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A high and increasing HPV prevalence in tonsillar cancers in Eastern Denmark, 2000–2010: The largest registry-based study to date

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The aim was to explore whether the incidence of tonsillar squamous cell carcinomas (TSCCs) increased in Eastern Denmark, 2000–2010, and whether human papillomavirus (HPV) could explain the increase, and to assess the association of HPV prevalence with gender, age, and origin (*i.e.*, the certainty of tonsillar tumor origin). We applied HPV DNA PCR and p16 immunohistochemistry to all TSCCs registered in the Danish Head and Neck Cancer Group (DAHANCA) and in the Danish Pathology Data Bank ($n = 632$). Pathologists reviewed and subdivided the tumors into two groups: specified and nonspecified TSCCs. Approximately 10% of HPV-positive tumors was genotyped by amplicon next-generation sequencing. The overall crude incidence of TSCCs increased significantly (2.7% per year) and was explained by an increasing incidence of HPV-positive TSCCs (4.9% per year). The overall HPV prevalence was 58%, with HPV16 being the predominant HPV type. In multivariate analysis, the HPV prevalence was associated with age (<55 *vs.* >60 years) (OR, 1.72; 95% CI 1.13–2.63) and origin (nonspecified *vs.* specified

Key words: oropharyngeal cancer, tonsillar cancer, HPV, p16

Abbreviations: APC: annual percentage change; bp: base pairs; BSCC: base of tongue squamous cell carcinoma; 95% CI: 95% confidence intervals; DAHANCA: Danish Head and Neck Cancer Group; DNA: deoxyribonucleic acid; FDA: US Food and Drug Administration; FFPE: formalin-fixed paraffin-embedded; HPV: human papillomavirus; IHC: immunohistochemistry; NCBI: National Center for Biotechnology Information; neg.: negative; ng.: nanogram; NGS: next-generation sequencing; no.: number of patients; NSTSCCs: nonspecified TSCCs; OPSCC: oropharyngeal squamous cell carcinoma; OR: Odds ratio; PCR: polymerase chain reaction; PIN: personal identification number; pos.: positive; SCC: squamous cell carcinoma; STSCCs: specified TSCCs; TNM: tumor, nodes, metastases; TSCC: tonsillar squamous cell carcinoma; T-site: tumor site

Additional Supporting Information may be found in the online version of this article.

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TSCCs) (OR, 0.15; 95% CI 0.11–0.22). The association of HPV prevalence with origin increased over time in specified TSCCs (OR per year, 1.10; 95% CI 1.01–1.19), whereas no change over time was observed among nonspecified TSCCs (OR per year, 0.99; 95% CI 0.90–1.08). In conclusion, the observed increase in the number of HPV-positive TSCCs can explain the increasing number of TSCCs in Eastern Denmark, 2000–2010. HPV prevalence was associated with younger age (<55 years) and a high certainty of tonsillar tumor origin.

What's new?

Are throat cancers on the rise in Denmark, as in other Western populations? These authors analyzed samples from the Danish Head and Neck Cancer Group, making this the largest non-selected cohort of tonsillar cancer cases studied to date. They found that during the years 2000–2010, the rate of tonsillar cancer increased, and that HPV could be to blame. The incidence of HPV-positive cancers rose over the study period, with most of the HPV-positive cancers harboring HPV-16. When they classified the tumors by whether they originated in the tonsillar tissue, they found that tumor origin was the strongest predictor of HPV status; specified tonsillar tumors contained HPV more often than those appearing to have originated elsewhere.

The incidence of oropharyngeal cancer has been reportedly increasing in the Western World, including Denmark,[1,2] and it is presumably due to infections with high-risk human papillomavirus (HPV).[3,4] HPV is a well-known carcinogen, identified in studies on cervical and anogenital cancers,[5,6] and recently HPV was also recognized as a risk factor for the development of oropharyngeal squamous cell carcinoma (OPSCC).[7] HPV causes a distinct class of OPSSCs with different epidemiology, clinical presentation, tumor biology, and pathological characteristics compared with those of HPV-negative OPSCCs, attributed to tobacco and alcohol consumption.[8–12] Additionally, HPV-positive OPSCCs have a substantially better prognosis regardless of treatment regimen.[13,14]

