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

assessment was only based on HPV DNA and/or $p16^{INK4a}$ overexpression ($p16^{high}$), but viral RNA, the gold standard for identifying HPV-driven tumours [31], was not considered.

In this retrospective multicentric study, we analysed 180 patients with NSCCUP from Germany, Italy and Spain. We assessed HPV mRNA transcripts in addition to HPV DNA and $p16^{INK4a}$ to identify HPV-driven NSCCUP. We compared the HPV prevalence among different centres and sampling periods (1988–2014) and evaluated the prognostic value of HPV.

2. Patients and methods

2.1. Patients

One hundred-eighty patients with neck lymph node metastasis and without evidence for a primary tumour (diagnostic workup described in supplements) were included. Forty-nine patients were from Heidelberg University Hospital, 59 from Hospital de Sant Pau in Barcelona, and 72 from Italy including 46 from Treviso Regional Hospital, 15 from Montebelluna Hospital and 11 from Trieste Hospital. This study was approved by the local Ethics Committees (see Supplements).

Formalin-fixed, paraffin-embedded (FFPE) tissues were available from 161 patients. Eleven patients from Heidelberg had an additional frozen biopsy, and 19 had only frozen biopsies.

2.2. Nucleic acid extraction

Sections were prepared as previously described (see Supplements) [17,32]. Genomic DNA was released from FFPE tissue sections by incubation in 200 µl proteinase K solution (1 mg/ml, 45 mM Tris–HCl, 0.9 mM ethylene diamine tetra acetic acid (EDTA), 0.45% Tween 20) for 16 h at 56 °C, followed by enzyme inactivation at 72 °C for 10 min. For polymerase chain reaction (PCR), 5 µl of supernatant was used.

RNA was extracted from tissue sections using the Pure-Link FFPE Total RNA Isolation Kit (Invitrogen, Carlsbad, CA). DNase (Qiagen, Hilden, Germany) treatment was performed prior to elution in 50 µl RNase-free water. For reverse transcription polymerase chain reaction (RT-PCR), 1 µl of RNA was used.

From frozen biopsies, DNA and RNA were isolated using the MagNA Pure 96 system (Roche) (see Supplements).

2.3. Multiplex HPV genotyping

Using Multiplex HPV genotyping (MPG), HPV DNA was detected by BSGP5+/6+ PCR capable of simultaneous amplification of 51 mucosal HPV types generating L1 amplimers of approximately 150 base pairs (bp) followed by hybridisation to type-specific probes coupled to beads (Luminex Corp., Austin, Texas) [33,34]. Human β-globin served as internal control. If neither HPV nor β-globin was detected, samples were incubated at 90 °C for 20 min to reverse formalin-induced cross-linking before retest.

2.4. HPV E6*I mRNA detection

E6*I mRNA, one of the spliced isoform RNAs of the E7 oncoprotein, was detected by RT-PCR followed by hybridisation as previously described [35]. Short E6*I (approximately 65 bp) and ubiquitin C (85 bp) amplicons were amplified to assess RNA integrity. We tested for mRNA of the HPV types detected by MPG, and all FFPE samples for HPV16.

2.5. Immunohistochemistry

By immunohistochemistry (IHC), $p16^{INK4a}$ was detected on FFPE sections using the mouse anti-human $p16^{INK4a}$ antibody G175–405 (BD Pharmingen, San Jose, United States of America (USA)) with the VENTANA BenchMark ULTRA system and OptiView DAB IHC Detection Kit (Roche). $p16^{high}$ was defined by moderate-to-strong and diffuse nuclear and cytoplasmic staining of >25% of tumour cells in the section (see Supplements).

2.6. Statistical analysis

Patient characteristics and follow-up data were obtained from clinical charts and were analysed in relation to HPV status. Overall survival (OS) time was calculated from the date of diagnosis to date of death (event) or end of follow-up (censored). Progression-free survival (PFS) time was calculated from the date of diagnosis to new lymph node or distant metastasis, malignancy of the head and neck region or outside, death (event), or end of follow-up without progression (censored).

