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Research Article

Cancer Prevention Research

Increasing Incidence rates of Oropharyngeal Squamous Cell Carcinoma in Germany and Significance of Disease Burden Attributed to Human Papillomavirus

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Abstract

Increasing incidences of head and neck cancers and rising proportions of these associated with human papillomavirus (HPV), especially in the oropharynx, have been reported in international studies. So far, the trends and contribution of HPV to the number of newly diagnosed cases of oropharyngeal squamous cell carcinomas (OPSCC) in Germany are uncertain. We investigated HPV association and incidence rates in a cohort of consecutively included patients with OPSCC in Giessen 2000–2017, and compared our results with regional (Giessen and the federal state of Hesse), national (Germany), and international (United States) databases. Regional data show a significant increase in the overall incidence rates of oropharyngeal cancers and in the incidence of HPV-associated cancers of the subsites tonsils and oropharynx, whereas other oropharyngeal subsites show no significant change. Analysis of national databases shows a significant incidence increase in Germany and in the United States. The rise in incidence is predominantly attributable to male patients in the US population, whereas in Germany rising OPSCC incidence is more associated with females. There is a significant elevation of OPSCC incidence rates in Germany, which corresponds to the recognized incidence increase of HPV-related oropharyngeal cancers based on experimental data from consecutively included patients of our cohort. Our investigation shows different patterns of this increase in Germany and in the United States, which demonstrates spatial heterogeneity and the need for population-based investigations regarding the role of HPV in oropharyngeal cancer.

Introduction

Worldwide, about 2.2 million new cancer cases were attributable to infectious agents in 2012 and next to Helicobacter pylori (770,000 cases), human papillomavirus (HPV, 640,000 cases) was the second most important infectious agent (1). HPV is a group of diverse and widespread DNA-viruses infecting epithelial cells of the skin and mucosa. HPV-induced lesions are often clinically inconspicuous and clear spontaneously, but persisting infection with high-risk HPV types is causative for malignancies at different anatomic locations in the anogenital and head and neck region (2). Worldwide, HPV types 16 and 18 contribute to 460,000/630,000 (73%) cases of all HPV-related cancers (3). In 2012, 29,000/96,000 (30.8%) cancers in the oropharynx worldwide were attributed to HPV (4). In the same year, 4,212 cases have been newly diagnosed with cancer of the oropharynx [International Classification of Diseases, 10th Revision (ICD10): C01, C09–10] in Germany (5).

HPV-attributable cancers in the head and neck region are most frequently located in palatine tonsils and rarely found outside the oropharynx (2, 6–8). The vast majority are oropharyngeal squamous cell carcinomas

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(OPSCC), distinct from HPV-negative OPSCC with respect to numerous molecular and clinical features (9–13). HPV-related OPSCC have been recently recognized as their own entity beside HPV-negative tumors with respective staging concepts introduced in 2018 (14). This issue has emerged because patients with HPV-associated OPSCC have a remarkably better prognosis (9, 15), but the HPV status has had no impact on the treatment decision to date. Therefore, patients with HPV-associated OPSCC might be overtreated and treatment deescalation is under investigation in clinical trials. New therapeutic concepts like checkpoint-inhibition immune therapy might be particularly suitable for HPV-associated OPSCC, as these viral-associated tumors harbor foreign antigens, which are excellent targets for reactivated immune cells.

Currently, there is no study evaluating the incidence and prevalence of OPSCC attributable to HPV on a population-based level, and HPV status is recorded neither by regional nor by national cancer registries in Germany so far. Comparable data among international studies is rare due to inconsistent concepts defining the anatomic region of interest. The "oropharynx" for example is a subsite in oropharyngeal cancer and in the literature, as well as in the recent tumor staging system (14); the oropharynx is summarized together with the tonsillar sublocalization.

The aim of this study was to investigate the incidence and prevalence of HPV in a cohort of consecutively included patients with OPSCC and to relate the data to regional, national, and international datasets. To support the need for a nationwide HPV vaccination program, particularly for boys, this study contributes a large-scale dataset on the impact of HPV in head and neck cancer.