The proportions of OPSCCs associated with HPV vary significantly in the literature.[15] In Europe, the reported HPV prevalence ranges from 3.2% in Northern Spain (1990–2009) to 79% in Sweden (2000–2007).[4,16–21] Recent reports from North America identified 72% HPV-positive OPSCCs in the United States (2000–2004),[3] 62% in Canada (2006–2011),[22] and 25% in Greenland (2002–2010).[23] Furthermore, in Australia, 47% of OPSCCs were HPV-positive in the time period 2001–2005.[24] These large variations in HPV prevalence have been attributed to geographic variation in HPV exposure, geographical differences in HPV infection susceptibility, variations in study periods, and limited sample sizes, with numbers of cases ranging from 26 to 314.[21,23]

HPV prevalence also depends on the specific site, that is, the origin of the tumors (T-site). Squamous cell carcinomas (SCCs) of the palatine tonsils [tonsillar SCC (TSCCs)] and the base of the tongue have a relatively high HPV prevalence compared with those in other oropharyngeal T-sites.[9] However, the oropharynx is a complex area, which implies a potential anatomical misclassification of tumors in clinical registries, which often comprise the basis for OPSCC studies.[25]

Another important issue is the definition of HPV positivity in OPSCCs.[26] The sensitivity of different HPV detection techniques may vary substantially, leading to diverse HPV prevalence.[27] Furthermore, some studies define HPV status by p16 immunohistochemical staining (IHC), even though p16 can be upregulated due to cellular perturbations other than HPV infection.[28]

Thus, HPV epidemiology in OPSCC calls for clarification. Based on this, we initiated the present Danish HPV study on TSCCs, the predominant OPSCC in Denmark. We included the largest, nonselected, consecutive cohort to date, consisting of all diagnosed tonsillar cancer patients in Eastern Denmark during an 11-year study period (2000–2010).

The aim of the study was to explore whether the incidence of TSCCs increased during the study period in Eastern Denmark and whether HPV was responsible for the increase. In addition, we examined HPV genotypes in HPV-positive tumors and assessed the association of HPV prevalence with gender, age, and tumor origin, that is, the certainty of tonsillar tumor origin. Furthermore, we explored the correlation of HPV DNA PCR and p16 immunohistochemistry (IHC).

Materials and Methods

Patients and tumor samples

Danish OPSCC patients are referred to and treated at regional Head and Neck Centers at University Hospitals. Due to the Danish public health care system, private treatment options do not exist for patients with head and neck cancer. Furthermore, it is very rare that Danish patients move to other Head and Neck Centers; it happens only in unusual cases of accumulation of patients at one center where they are offered earlier treatment at another center.

All Danish residents are registered in the computerized Civil Registration System with a unique personal identification number (PIN).[29] The PIN is used in all national registries, facilitating accurate linkage. Using the PIN as a key

identifier, we linked two nationwide Danish registries [the Danish Head and Neck Cancer Group (DAHANCA) database and the Danish Pathology Data Bank] to identify and validate our cohort of tonsillar cancers in Eastern Denmark. The DAHANCA collects information on all head and neck cancer patients from the time of diagnosis and during follow-up (www.dahanca.dk). The Danish Pathology Databank contains data on all pathoanatomical examinations done in Denmark, reported by the pathology departments through an online, real-time system.[30] First, we identified all patients diagnosed with tonsillar cancer in the Capital Region of Denmark and the Region of Zealand (covering ca. 40% of the Danish population, 2.2 million inhabitants) as registered in the DAHANCA database during 2000–2010. Second, we linked our cohort to the Danish Pathology Data Bank. We obtained information on the patients' tumor tissue and collected specimens. See Supporting Information for a map of the Danish Regions including their populations. TSCCs represent ca. 60% of Danish OPSCCs.[1]

The study was conducted according to the Helsinki Declaration and approved by the Regional Scientific Ethical Committee (H-C-2008-080) and the Danish Data Protection Agency.