The Kaplan–Meier method was used to assess survival distributions. Curves were compared using log-rank tests. To assess the effect of HPV on OS and PFS and adjust for possible confounders, multivariable Cox proportional hazard regression models were fitted. Models were stratified by country and included the following covariates: HPV status, age at diagnosis, gender, N stage (categorised into 1/2a, 2b and 2c/3), extracapsular spread, alcohol and tobacco consumption (current/former versus never) and treatment (multimodal versus single). One hundred sixty-four cases (91%) could be included based on complete clinical data. The proportional hazards assumption was met after modelling a time-dependent treatment effect. P values below 0.05 were considered statistically significant. For statistical analyses, R (version 3.2.3) was used.

3. Results

3.1. Study population

Patients were predominantly male (88%) with a median age of 62 years (Table 1). Median year of diagnosis for all cases was 2004: 2002 in Spain, 2004 in Italy and 2008 in Germany (recoded as early cases: 1988–2004, and late: 2005–2014). Tumours were mainly N stage 1, 2a or 2b (70%), and 71% of the tumours showed extracapsular spread. Tobacco use (85%) and alcohol consumption (70%) were common, but decreased with year of diagnosis (p_{trend} = 0.006 and 0.008, respectively). Most patients received multimodal treatment (30% of the patients, postoperative radiotherapy and 47% of them, chemoradiation). In Spain, cases had a significantly higher N stage and more patients received chemoradiation ($p < 0.01$).

3.2. HPV prevalence in NSCCUP

Three markers, associated with HPV, were analysed: HPV DNA, E6*I mRNA and $p16^{high}$. HPV-driven cases were defined by presence of E6*I mRNA together with at least one additional marker. Twenty-eight (16%) patients of the 180 NSCCUP were HPV-driven. The percentage was highest in Germany (10/49, 20%), followed by Italy (12/72, 17%) and lowest in Spain (6/59, 10%). The proportion of HPV-driven cases increased significantly with sampling time (p_{trend} = 0.004), in total from 9% (1988–2004, n = 93) to 23% (2005–2014, n = 87; Fig. 1). In Spain, the HPV-driven proportion increased only marginally from 8% to 13%, while it approximately tripled in Germany (10% to 28%) and Italy (8% to 26%).

The most common HPV type was HPV16 (89%). Single cases each was driven by HPV18, 33 or 35. Two further cases were infected with DNA from HPV90, and both HPV52 and 53, but neither E6*I mRNA of those types (no assay available for HPV90) nor $p16^{high}$ was detected.

Compared to the non-HPV-driven patients, among the HPV-driven NSCCUP patients, there were more women (25% versus 10%, p = 0.02), less smokers (64% versus 88%, p = 0.002) and less drinkers (44% versus 74%, p = 0.002). HPV-driven tumours less likely present with high N stage (4% N2c/3) compared to non-HPV-driven tumours (35%, p = 0.002). Regarding study centre, age at diagnosis, extracapsular spread and treatment, there were no significant differences between HPV-driven and non-HPV-driven cases (Table 2).

Table 1
Characteristics of NSCCUP patients from Germany, Spain and Italy, collected between 1990 and 2014.