Materials and Methods

Patients diagnosed with primary cancers of the oropharynx (C09, C10 according to ICD10) and treated between 2000 and 2017 at the Department of Otorhinolaryngology, Head and Neck Surgery of the University of Giessen [ear, nose, and throat (ENT)-Giessen, Germany] were included in the retrospective cohort for HPV prevalence analysis. Written informed consent was obtained from all patients and tumor material was used in accordance with the local ethics committee.

Archived formalin-fixed, paraffin-embedded samples containing tumor tissue according to routine histopathologic reports were selected for preparation of consecutive sections for DNA extraction and immunohistologic detection of p16INK4A expression. DNA extracted from sections was analyzed for mucosal high-risk HPV DNA as described previously (7, 16). p16INK4A expression was detected using the CINtec Histology Kit (Roche mtm Laboratories) according to antibody suppliers' and standard protocols (17). Presence of tumor cells was verified and p16INK4A expression was determined by members of the Department of Applied Tumor Biology, Institute of Pathology, University Hospital Heidelberg (Heidelberg, Germany). Cancer samples were defined to be HPV-associated when positive for both, high-risk HPV-DNA detection, and strong, diffuse p16INK4A expression in >70% of tumor cells as described previously (7, 16).

Incidence data for the United States and Germany was retrieved from public national databases (SEER 18, 2000–2014: https://seer.cancer.gov/faststats, RKI 1999–2014: https://www.krebsdaten.de; refs. 5, 18), both accessed on April 17, 2018. Regional data are available only from 2008 onwards and were provided from the Hesse (Hessen) Cancer Registry (Landesauswertungsstelle des Hessischen Krebsregisters beim Hessischen Landesprüfungs- und Untersuchungsamt im Gesundheitswesen), database version 12/2017. Incidence rates were queried for equal coverage of major oropharyngeal cancer subsites and invasive cervical cancer in all databases using cancer site selections as follows: Tongue: SEER (ICD-O-3): C019-029; RKI/Hesse (ICD10) C01 and C02. Gum & other mouth: SEER (ICD-O-3): C030-039, C050-069; RKI/Hesse (ICD10): C03, C05, and C06. Oropharynx & Tonsil: SEER (ICD-O-3): C090-109; RKI/Hesse (ICD10): C09 and C10. Cervix Uteri: SEER (ICD-O-3): C530-C539; RKI (ICD10): C53. Incidence rates were age-adjusted to the US population as recorded in the 2000 census for SEER, RKI, and Hesse data. Data of patients diagnosed and treated at the University of Giessen were received from the local cancer registry. Statistical analyses were conducted using SPSS Statistical Software (IBM SPSS 24.0) and significance was considered for $P \leq 0.05$ for all tests.

Results

We have examined the HPV status of all primary OPSCC cases with sufficient tumor material available from a consecutive cohort of patients, diagnosed and treated in Giessen between 2000 and 2017 (Fig. 1A). In total, we analyzed $n = 730$ OPSCC. HPV association was determined in $n = 198$ cancers representing a total prevalence of 27.1% of HPV-related oropharyngeal cancers in Giessen. The total number of OPSCC diagnosed and treated at the ENT-Giessen significantly increased over time with 0.72 cases annually (Table 1). A highly significant trend is observed for HPV-associated OPSCC, with an increase of approximately 1 case annually in the analyzed period ($P < 0.001$; Table 1). HPV prevalence in OPSCC has roughly doubled in the past ten years in Giessen (Fig. 1b). At the same time, annual cases of HPV-negative OPSCC decreased, although not significantly (Fig. 1A).