Review of histology

Specialized head and neck pathologists, according to our author list, validated the diagnoses of all histological specimens, assessed the expression of p16 by IHC in the tumor slides, and categorized all tumors as specified TSCCs (STSCCs) or nonspecified TSCCs (NSTSCCs).

p16 immunohistochemistry. Immunohistochemistry for p16 was carried out on formalin-fixed paraffin-embedded (FFPE) tissue sections on the Ventana BenchMark ULTRA platform (Roche, Basel, Switzerland) with the OptiView Detection Kit (Roche) and the p16 monoclonal antibody E6H4 (Roche), according to standard protocols. Based on p16 positivity, tumors were categorized into five groups[31]: 0, 0% positive tumor cells; 1, 1–25% positive tumor cells; 2, 26–50% positive tumor cells; 3, 51–75% positive tumor cells; and 4, 76–100% positive tumor cells. Tumors in group 4 (76–100% p16-positive tumor cells) exhibiting strong diffuse nuclear and cytoplasmic staining were defined as p16-positive.[32]

Division of tumors into "specified TSCCs" and "nonspecified TSCCs." Tumors were divided into STSCCs and NSTSCCs based on a combination of clinical information obtained in the Danish Pathology Data Bank, reported by the clinician who performed the biopsy, and review of tissue-specific structures in the tumor sections. Tumors were reclassified as STSCCs if they exhibited tonsillar tissue (tonsillar crypts and tonsillar lymphoid tissue with germinal centers) in the biopsy section or the tumor, according to clinical information originated in the tonsils. Tumors were reclassified as NSTSCCs if they did not exhibit tonsillar tissue, and the tumor, according to the clinical information, originated in oropharyngeal T-sites other than the tonsil. NSTSCCs were often tumors involving multiple oropharyngeal T-sites. In case of doubt, further clinical information was obtained from the patient's file or from analysis of additional tumor sections. None of the tumors was assessed as arising from the base of the tongue. Thirty-six tumor biopsies (6% of total) contained only tumor tissue, and clinical information on T-site was insufficient. These were reclassified as NSTSCCs. The group of pathologists held regular meetings to secure a uniform classification of STSCCs versus NSTSCCs.

HPV DNA analysis

The HPV DNA analysis was blinded with regard to the review of histology. Each FFPE tumor specimen was handled at the Department of Pathology according to standard PCR protocol. For every five tumor specimens, one negative control (consisting of skeletal muscle) was cut and processed the same way as the tumor samples, to exclude cross-contamination in the DNA isolation and the HPV PCR analysis.

DNA isolation and HPV PCR and HPV genotyping were performed at the Center for Genomic Medicine, Rigshospitalet: DNA was isolated from four 10-μm sections with the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the instruction manual. We performed HPV DNA PCR using the general primers GP5+/GP6+.[33] The DNA quality of specimens with a negative GP5+/GP6+ PCR was validated with PCR for the housekeeping gene GAPDH, based on an amplicon of approximately 200 base pairs (bp).

HPV genotyping

We performed amplicon next-generation sequencing (NGS) on 34 blindly selected GP5+/GP6+ positive samples (approximately 10% of the positive samples). Fifty-nanogram quantities of DNA of each sample were pooled. The pooled amplicons were purified with 1,2x sample volume Agentcourt AMPure XP beads (Beckman Coulter, Brea, CA), which excluded DNA fragments of fewer than 100 bp, followed by amplicon size analysis on a Bioanalyzer (Agilent Technologies, Santa Clara, CA). Next we performed emulsion PCR according to the emPCR Amplification Method Manual-Lib-A, GS Junior Titanium Series (Roche), and the amplicons were sequenced on the GS Junior platform (Roche) according to the manufacturer's Sequencing Methods Manual, GS Junior Titanium Series.

We analyzed the variants in a GS Amplicon Variance Analyzer (Roche), and the identified sequences were blasted in a NCBI Nucleotide Blast program (http://blast.ncbi.nlm.nih.gov) to determine HPV types.

Statistical analysis

Statistical analysis was done in IBM SPSS 19 (IBM SPSS, Chicago, IL) and SAS (SAS 9.3, SAS Institute, Cary, NC). The Pearson Chi-square test was used to compare included