	Germany (n = 49)	Spain (n = 59)	Italy (n = 72)	Total (n = 180)	p[a]
Gender					
Male	43 (88%)	52 (88%)	63 (88%)	158 (88%)	1.0
Female	6 (12%)	7 (12%)	9 (13%)	22 (12%)	
Age, range (median)	43–85 (61)	36–81 (61)	39–88 (63)	36–88 (62)	0.7
Sampling period					
1988–2004	20 (41%)	36 (61%)	37 (51%)	93 (52%)	0.1
2005–2014	29 (59%)	23 (39%)	35 (49%)	87 (48%)	
N stage					
1, 2a	21 (45%)	12 (20%)	14 (19%)	47 (26%)	**<0.01**
2b	14 (30%)	24 (41%)	39 (54%)	77 (43%)	
2c, 3	12 (26%)	23 (39%)	19 (26%)	54 (30%)	
N/A	2			2	
Extracapsular spread					
Yes	27 (63%)	43 (75%)	51 (72%)	121 (71%)	0.4
No	16 (37%)	14 (25%)	20 (28%)	50 (29%)	
N/A	6	2	1	9	
Tobacco					
Smokers	33 (73%)	52 (88%)	63 (88%)	148 (84%)	0.07
Non-smokers	12 (27%)	7 (12%)	9 (13%)	28 (16%)	
N/A	4			4	
Alcohol					
Drinkers	30 (68%)	43 (73%)	48 (67%)	121 (69%)	0.7
Non-drinkers	14 (32%)	16 (27%)	24 (33%)	54 (31%)	
N/A	5			5	
Treatment					
Surgery only	8 (18%)	10 (17%)	21 (29%)	39 (22%)	**<0.01**
pRT	16 (36%)	1 (2%)	37 (51%)	54 (31%)	
pCT/pRCT	20 (45%)	47 (81%)	14 (19%)	81 (47%)	
N/A	5	1		6	
Median follow-up, years	1.7	3.3	2.9	2.5	

Significant values are displayed in bold.
N/A = not available, pRT = postoperative radiotherapy (2 cases pRT only), pCT = postoperative chemotherapy, pRCT = postoperative chemoradiation.

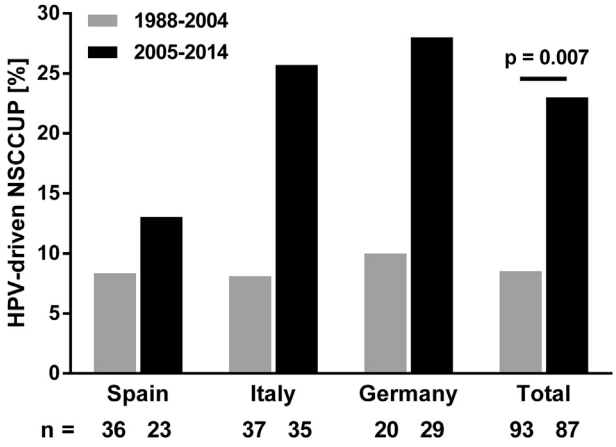

Fig. 1. **Time trend of HPV-driven NSCCUP**. The proportion of HPV-driven NSCCUP in 1988–2004 (grey) is compared to 2005–2014 (black) for all 180 cases and all centres. The number of cases analysed in each sampling period is depicted below the graph. Chi-squared test for trend in proportion was used to calculate the p value.

Table 2
Clinical parameters in relation to HPV status.

	n	HPV-driven (n = 28)	non-driven (n = 152)	p[a]
Gender				
Male	158	21 (75%)	137 (90%)	**0.02**
Female	22	7 (25%)	15 (10%)	
Age, years (median)		39–79 (59)	36–88 (62)	0.2
Country				
Germany	49	10 (36%)	39 (26%)	0.3
Italy	72	12 (43%)	60 (39%)	
Spain	59	6 (21%)	53 (35%)	
Sampling time				
1988–2004	93	8 (29%)	85 (56%)	**<0.01**
2005–2014	87	20 (71%)	67 (44%)	
Tobacco				
Ever	148	18 (64%)	130 (88%)	**<0.01**
Never	28	10 (36%)	18 (12%)	
N/A	4			
Alcohol				
Ever	121	12 (44%)	109 (74%)	**<0.01**
Never	54	15 (56%)	39 (26%)	
N/A	5			
N status				
1, 2a	47	12 (43%)	35 (23%)	**<0.01**
2b	77	15 (54%)	62 (41%)	
2c, 3	54	1 (4%)	53 (35%)	
N/A	2			
Extracapsular spread				
Yes	121	18 (64%)	103 (72%)	0.4
No	50	10 (36%)	40 (28%)	
N/A	9			
Treatment				
Single	41	9 (32%)	32 (22%)	0.2
Multimodal	133	19 (68%)	114 (78%)	
N/A	6			

N/A = not available, single = surgery or radiotherapy, multimodal = postoperative radiotherapy/chemotherapy/chemoradiation.
Significant values are displayed in bold.
[a] Pearson chi-squared test (age categories: ≥62 versus <62).