Overall and for the major subsites of the oropharynx (oropharyngeal and tonsillar cancers), data from the

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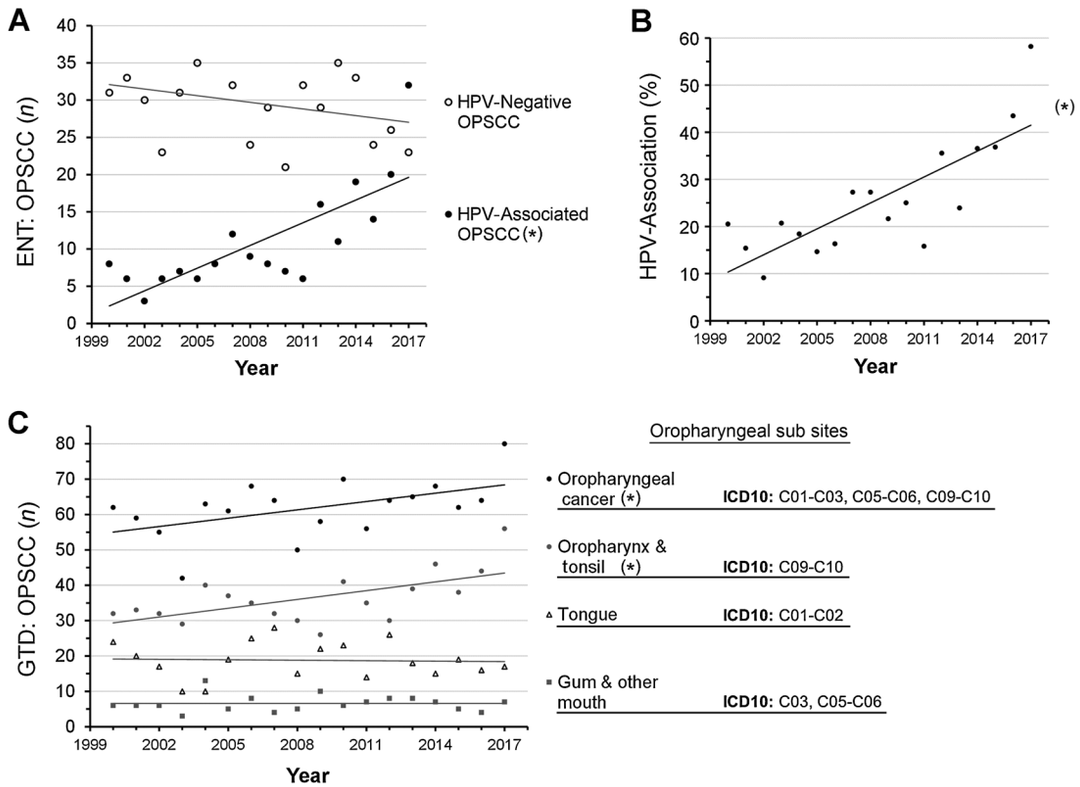

Figure 1.

OPSCC and proportion of HPV-associated cases in Giessen between 2000 and 2017. **A,** Annual number of HPV-negative and HPV-associated OPSCC diagnosed and treated at ENT-Giessen. **B,** Percentage of HPV-associated OPSCC treated at ENT-Giessen by year. **C,** Total number of oropharyngeal cancers and major subsites annually recorded by the local cancer registry in Giessen (GTD). Trend (lines) was estimated by linear regression, inclination- ("B") and *P* values are listed in Table 1, significant deviation in inclination from constant values is indicated (*).

local cancer registry display a significant increase of the annual number (Table 1). With about 0.8 cases per year for oropharyngeal and tonsillar cancers and overall, this increase is similar to the increase we observe in data from ENT-Giessen. However, the annual number of other oropharyngeal subsites remains constant over time (Fig. 1C; Table 1).

Next, we compared regional (Hesse), national (Germany), and international (United States) trends in the incidence of oropharyngeal and cervical cancers. Invasive cancer of the uterine cervix is almost exclusively related to infections with high-risk HPV (1, 4) and in Germany as well as in the United States, a significant decrease in incidence is observed (Fig. 2; Table 2). The annual reduction is similar in the United States (−0.136 cases/100,000) and in Germany (−0.113 cases/100,000; Table 2). In contrast, the incidence of oropharyngeal cancer is significantly increased in the United States ($P < 0.001$) and in Germany ($P = 0.004$) with higher rates observed in the United States (+0.101 vs. +0.037 cases/100,000; Fig. 2; Table 2). Remarkably, the incidence rates of cervical and

Table 1. HPV-prevalence and annual change in number of patients with oropharyngeal cancers and HPV-associated OPSCC in Giessen