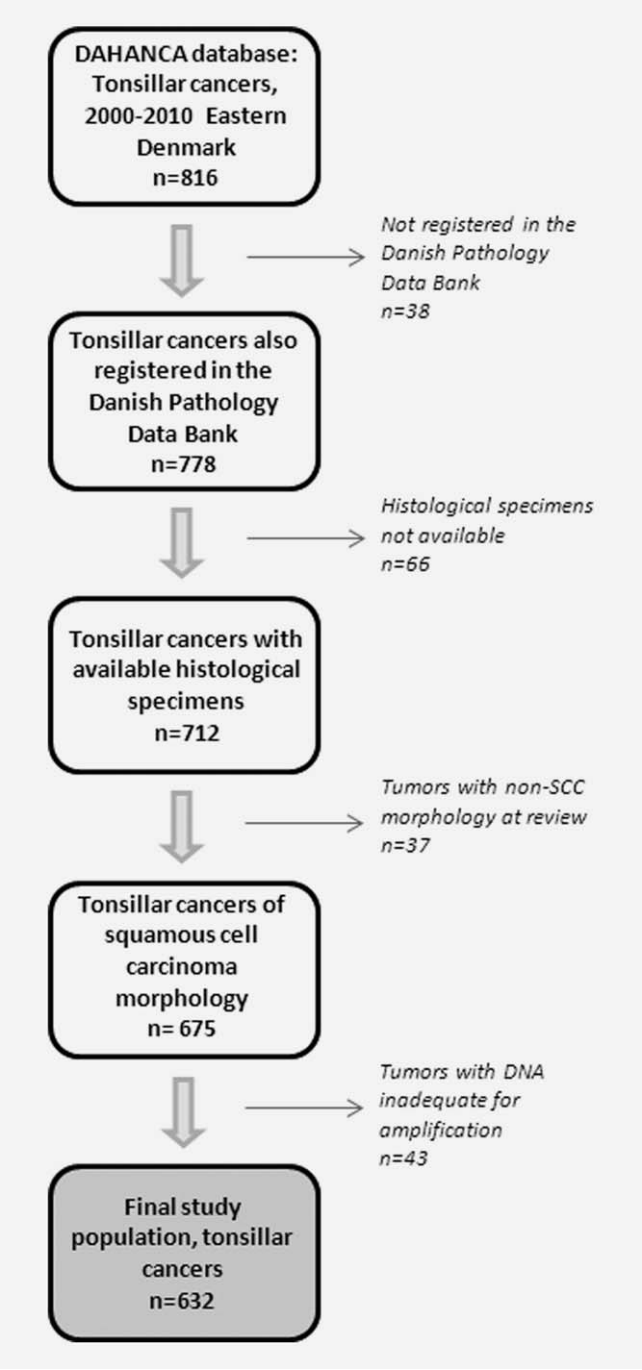

Figure 1. Overview of exclusions and final study population. Abbreviations: DAHANCA, Danish Head and Neck Cancer Group; SCC, squamous cell carcinoma; n, number of patients.

patients with excluded patients according to age, gender, and year of diagnosis, as well as proportions of HPV+/p16- and HPV-/p16+ in STSCCs versus NSTSCCs. Cohen's kappa was calculated to assess the correlation of HPV DNA PCR and p16 IHC, including 95% confidence intervals (95% CI). Poisson regression analysis was used to estimate the average annual percentage change (APC) in the incidence of all TSCCs as well as in groups according to HPV status. The

Table 1. Comparison of tonsillar cancers in Eastern Denmark (2000–2010) included and excluded in the study

Total number of tonsillar cancers in Eastern Denmark (2000–2010) identified from DAHANCA (n = 816)					
	Excluded (n = 184)		Included (n = 632)		
	no.	(%)	no.	(%)	*p*-value
Year					0.47
2000–2003	65	(35)	208	(33)	
2004–2007	61	(33)	241	(38)	
2008–2010	58	(32)	183	(29)	
Gender					0.29
Women	44	(24)	176	(28)	
Men	140	(76)	456	(72)	
Age					0.41
<55 years	50	(27)	195	(31)	
55–60 years	46	(25)	169	(27)	
>60 years	88	(48)	268	(42)	

Abbreviations: DAHANCA, Danish Head and Neck Cancer Group; no., number of patients; %, percentage of total

HPV prevalence according to gender, age, and origin was investigated in a multivariate logistic regression analysis, with associations presented as odds ratios (OR) with 95% CI. Variables were mutually adjusted and furthermore adjusted for year of diagnosis (continuous).

Results

Patient population

We identified a total of 816 tonsillar cancer patients in the DAHANCA database during 2000–2010 covering Eastern Denmark (Fig. 1), and 778 (95%) of these were also registered in the Danish Pathology Data Bank. We were able to retrieve histological specimens for 712 patients from the pathology archives, and during review of the tumor slides, our pathologists identified 675 of these as SCCs. The final study population was comprised of 632 patients, with specimens adequate for HPV PCR analysis. Importantly, patients included in our study were similar in age, gender, and year of diagnosis compared with those excluded (Table 1). During the study period, HPV was present in 366 (58%) out of 632 TSCCs. The HPV prevalence in STSCCs (77%) was significantly higher than that in NSTSCCs (32%) ($p < 0.0001$).

