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ORIGINAL ARTICLE

Timing of HPV16-E6 antibody seroconversion before OPSCC: findings from the HPVC3 consortium

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Background: Human papillomavirus type 16 (HPV16)-E6 antibodies are detectable in peripheral blood before diagnosis in the majority of HPV16-driven oropharyngeal squamous cell carcinoma (OPSCC), but the timing of seroconversion is unknown.

Patients and methods: We formed the HPV Cancer Cohort Consortium which comprises nine population cohorts from Europe, North America and Australia. In total, 743 incident OPSCC cases and 5814 controls provided at least one pre-diagnostic blood sample, including 111 cases with multiple samples. Median time between first blood collection and OPSCC diagnosis was 11.4 years (IQR = 6–11 years, range = 0–40 years). Antibodies against HPV16-E6 were measured by multiplex serology (GST fusion protein based Luminex assay).

Results: HPV16-E6 seropositivity was present in 0.4% of controls (22/5814; 95% CI 0.2% to 0.6%) and 26.2% (195/743; 95% CI 23.1% to 29.6%) of OPSCC cases. HPV16-E6 seropositivity increased the odds of OPSCC 98.2-fold (95% CI 62.1–155.4) in whites and 17.2-fold (95% CI 1.7–170.5) in blacks. Seropositivity in cases was more frequent in recent calendar periods, ranging from 21.9% pre-1996 to 68.4% in 2005 onwards, in those with blood collection near diagnosis (lead time <5 years). HPV16-E6

seropositivity increased with lead time: 0.0%, 13.5%, 23.7%, and 38.9% with lead times of >30 years ($N = 24$), 20–30 years ($N = 148$), 10–20 years ($N = 228$), and <10 years ($N = 301$ cases) (p-trend < 0.001). Of the 47 HPV16-E6 seropositive cases with serially-collected blood samples, 17 cases seroconverted during follow-up, with timing ranging from 6 to 28 years before diagnosis. For the remaining 30 cases, robust seropositivity was observed up to 25 years before diagnosis.

Conclusions: The immune response to HPV16-driven tumorigenesis is most often detectable several decades before OPSCC diagnosis. HPV16-E6 seropositive individuals face increased risk of OPSCC over several decades.

Key words: HPVC3, OPCSCC, oropharyngeal squamous cell carcinoma

Introduction

The chief causes of oropharyngeal squamous cell carcinoma (OPSCC) are tobacco and alcohol use, as well as infection with human papillomavirus type 16 (HPV16) [1]. In the United States (US) and some other high-income western countries, the attributable fraction of OPSCC due to HPV now exceeds 70% [1]. Further, the incidence and burden of HPV-related OPSCC is increasing, particularly for men [2–5], warranting potential methods for early detection to be considered.

We initially reported that HPV16-E6 antibodies are detectable in the circulation before diagnosis in a considerable proportion of OPSCC cases in a European population, whilst being nearly absent in controls [6]. We subsequently replicated those results in a US cohort, and demonstrated that HPV16-E6 antibody levels are stable up to a decade before diagnosis [7]; however, due to the rarity of OPSCC, there were insufficient serial samples to describe the timing of HPV16-E6 seroconversion among HPV16-driven OPSCC cases.

To overcome this constraint, we formed the HPV Cancer Cohort Consortium (HPVC3) to investigate HPV antibodies and risk of all HPV-driven cancers [8], including oropharyngeal, anal, cervical, vaginal, vulvar, and penile cancers. Nine prospective cohort studies participated, together providing a study base exceeding 1.3 million participants from Europe, North America, and Australia within which blood was collected up to 40 years before cancer diagnosis. HPV serologic testing was conducted for incident cancer cases and matched controls simultaneously for antibodies against multiple HPV genotypes and antigens. In this first HPVC3 manuscript where we focus on the HPV16-E6 antibodies before diagnosis of OPSCC and considers different lead times and periods of calendar time, which is important given the temporal increases in the HPV16-attributable fraction of OPSCC [9].

Methods

Prospective cohorts participating in the US National Cancer Institute (NCI) cohort consortium were invited to participate in HPVC3. Nine individual cohorts agreed to participate, including ATBC [10] (Years of enrollment, 1985–1988), CLUE [11] (1974–1989), EPIC [12] (1991–1999), HUNT [13] (1995–2008), JANUS [14] (1972–2004), MCCS [15] (1990–1994), PLCO [16] (1993–2001), SCCS [17] (2002–2009), and WHI [18, 19] (1994–1998), representing a total study base of over 1.3 million study participants. Details on design of the cohorts and their follow-up procedures are provided in the supplementary Methods (available at *Annals of Oncology* online). All study participants provided written informed consent for their samples to be used for research purposes and the research project was approved by the Ethical Committees and/or Institutional Review Board of each participating cohort.