	2000–2017		Annual change	
	Total (n; %)	Annual cases Mean (n)	B (Cases/year)	*P*
Local cancer registry				
All subsites	1111 (100.0%)	61.7 (±8.2)	+0.787	**0.029**
Tongue	338 (30.4%)	18.8 (±5.2)	−0.041	0.866
Gum & other mouth	118 (10.6%)	6.6 (±2.4)	+0.000	1.000
Oropharynx & tonsil	655 (59.0%)	36.4 (±7.3)	+0.829	**0.007**
Treated at ENT-Giessen				
All oropharyngeal cancers	730 (100.0%)	40.6 (±7.2)	+0.718	**0.029**
HPV-negative	532 (72.9%)	29.6 (±5.1)	−0.297	0.221
HPV-associated	198 (27.1%)	11.0 (±6.9)	+1.015	**<0.001**

Abbreviations: B, Annual change in incidence (cases/year); *P, P* value calculated by linear regression, significant values in bold.

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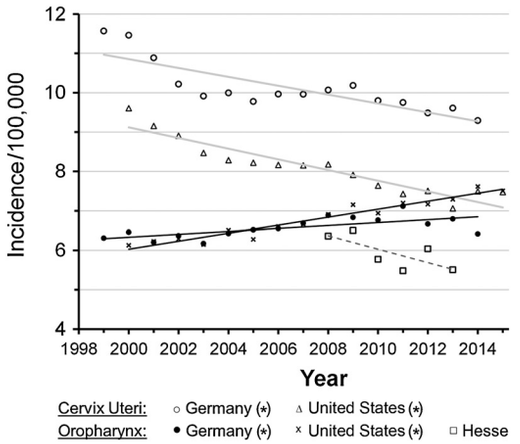

Figure 2.

Age-adjusted annual incidence rates of oropharyngeal and cervical cancer in the United States and in Germany/Hesse. Trend (lines) was estimated by linear regression, inclination- ("B") and *P* values are listed in Table 2, significant deviation in inclination from constant values is indicated (*).

oropharyngeal cancers have crossed in 2013 in the United States, while this can be first expected in 2030/2031 in Germany. Regional data show a declining trend for oropharyngeal cancer in Hesse, which is not statistically significant ($P = 0.096$; Fig. 2; Table 2).

To investigate potential differences in incidence trends in Germany and in the United States, we analyzed the data separated by gender and major subsites of the oropharynx (Fig. 3). Interestingly, incidence rates are increasing more in males than in females in the United States and vice versa in Germany. This results in opposing trends in the male-to-female ratio, which is significantly increasing in the United States and significantly decreasing in Germany (Fig. 3A; Table 2). The declining male-to-female ratio in Germany is reflected in regional data from Hesse, although, the observed regional trend is not significant (Fig. 3A; Table 2).

Concerning the major oropharyngeal subsites, the incidence increase of OPSCC is related to the oropharynx and tonsils (ICD10: C09, C10) and tongue (ICD10: C01, C02) in male patients in the United States. An increase in Germany cannot be attributed to specific subsites, but appears to be more pronounced in females (Fig. 3B; Table 2). Interestingly, incidence trend does not significantly change for the subsite oropharynx and tonsil in Germany (although a trend is observed for females ($P = 0.088$; Table 2)).

Discussion

We found a significant increase in HPV-associated cancers in a continuous cohort of patients with OPSCC. A rising incidence of HPV-positive OPSCC has been reported for the United States. This was estimated on the basis of experimental HPV-data for $n = 271$ OPSCC diagnosed from 1984 to 2004 in Hawaii, Iowa, and Los Angeles, and it was concluded that HPV-associated OPSCC will constitute a majority of all head and neck cancers in the United States in the next 20 years (19). This forecast has been confirmed by epidemiologic data for the United States in several studies (20–22). Nevertheless, experimental data for the HPV status is still limited and mainly extrapolated from analysis of study populations, which are often limited in sample size or inconsistent concerning the time period, patient residence, or included anatomic regions. Several studies on trends in HPV-related OPSCC do not consider experimental data and define HPV-related OPSCC according to the affected anatomic subsite (23–25).