Trends in overall TSCC incidence in Eastern Denmark, 2000–2010

The total number of TSCC patients increased significantly, from 37 patients in 2000 to 62 patients in 2010, with a peak of 84 patients in 2009 (APC, 2.7%; 95% CI, 0.20%–5.3%) (Fig. 2). This increase was accounted for by an increasing number of HPV-positive patients (APC, 4.9%; 95% CI, 1.5%–8.3%), from 23 patients in 2000 to 39 patients in 2010, peaking in 2009 with 55 patients. In contrast, the number of

HPV-negative patients did not increase during the study period (APC, −0.23%; 95% CI, −4.3–3.6).

Factors playing a role in HPV prevalence in overall TSCC

We performed a multivariate analysis with the aim of assessing the role of *a priori* selected factors in the prevalence of HPV in the TSCCs included in our study (Table 2). High HPV prevalence was significantly associated with patients <55 years compared with those >60 years (OR, 1.72; 95% CI 1.13–2.63). We did not find an association between HPV prevalence and gender (OR men *vs.* women, 0.88; 95% CI 0.59–1.31). The strongest predictor of HPV prevalence was origin, demonstrating a significantly lower HPV prevalence in NSTSCCs compared with STSCCs (OR, 0.15; 95% CI 0.11–0.22; $p < 0.0001$). Subsequently, we assessed the association of HPV prevalence with calendar time in STSCCs and NSTSCCs, respectively, in a stratified analysis (Table 3). The HPV prevalence in STSCCs was significantly associated with calendar time, showing an increased OR per year over time (OR per year, 1.10; 95% CI 1.01–1.19; $p = 0.023$). In contrast, the HPV prevalence in NSTSCCs did not exhibit any association with calendar time (OR per year, 0.99; 95% CI 0.90–1.08; $p = 0.8$).

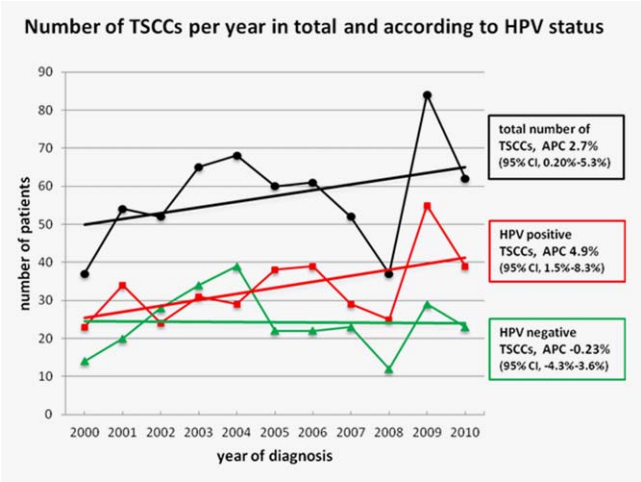

Figure 2. Number of TSCCs, total and according to HPV status. The black circles represent the number of total TSCCs per year, and the black line is an estimated regression line showing a significant increasing number of TSCC patients (2.7% per year). The red squares represent the number of HPV-positive patients per year, and the red line is an estimated regression line showing a significant increasing number of HPV-positive TSCC patients (4.9% per year). The green triangles represent the number of HPV-negative patients per year, and the green line is an estimated regression line showing no increase in the number of HPV-negative patients (−0.23% per year). Abbreviations: APC, annual percentage change; CI, confidence interval; TSCCs, tonsillar squamous cell carcinomas.

HPV genotyping, overall TSCC

By NGS, we identified 13 different GP5+/GP6+ amplicon sequences in overall TSCCs (see Supporting Information for sequences). They were all blasted with alignment scores between 80 and 100. Twelve sequences, corresponding to 99.7% of the amplified sequences, were identified as HPV16. One sequence, corresponding to 0.3% of the amplified sequences, was identified as HPV33.

