate compared with HPV-negative patients [14–17]. Ongoing clinical trials assess whether HPV-positive OPSCC patients might benefit from deintensified treatment [18].

In a systematic review, we evaluated 18 studies including 659 patients with NSCCUP for the impact of HPV [19]. HPV prevalence varied between 0% and 85% (mean = 36%) with positivity for HPV DNA and $p16^{INK4a}$. Several studies showed that HPV might predict an oropharyngeal primary site [20–26] and that HPV-positive patients have a better prognosis than HPV-negative patients [21,23,24,26–30]. However, those studies were small and heterogeneous regarding patient selection and HPV detection methods. HPV