3.3. HPV marker concordance

The three markers, i.e. HPV DNA, mRNA and $p16^{high}$, were concordant in 90 (88%) of the 102 cases with valid result for all markers. Among the 26 HPV-driven cases tested for all markers, there was a single case without $p16^{high}$ (Fig. 2). In the two HPV-driven frozen biopsies, $p16^{INK4a}$ expression could not be evaluated, but both were HPV DNA-positive and mRNA-positive. Seven cases (7%) showed $p16^{high}$ without presence of HPV DNA or mRNA. In three cases (3%) only DNA was detected and in one case (1%) both DNA and $p16^{high}$ were present, but no mRNA.

3.4. Prognostic value of HPV

Patients with HPV-driven NSCCUP showed better OS and PFS in univariable analyses ($p = 0.002$ and $p = 0.0006$, log-rank test; Fig. 3). Only seven (26%) of 27 HPV-driven patients died and eight (30%) progressed, while among 137 non-HPV-driven patients, 89 (65%) died and 99 (72%) progressed. Multivariable Cox regression analysis confirmed the prognostic role of HPV with hazard ratios of 0.3 for OS and 0.27 for PFS ($p = 0.008$ and $p = 0.003$, Wald test). Increased age, extracapsular spread and high N stage were independent risk factors (Table 3). Multimodal treatment was significantly associated with better overall survival, but a time-dependent effect of treatment needs to be considered. Gender, tobacco use and alcohol consumption had no statistically significant influence on survival.

4. Discussion

This multicentre European study revealed an HPV prevalence in NSCCUP of 16%, defined by presence of HPV E6*I mRNA together with HPV DNA and/or $p16^{high}$. HPV prevalence was lowest in Spain, which is in line with a low prevalence of 6–9% in OPSCC reported by systematic reviews across several countries [9,12,36]. The highest HPV prevalence (91%) in NSCCUP was reported in the USA; however, 60% of cases were finally diagnosed as OPSCC [37]. The largest German study found 37% (23/63) cases with HPV DNA and $p16^{high}$

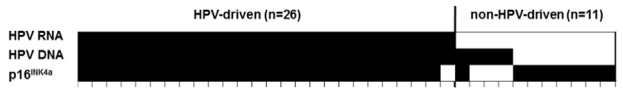

Fig. 2. **Marker distribution among the 37 cases positive for at least one marker with valid result for all markers**. The 26 HPV-driven cases on the left are defined by presence of E6*I mRNA (HPV RNA) with at least one additional marker. The result for each marker is represented as a black box (positive), or white box (negative). Not shown are the 65 cases negative for all three markers, DNA-invalid cases (n = 58), and cases not tested for RNA (n = 1) or p16 (n = 19, including 2 HPV-driven cases).