Selection of cases and controls for HPVC3

Anogenital and head and neck cancer sites with an etiologic link to HPV infection [8] were included in the HPVC3 study and classified based on the International Classification of Diseases for Oncology. In total, 2808 anogenital and head and neck cancer cases with available plasma or serum samples were identified within the participating cohorts. For each HPVC3 eligible anogenital and head and neck cancer case, two controls were randomly chosen from risk-sets consisting of all cohort members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis of the index case. Matching criteria included cohort (and study center where relevant), sex, date of blood collection (±6 months, relaxed to ±12 months for sets without available controls, $N = 68$), and date of birth (±1 year, relaxed to ±5 years, $N = 52$). A total of 5814 matched controls with available plasma or serum samples and relevant data were included in serologic analyses.

Of the 2808 eligible cancer cases in HPVC3, 825 were defined as oropharyngeal cancer (OPC) cases of any histologic type (International Classification of Diseases 10th Revision Clinical Modification; ICD-10-CM: C01, C02.4, C02.8, C05.1, C05.2, C09.0, C09.1, C09.8, C09.9, C10.0, C10.1, C10.2, C10.3, C10.4, C10.8, C10.9; supplementary Table S1, available at *Annals of Oncology* online). We focused this analysis on 743 (90.1%) cases classified as squamous cell carcinoma subtypes (OPSCC) (ICD-03; 8051/3, 8052/3, 8070/2, 8070/3, 8071/3, 8072/3, 8073/3, 8074/3, 8076/3, 8082/3, 8083/3, 8094/3).

The current analysis was based on plasma or serum analysis from 743 OPSCC cases and all 5814 matched controls (n.b. use of all the controls, and not just matched controls, is justified by the exceptionally low HPV16 E6 seroprevalence in people without cancer, and its lack of associations with covariates [20]; analyses subset to only matched controls provided the same results albeit with wider confidence intervals). We previously published data from EPIC [6] and PLCO [7] OPC cases ($n = 210$); updated cancer linkages resulted in seven additional cases from the PLCO study and 526 cases from the newly-added cohorts.

Serological analyses

Serum/plasma samples were sent on dry ice to the German Cancer Research Center (DKFZ, Heidelberg, Germany) and testing was carried out using multiplex serology by laboratory staff blinded to the case–control status of the subjects. Antigens were affinity-purified, bacterially-expressed fusion proteins with N-terminal Glutathione S-transferase [21]. Samples were analyzed for antibodies to the early oncoprotein HPV16-E6. Median fluorescence intensity (MFI) values were dichotomized as antibody positive or negative using a pre-defined cut-off value of 1000 MFI as in our previous PLCO study [7]. Based on 72 subjects with serum assayed in duplicate, the intra-class correlation coefficients (ICC) for HPV16-E6 was 0.97 (95% CI 0.95–0.98).

Statistical analyses

Baseline characteristics of the cancer cases and controls were initially tabulated and evaluated. Noted imbalances were the result of using all available control data. Our pre-specified main analyses involved estimating HPV16-E6 seroprevalence for OPSCC cases overall, stratified by

anatomical subsites within the oropharynx, and for controls. To provide the most relevant representation of HPV16-E6 status for the OPSCC cases, and considering the hypothesis that HPV16-E6 seroconversion can occur both close to (i.e. within a few years) and long before (i.e. over 10 years) the clinical diagnosis of HPV-related OPSCC, we based our main analysis on the HPV16-E6 antibody measure from the sample collected closest to diagnosis for subjects with multiple pre-diagnostic blood samples. Unadjusted (crude) odds ratios were calculated for OPSCC overall and by anatomic subsites across all participating cohorts using unconditional logistic regression. Of note, conditional or multivariable logistic regression was not possible due to some cohorts lacking seropositive controls. HPV16-E6 seropositivity was stratified on sex and, given suggestions in the literature of heterogeneity in the fraction of HPV-related OPSCC [22], by race (US cohorts only; non-US cohorts included only white study participants).