Correlation between HPV and p16

p16 positivity was demonstrated in 365 (58%) out of 632 TSCCs. The agreement between HPV DNA PCR and p16 IHC status was 86% in total TSCCs, 87% in STSCCs, and 84% in NSTSCCs, corresponding to good intersample agreement (Table 4). The largest discrepancy was recognized in the oldest samples (data not shown). Among STSCCs, the majority (72%) were positive by both tests; in contrast, among NSTSCCs, the majority (62%) were negative by both tests. When we compared the discordant groups (HPV+/p16− and HPV−/p16+), we observed that the proportion of HPV+/p16− was lower in STSCCs (5%) compared with that in NSTSCCs (10.5%) ($p < 0.01$), while there was no difference in the proportions of HPV−/p16+ between STSCCs and NSTSCCs.

Discussion

The present study represents a nonselected, consecutive cohort of more than 600 DAHANCA TSCC patients obtained in a

Table 2. *A priori* selected factors and their association with HPV prevalence, including age, gender, and origin

Factors associated with HPV prevalence	Levels	no.	HPV prevalence	OR[1]	95% CI	*p*-value
Age	<55 years	195	69%	1.72	(1.13;2.63)	0.016
	55–60 years	142	52%	0.92	(0.58;1.44)	
	>60 years	295	53%	1 (ref.)	–	
Gender	Women	176	53%	0.88	(0.59;1.31)	0.53
	Men	456	60%	1 (ref.)	–	
Origin	NSTSCCs	267	32%	0.15	(0.11;0.22)	<0.0001
	STSCCs	365	77%	1 (ref.)	–	

Data are presented as number of patients (no.), HPV prevalence, OR including 95% CI, and *p*-value.
Abbreviations: NSTSCCs, nonspecified TSCCs; STSCCs, specified TSCCs; no., number of patients; OR, odds ratio; CI, confidence interval.
[1]Mutually adjusted and furthermore adjusted for year of diagnosis (continuous).

Epidemiology

Table 3. The association of HPV prevalence with origin and calendar time

Association of HPV prevalence with origin and calendar time	Levels	no.	HPV prevalence	OR	(95% CI)	*p*-value
Nonspecified TSCCs	2000–2003	92	36%	1.16	(0.59–2.29)	
	2004–2007	111	28%	0.79	(0.40–1.54)	
	2008–2010	64	34%	1 (ref.)	-	
	per year			0.99	(0.90–1.08)	0.8
Specified TSCCs	2000–2003	116	68%	0.46	(0.25–0.85)	
	2004–2007	130	80%	0.94	(0.50–1.78)	
	2008–2010	119	82%	1 (ref.)	-	
	per year			1.1	(1.01–1.19)	0.023

Calendar time is presented both as periods (2000–2003, 2004–2007, 2008–2010) and per year.
Abbreviations: no., number of patients; OR, odds ratio; CI, confidence interval.

Table 4. Distribution of patients according to HPV DNA PCR and p16 immunohistochemistry in STSCCs versus NSTSCCs

Specified TSCCs Kappa 0.61 (95% CI 0.50–0.70)	HPV-neg. (no./%)	HPV-pos. (no./%)
p16 neg. (no./%)	55 (15%)	18 (5%)*
p16 pos. (no./%)	30 (8%)	262 (72%)
Nonspecified TSCCs Kappa 0.62 (95% CI 0.51–0.73)	**HPV-neg. (no./%)**	**HPV-pos. (no./%)**
p16 neg. (no./%)	166 (62%)	28 (10.5%)*
p16 pos. (no./%)	15 (5.5%)	58 (22%)

The 2 × 2 tables show the number of patients with the corresponding HPV and p16 profile and the percentage of total, as well as the kappa value for STSCCs and NSTSCCs, respectively, including 95% CI.
Abbreviations: neg., negative; pos., positive; no., number; %, percentage of total; CI, confidence interval.

well-described, extensive area during an 11-year study period, with all histological tumor slides being reviewed by expert pathologists. Due to the large size of our study, we were able to demonstrate an increasing TSCC incidence in our cohort and link it directly to an increasing number of HPV-positive TSCC patients. To our knowledge, this has not been done previously. The overall HPV prevalence was 58%, with HPV16 as the predominant HPV type. HPV prevalence in overall TSCCs was associated with younger patients (<55 years) and origin (nonspecified TSCCs *vs.* specified TSCCs), with origin as the strongest predictor, demonstrating a much higher HPV prevalence (77%) in specified TSCCs compared with nonspecified TSCCs (32%). Furthermore, the association of HPV prevalence with origin over time showed an increased odds ratio per year in specified TSCCs and no association with calendar time in nonspecified TSCCs.