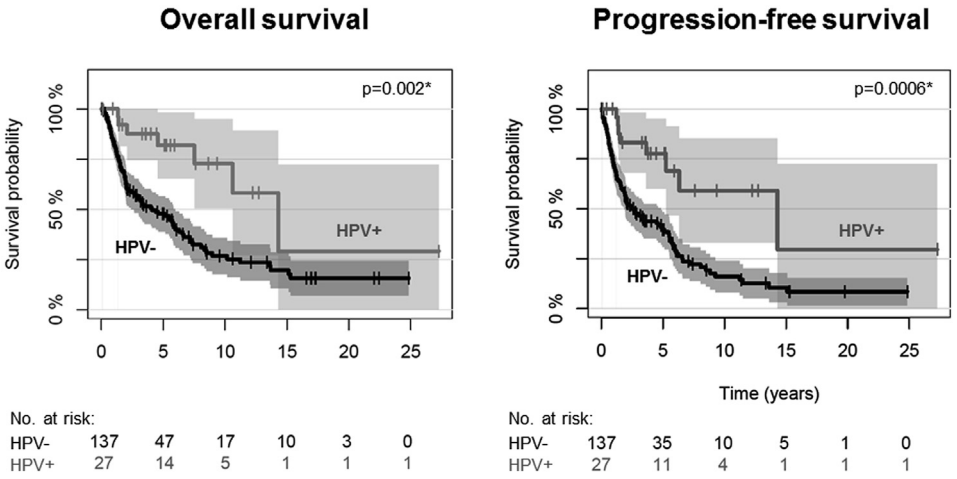

Fig. 3. **Survival in relation to HPV status**. Kaplan−Meier curves for overall survival (left) and progression-free survival (right) with 95% confidence interval are compared for HPV-driven (HPV+, grey) cases versus non-HPV-driven (HPV−, black) cases. Only patients with all covariate information available were included (n = 164). The numbers at risk are depicted for each group below the graph. An asterisk (*) indicates a significant difference (log-rank test).

Table 3
HPV status and clinical parameters in relation to survival.

Multivariable analysis (n = 164)[a]	OS		PFS	
	HR (95% CI)	p[c]	HR (95% CI)	p[c]
HPV-driven (yes/no)	**0.30 (0.12−0.73)**	**0.008**	**0.27 (0.11−0.65)**	**0.003**
Gender (female/male)	0.71 (0.31−1.65)	0.4	0.48 (0.21−1.11)	0.09
Age at diagnosis	**1.03 (1.01−1.06)**	**0.003**	**1.03 (1.01−1.05)**	**0.01**
N stage				
2b versus 1, 2a	**2.02 (1.05−3.91)**	**0.04**	1.71 (0.93−3.14)	0.08
2c, 3 versus 1, 2a	**2.31 (1.16−4.57)**	**0.02**	**2.69 (1.44−5.04)**	**0.002**
Extracapsular spread (yes/no)	**2.61 (1.41−4.85)**	**0.002**	**2.20 (1.26−3.83)**	**0.005**
Tobacco (ever/never)	1.00 (0.48−2.10)	1.0	1.10 (0.53−2.26)	0.8
Alcohol (ever/never)	1.00 (0.57−1.75)	1.0	1.12 (0.66−1.91)	0.7
Treatment (multimodal/single)	0.09 (0.01−1.46)	0.09	**0.12 (0.03−0.43)**	**0.001**
Treatment time[b]	2.18 (0.56−8.51)	0.3	**3.20 (1.25−8.18)**	**0.02**

Significant values are displayed in bold.
OS = overall survival, PFS = progression-free survival, HR = hazard ratio, CI = confidence interval, single = surgery or radiotherapy, multimodal = postoperative radiotherapy/chemotherapy/chemoradiation.
[a] Stratified by country.
[b] Modelling of time-dependent treatment effect.

[38]. An Italian study detected HPV16/18 E6/E7 mRNA in 45% (10/22) of NSCCUP [39]. Compared to those studies, we found a slightly lower HPV prevalence. However, those patients were diagnosed more recently (German patients: 2002−2011; Italian patients: 2010−2012).

A strong, about 2.5-fold increase of the HPV prevalence was observed in our study in recently diagnosed NSCCUP (2005−2014) versus those diagnosed earlier (1988−2004). Increasing HPV prevalence has also been reviewed for OPSCC from pre-1995 till 2013 [36]. This trend could explain the lower prevalence in our study compared to studies analysing more recent cases. However, we cannot conclude whether the increase in HPV prevalence is an absolute or a relative increase due to lower number of alcohol- and smoking-related cancers, taking into account the decreasing number of alcohol and tobacco consumers ($p_{trend} = 0.006$ and $p_{trend} = 0.008$).