The overall HPV prevalence in cancers of the head and neck region has been estimated to range between 25% and 31% worldwide (3, 26), with higher tumor site–specific HPV prevalence among studies from North America compared with Europe and Asia (26). Varying HPV prevalence is reported in European countries, with higher rates in Northern countries in general. Considering OPSCC in the first decade of this century (roughly 2000–2010), rates for HPV-driven cancers have been reported for North-East Italy: 27% (27), the Netherlands: 38% (28), and Eastern Denmark: 49% (29). The proportion of HPV-associated OPSCC increased to 62% in Eastern Denmark in the following period (2011–2014; ref. 29), which is still lower compared with Norway (77% in a population cohort in 2010–2011; ref. 30). A rise in HPV prevalence was also shown in the Netherlands (1980–1989: 28%, 1990–1999: 38%, and 2000–2009 38%), although data only relies on randomly selected patients from three periods (28). We found a total prevalence of 27.1% of HPV-associated OPSCC in our continuous cohort of patients from central Germany between 2000 and 2017, which is relatively low when compared with other European countries. However, the HPV prevalence has roughly doubled within ten years and currently is approximately 40% in Giessen, which is higher than the global prevalence (30.8%) estimated for 2012 (4). Considering the continuous increase in Giessen, HPV prevalence of about 40% for OPSCC appears reasonable for this central region of Germany, which corresponds to the geographical trend in Europe.

A problem in comparing international studies is that the "oropharynx" is a subsite in oropharyngeal cancer due to anatomic naming definition. Tumor borders are often hard to define in clinical practice, error probability might be high in the anatomic classification and different classification systems exist. Frequently in literature, as well as in the recent tumor staging system (14), the oropharynx is summarized together with the tonsillar sublocalization.

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Table 2. Incidence trends of cervical and oropharyngeal cancers in the United States and Germany

Figure	Cancer site	Country	B	*P*
2	Cervix Uteri	United States	−0.136	<**0.001**
		Germany	−0.113	<**0.001**
	Oropharynx	United States	+0.101	<**0.001**
		Germany	+0.037	**0.004**
		Hesse (Germany)	−0.169	0.096

	Subsite	Country	Gender	B	*P*
3A	Oropharynx (all subsites)	United States	Males	+0.186	<**0.001**
			Females	+0.023	<**0.001**
			Ratio males/females	+0.032	<**0.001**
		Germany	Males	+0.021	0.190
			Females	+0.046	<**0.001**
			Ratio males/females	−0.032	<**0.001**
		Hesse (Germany)	Males	−0.276	0.084
			Females	−0.057	0.527
			Ratio males/females	−0.034	0.505
3B	Tongue	United States	Both sex[a]	+0.057	<**0.001**
			Males	+0.097	<**0.001**
			Females	+0.020	<**0.001**
			Ratio males/females	+0.026	<**0.001**
		Germany	Both sex[a]	+0.023	**0.001**
			Males	+0.022	**0.018**
			Females	+0.022	<**0.001**
			Ratio males/females	−0.032	**0.001**
	Oropharynx & tonsil	United States	Both sex[a]	+0.054	<**0.001**
			Males	+0.102	<**0.001**
			Females	+0.010	<**0.001**
			Ratio males/females	+0.069	<**0.001**
		Germany	Both sex[a]	+0.002	0.628
			Males	−0.002	0.779
			Females	+0.007	0.088
			Ratio males/females	−0.023	0.093
	Gum & other mouth	United States	Both sex[a]	−0.010	**0.003**
			Males	−0.013	**0.006**
			Females	−0.007	**0.013**
			Ratio males/females	−0.002	0.518
		Germany	Both sex[a]	+0.011	**0.019**
			Males	+0.003	0.668
			Females	+0.017	<**0.001**
			Ratio males/females	−0.030	<**0.001**

Abbreviations: B, Annual change in incidence (per 100,000) or ratio (males/females); United States, SEER-database; Germany, RKI-database; *P*, *P* value calculated by linear regression, significant values in bold.

[a]Not displayed in figure.

Thus, trends in biologically distinct entities could be masked. This is demonstrated by our data, showing that the observed overall increase is restricted to specific subsites of the oropharynx (oropharyngeal and tonsillar cancers), but not to other subsites (Fig. 1C).