Further pre-specified analyses included evaluating differences in the fraction of seropositive cases by lead time and by calendar time. To assess lead time differences, we restricted cases to the most recent category of calendar time (>2005) to control for known changes in HPV16-E6 DNA positivity over time [9]. Similarly, to assess calendar time differences in the fraction of seropositive cases, we restricted cases to those with the shortest lead time (0–5 years). Trends in HPV16-E6 seroprevalence were tested using simple logistic regression including the variable (e.g. calendar time) as an ordinal covariate.

For OPSCC cases with multiple blood samples available before diagnosis, patterns of HPV16-E6 MFI levels were described graphically by time before diagnosis and age, separately for three groups: cases who were HPV16-E6 seronegative throughout follow-up, cases who were HPV16-E6 seropositive throughout follow-up, and cases who seroconverted during follow-up. Seroconversion was defined as a change from below to above the assay cut-off for seropositivity of 1000 MFI. We described the longest and shortest lead times for individual samples and additionally computed the median lead time as the average between the time points just before and after the observed seroconversion. As this assumes linearity in the immune response, we did sensitivity analyses where the timing of seroconversion used the time point before as well as the time point after seroconversion. Timing of HPV16-E6 seroconversion was also evaluated using age as the time scale. All statistical analyses were conducted using R version 3.5.0.

Results

Baseline characteristics

OPSCC cases and 5814 controls were identified from nine participating cohorts (Table 1; supplementary Table S2, available at *Annals of Oncology* online). The median age at study enrollment and, therefore, first blood draw was 46 years [interquartile range (IQR) 41–55]. The vast majority (~94%) of study participants were white, male (72.9% of cases compared with 62.2% of controls) and ever smokers (83.1% of cases and 65.0% of controls).

Among cases, median calendar year of diagnosis was 2004 (IQR 1999–2009) and median age at diagnosis was 62 years (IQR 57–68). Based on the sample collected closest to diagnosis, median lead time was 11.4 years (IQR 6–19; range 0–40 years); 43.1% of cases had samples collected <10 years before diagnosis, 32.4% between 10 and 20 years, 20.4% between 20 and 30 years, and 3.4% had ≥30 years. Fifteen percent of cases had multiple pre-diagnostic samples including subjects with 10 ($n=1$), 8 ($n=1$), 7 ($n=3$), 6 ($n=18$), 5 ($n=12$), 4 ($n=14$), 3 ($n=19$), and 2 ($n=43$) samples, as well as 590 (85%) subjects who provided a single blood sample.

HPV16-E6 seropositivity in cases and controls

HPV16-E6 seropositivity was present in 22 of 5814 controls (0.4%; 95% CI 0.2% to 0.6%); thus, the marker specificity for cancer was 99.6% (95% CI 99.4% to 99.8%). HPV16-E6 seropositivity was present before diagnosis in 27.2% of white OPSCC cases (191 of 701; 95% CI 24.0% to 30.7%) and 7.7% of black OPSCC cases (3 of 39; 95% CI 2.0% to 21.7%; p for difference between white and black OPSCC cases <0.001; Table 2). Thus, HPV16-E6 seropositivity was associated with a 98.2-fold increase in the odds of OPSCC in whites (95% CI 62.1–155.4) and 17.2-fold increase in odds in blacks (95% CI 1.7–170.5). HPV16-E6 seroprevalence was 28.0% (95% CI 24.3% to 32.0%) in males and 21.4% (95% CI 15.9% to 27.7%) in females ($p_{\text{difference}}=0.07$). The median age at diagnosis was 62 years (95% CI 56–68) for HPV16-E6 seropositive cases and 68 years (95% CI 62–73) for HPV16-E6 seronegative cases.

By anatomic subsite within the oropharynx, HPV16-E6 seropositivity was present before diagnosis in 36.9% of 328 tonsillar cancers, 25.2% of 218 bases of tongue cancers, and 9.7% of 170 cases with cancer at other OPSCC anatomic subsites.