The results of our comprehensive study are likely to be representative of Danish tonsillar cancer patients in general. In addition, analysis of our data confirms (58% HPV-positive overall TSCCs, 77% HPV-positive specified TSCCs) that a high proportion of TSCCs in Scandinavia are HPV-positive, in accordance with reports from earlier studies on smaller patient series (79% HPV-positive in 120 TSCCs in Sweden, 2000–2007; 64% HPV-positive in 137 TSCCs from Norway, 1985–1996).[4,34]

Our results are also in agreement with recent reports from Northern and Western Europe and North America,[3,18–22] supporting HPV as highly prevalent and increasing over time in OPSCCs. However, these studies were smaller, and many of them were comprised of selected patient series, for example, patients enrolled in trial protocols, patients admitted to certain hospitals, or patients belonging to a restricted geographical area.

It is an advantage of our study that the review of tumor origin led to a categorization of tumors into either a specified group (STSCCs) or an unspecified group (NSTSCCs). We thereby obtained a pathoanatomically well-defined group of TSCCs. To our knowledge, this has not been done before. Many studies have not focused on T-site in relation to HPV prevalence and included all OPSCCs, even though OPSCCs comprise a heterogeneous tumor group. Our results support a strong association of HPV with tonsillar tumor origin and indicate lesser involvement of HPV in tumors of unspecific T-sites.[35] The explanation for these findings could be that productive HPV infection has been localized to the specialized tonsillar crypt epithelium.[36,37] Therefore, we emphasize an accurate T-site classification relative to HPV OPSCC studies. Furthermore, our results imply that audit of clinical registries is necessary.

Another strength of the present study is that we used validated HPV DNA PCR primers (GP5+/GP6+)[8,33,38] and a well-defined cut-off for p16 expression by IHC[25,31] to obtain standardized HPV results. The two tests demonstrated good intersample agreement, with the highest occurrence of both tests being positive among the STSCCs. In contrast, the proportion of the discordant outcome, HPV+/p16−, was significantly higher in the NSTSCCs compared with the STSCCs.[39] Further studies are needed to clarify the biological and prognostic significance of the four tumor groups (HPV+/p16+, HPV+/p16−, HPV−/p16+, HPV−/p16−) obtained by combining HPV and p16.[28]

However, there were some limitations to our study. We were able to obtain data for only 632 patients out of 816;

nevertheless, the missing patients were randomly distributed with regard to gender, age, and year of diagnosis, which are important factors for HPV positivity. Thirty-six NSTSCCs contained only tumor tissue, and clinical information on T-site was lacking. We cannot exclude that some of these could still be misclassified. Still, pathological examination has the highest certainty factor of diagnostic measures according to the TNM system,[40] but other diagnostic options could be a morphologic subclassification of the included TSCCs,[41] or microRNA profiling, which has been proven to be highly tissue-specific.[8,38]

The present study demonstrates a significant increase in the number of HPV-positive TSCCs in Eastern Denmark during a recent, 11-year period. In contrast, the incidence of cervical cancers (covering all of Denmark) has decreased from 391 cases in 2000 to 357 cases in 2010 as a result of the screening program,[42,43] and with the introduction of female HPV vaccination in 2009, provided for free to girls and women up to 27 years, a further reduction is expected.[44] In line with previous reports, we identified that younger patients (<55 years) are at particular risk for HPV-positive TSCCs, and that HPV16 is the predominant HPV type.[4,8] Secondary prophylaxis is currently not possible, since precursor lesions are not recognized in HPV-positive OPSCCs.[45] This implies that primary prophylaxis by the FDA-approved HPV vaccination, could be relevant for Danish boys, even though the HPV vaccination is not approved for the prevention of HPV-positive OPSCC. Still, HPV vaccination has been proven to be highly efficacious in preventing extracervical infections in both women and men, for example, development of anal intraepithelial neoplasia,[46] external genital lesions,[47] and reducing the prevalence of oral HPV infection,[48] which is highly associated with HPV-positive OPSCCs.[9] Interestingly, Austria and Australia are introducing male HPV vaccination as part of their national immunization programs.[49,50]

In conclusion, we identified HPV16 as a major risk factor contributing to an increasing number of TSCCs in Eastern Denmark during 2000–2010. High HPV prevalence was associated with younger age (<55 years) and high certainty of tonsillar tumor origin. HPV DNA PCR and p16 IHC appeared to have good intersample agreement; however, the prognostic importance of HPV and p16, respectively, remains to be elucidated.

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