Estimating the prevalence of HPV in oropharyngeal cancer in Germany based on our data might be sensible. However, care should be taken because our data only reflect the population of a region that can be considered in-between rural and urban regions and factors influencing HPV prevalence may vary regionally. For example, there is a remarkable difference in the smoking prevalence among the different federal states of Germany. Hesse has the lowest prevalence (18.1%) compared with other federal states (e.g., Brandenburg: 42.6% and North Rhine-Westphalia: 30.3%; ref. 31).

The population-based data from Germany and the United States consistently show an increase of OPSCC and a decrease of invasive cervical cancer, with slightly higher rates in the United States (Fig. 2). The increase of HPV prevalence in oropharyngeal cancer is in contrast to invasive cancer of the uterine cervix, which is exclusively related to HPV. An increase in the *in situ* tumors of the uterine cervix cannot be excluded. However, the observed decrease might be an effect of better screening for cervical cancer rather than an actual decrease in its onset. With the introduction of the HPV vaccination programs for both girls and boys, prevalence patterns may differ even further depending upon the adoption rates of the vaccination, which varies greatly between countries (32, 33).

Differences between Germany and the United States are observed with respect to incidence rates in sublocalizations of oropharyngeal cancer and trends in the male-to-female ratio, which may relate to national differences in lifestyle risks and socioeconomic factors influencing both populations. Smoking prevalence has decreased from 20.9% in 2005 to 15.1% in 2015 in the United States (34), indicating that the oropharyngeal cancers increase independently of

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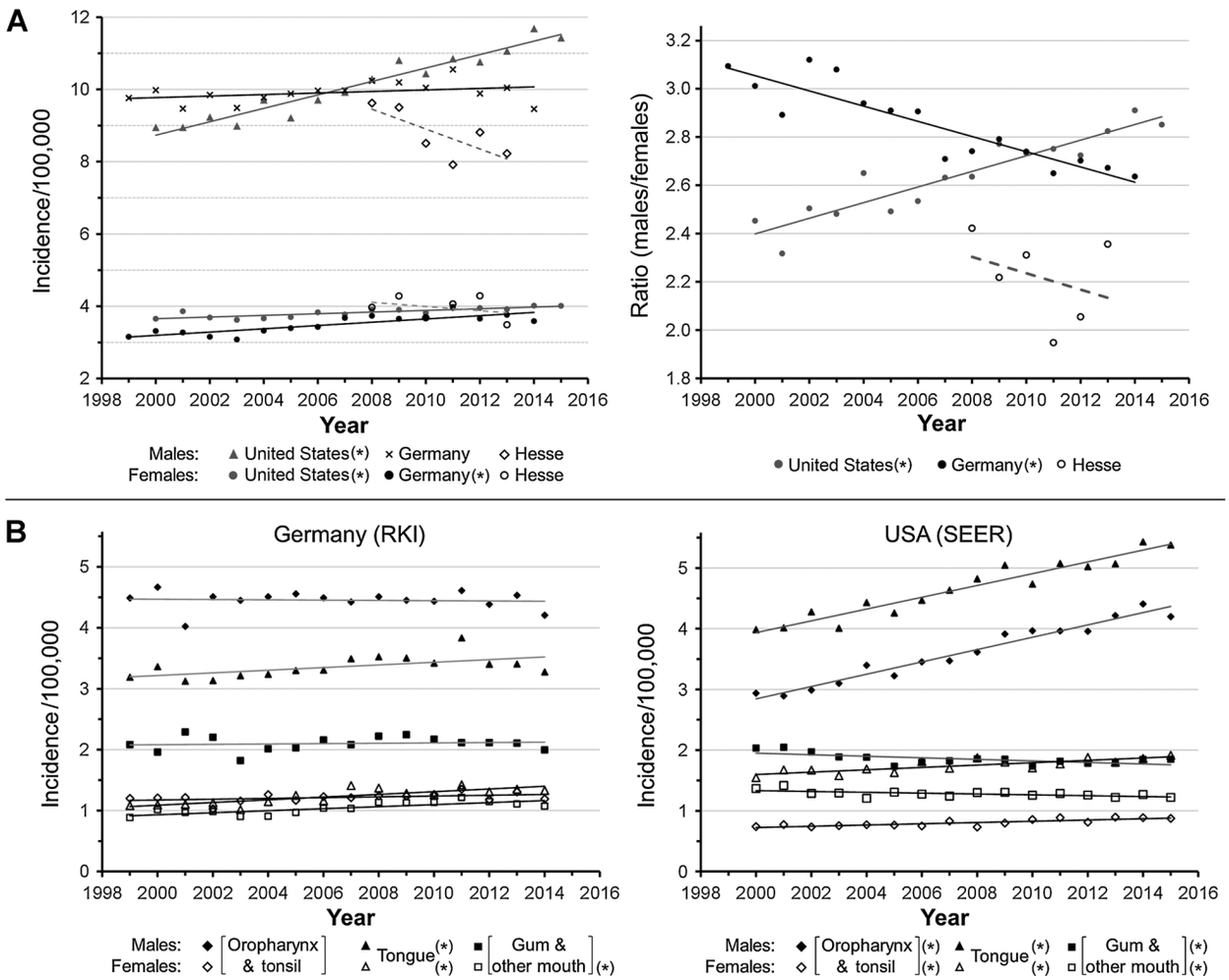