The clinically relevant finding of this study is the prognostic role of HPV in NSCCUP. HPV-driven cases had better OS and PFS rates with a hazard ratio of 0.3 ($p \leq 0.008$). A similar prognostic finding was recently reported by a Danish study including 60 patients (2000−2011) with an even lower hazard ratio of 0.16 for OS of HPV-positive patients defined by HPV DNA and $p16^{high}$ [27]. Besides HPV status, the independent risk factors such as age, extracapsular spread and N stage should be considered. As suggested by previous NSCCUP and OPSCC studies, smoking also might be considered, since survival curves stratified by HPV and smoking status show poorer survival rates of

HPV-driven smokers compared to HPV-driven non-smokers (Supplementary Fig. 1).

With regard to the rising prevalence of HPV-driven cases, HPV assessment might become clinically relevant, as it may help to identify the primary tumour and deliver a more targeted therapy that, in HPV-driven patients, may result in a better prognosis and quality of life [21–26]. Transoral robotic surgery (TORS) is an innovative tool to facilitate resection of oropharyngeal subsites. In a United States (US) study, TORS revealed small occult base of tongue carcinomas in 9/10 (90%) patients [40]. This tool may be particularly beneficial for HPV-positive patients. The role of deintensified treatment of HPV-driven NSCCUP could be assessed in prospective clinical trials. Promising results from studies on HPV-driven OPSCC raise hope for reducing treatment toxicity and improving tolerance [18].

A challenge faced in this study was the limited HPV DNA assessment from archived FFPE tissues. Invalid DNA results (HPV-negative and β-globin-negative) were obtained in 102 cases (63%). A large β-globin amplicon (208 bp) was designed to ensure prior amplification of the shorter HPV amplicons (approximately 150 bp), if present, and to exclude false HPV-negative results due to DNA fragmentation. Cross-linking between nucleic acids and proteins hampers amplification of large amplicons. After incubating invalid DNA to reverse cross-linking, valid results were obtained in 44 cases (43%), and in eight cases DNA status could be assessed in frozen biopsies from the same tumour. The remaining 50 DNA-invalid cases were positive for ubiquitin C mRNA, but HPV16 mRNA-negative (the nine $p16^{high}$ cases were also HPV18, 33 and 35 mRNA-negative), and were thus defined as non-HPV-driven. To ensure that we do not miss HPV-driven cases in the early sampling period due to technical problems, all FFPE tissues were tested for $p16^{high}$ and for HPV16 mRNA independent of their HPV DNA status. All cases were positive for ubiquitin C mRNA, which validates RNA integrity.

The best marker to identify truly HPV-driven cases is supposed to be detection of HPV transcripts [31]. All 28 HPV mRNA-positive cases were also positive for HPV DNA, and only one case did not overexpress $p16^{INK4a}$. However, another case was HPV DNA-positive and showed $p16^{high}$ but was HPV mRNA-negative. This latter case might reflect detection of biologically non-relevant HPV DNA and unspecific $p16^{high}$. Given its role in cell cycle regulation, overexpression of $p16^{INK4a}$ without HPV mRNA might be triggered by other signalling pathways.

An as yet open question is which marker, or combination of markers, would be suitable for routine HPV status assessment in NSCCUP patients. If mRNA analysis is not feasible, HPV DNA in combination with $p16^{high}$ might be the best alternative. But this marker combination would have identified one case without HPV mRNA and would have missed one HPV-driven case. Applying the commonly used $p16^{high}$ cut-off of >70% stained tumour cells (instead of 25%, see supplements) would have missed five HPV-driven cases. Thus there is a need for prospective studies using simple, rapid and cost-effective assays for HPV detection in easily accessible biological samples.

In conclusion, this is so far the largest NSCCUP study analysing the prevalence of HPV and its prognostic impact, and the first study assessing HPV RNA in addition to HPV DNA and $p16^{INK4a}$ overexpression. HPV status assessment should be implemented in NSCCUP standard diagnostic workup as a substantial proportion (16%) of NSCCUP with increasing trend is HPV-driven. A better OS and PFS of HPV-driven NSCCUP patients may allow for deintensified treatment, which needs to be evaluated in prospective trials. Identification of HPV-driven NSCCUP and a change in management of those patients might hopefully result in a better tolerance and quality of life.

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Conflict of interest statement

The authors disclose no potential conflicts of interest.

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