Figure 3.

Incidence trends in Germany, Hesse, and in the United States displayed according to gender (**A**) and major subsites of the oropharynx (**B**). Trend (lines) was estimated by linear regression, inclination- ("B") and *P* values are listed in Table 2, significant deviation in inclination from constant values is indicated (*).

smoking. This has been shown by a large meta-analysis of 148 studies, confirming that HPV (DNA) prevalence significantly varies by anatomic site, geographic region, but not by gender or tobacco or alcohol consumption (6). Nevertheless, smoking prevalence is still high in Germany (23%–28%; refs. 31, 35) compared with the United States (15%; ref. 34), and it is important to notice that huge differences exist among the federal states of Germany and between males and females, with the latter exhibiting a much slower decline (31). Although not directly related to rising OPSCC incidence rates, this might explain differences among anatomic subsites and geographical regions. However, more attention should be given to experimental data to verify findings exclusively based on cancer registry data and inclusion of (risk) factors like HPV association should be considered for cancer registries.

In summary, there has been no nationwide, population-based investigation regarding the role of HPV in oropharyngeal cancer in Germany until now and still, estimates are based on inconsistent cohorts. We provide evidence for rising incidence rates of HPV-associated OPSCC for a local, but temporally and geographically continuous population in Germany, which is consistent with trends in other European countries and the United States. Although local and regional differences exist, the overall incidence rates of OPSCC are rising and at the same time declining for invasive cervical cancer in Germany and the United States, supporting the global trends in both entities. On the basis of current data and the still insufficient adoption of HPV vaccination programs, a continuing increase of HPV-associated OPSCC can be expected for Germany and many other countries.

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Disclosure of Potential Conflicts of Interest

E.-S. Prigge has received speakers bureau honoraria from MSD Sharp & Dohme GmbH (MSD). N. Wuerdemann reports receiving commercial research grant from MSD. J.P. Klussmann is a consultant/advisory board member and reports receiving commercial research grant from MSD. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Wittekindt, S. Wagner, J.P. Klussmann
Development of methodology: S. Wagner
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Wittekindt, S. Wagner, A. Bushnak, E.-S. Prigge, M. Doeberitz, K. Bernhardt, J.P. Klussmann
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Wagner, A. Bushnak, E.-S. Prigge, K. Bernhardt, J. Pons-Kühnemann, C. Maulbecker-Armstrong, J.P. Klussmann
Writing, review, and/or revision of the manuscript: C. Wittekindt, S. Wagner, E.-S. Prigge, N. Würdemann, C. Maulbecker-Armstrong, J.P. Klussmann
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Wittekindt, S. Wagner, E.-S. Prigge, M. Doeberitz, J.P. Klussmann
Study supervision: S. Wagner, J.P. Klussmann

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Cancer Prevention Research

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