Trends in HPV16-E6 seropositivity in OPSCC cases by lead time

Restricting to cases diagnosed most recently in calendar time (>2005), overall HPV16-E6 seroprevalence was 27.9% (87 of 312 cases; 95% CI 23.1% to 33.3%). Amongst these 312 cases, those with shorter lead time had incrementally higher HPV16-E6 seroprevalence: 68.4% (95% CI 43.5% to 83.4%) of 19 cases with lead time <5 years, 45.0% (95% CI 29.6% to 61.3%) of 40 cases with lead time 5–10 years, 34.3% (95% CI 25.6% to 44.1%) of 108 cases with lead time 10–20 years, 15.2% (95% CI 9.6% to 23.0%) of 125 cases with lead time 20–30 years, and 0% (95% CI 0.0% to 20.0%) of 20 cases with lead time >30 years (p-trend < 0.001; Figure 1 and supplementary Table S3, available at *Annals of Oncology* online).

Trends in HPV16-E6 seropositivity in OPSCC cases and controls by calendar time

Restricting to cases with shortest lead time (<5 years), the overall HPV16-E6 seroprevalence was 43.0% (61 of 142 cases; 95% CI 34.8% to 51.2%); however, seroprevalence decreased from 68.4% (95% CI 43.5% to 83.4%) of 19 cases diagnosed after 2005, to 45.1% (95% CI 34.7% to 55.8%) of 91 cases diagnosed between 1995 and 2005, and further to 21.9% (95% CI 9.9% to 40.4%) of 32 cases diagnosed before 1995 (p-trend < 0.001; Figure 1 and supplementary Table S3, available at *Annals of Oncology* online).

HPV16-E6 seropositivity was present in 0.2% (95% CI 0.1% to 0.4%) of 3466 controls with blood drawn before 1995, 0.7% (95% CI 0.4% to 1.3%) of 1808 controls with blood drawn between 1995 and 2005, and in 0.0% (95% CI 0.0% to 1.8%) of 23 controls with blood drawn after 2005.

Table 1. Characteristics of OPSCC cases and controls in the HPV Cancer Cohort Consortium (HPVC3)

	OPSCC (*N* = 743)	Controls (*N* = 5814)
Race, *N*		
Whites	701 (94.3)	5509 (94.8)
Blacks	39 (5.2)	208 (3.6)
Others	3 (0.5)	97 (1.7)
Sex, *N*(%)		
Male	542 (72.9)	3619 (62.2)
Female	201 (27.1)	2195 (37.8)
Smoking, *N*(%)[a]		
Current	356 (54.4)	1739 (30.9)
Former	188 (28.7)	1919 (34.1)
Never	110 (16.8)	1969 (35.0)
Alcohol drinking, *N*(%)[a]		
Current	304 (79.6)	3235 (79.6)
Former	68 (17.8)	691 (17.0)
Never	10 (2.6)	139 (3.4)
Age at blood draw, *N*(%)[a]		
≤40	143 (20.2)	696 (13.1)
41–50	226 (31.9)	1130 (21.2)
51–60	183 (25.8)	1490 (28)
61–70	128 (18.1)	1625 (30.5)
≥70	29 (4.1)	388 (7.3)
Case-only characteristics[b]		
Calendar year at diagnosis		
Median (IQR)	2004 (1999, 2009)	–
Age at diagnosis		
Median (IQR)	62 (57, 68)	–
Years between blood draw and diagnosis		
At the case level[a]		
Median (IQR; range)	11.4 (6–19; 0–40)	
N(%)		
[0–10)	301 (42.94)	–
[10–20)	228 (32.52)	–
[20–30)	148 (21.11)	–
≥30	24 (3.42)	–
At the specimen level[c]		
Median (IQR; range)	11.4 (6–20; 0–42)	

[a]Latest date of blood draw for serial samples was used for calculations.
[b]Calculations restricted to white ethnicity.
[c]Calculations including all samples for OPSCC cases with several blood draws (HUNT, JANUS, MCCS, and PLCO).
OPSCC, oropharyngeal squamous cell carcinoma.

HPV16-E6 seroconversion

In assessment of 111 OPSCC cases with serial blood samples, 30 cases were robustly HPV16-E6 seropositive throughout the follow-up up to 25 years before clinical diagnosis (Figure 2A) and 17 seroconverted during the follow-up, with only one case fluctuating around the threshold for HPV16 E6 seropositivity (Figure 2B). In these 17 cases, seroconversion took place up to 28 years before diagnosis but not <6 years before diagnosis (supplementary Table S4, available at *Annals of Oncology* online). Using the average of the time points before and after the observed seroconversion, the median lead time was 11.5 years (IQR 6.5–14.3). In the sensitivity analyses, the median lead time based on the time point just before seroconversion was 17.9 years (IQR 13.0–19.8) and based on the time point after observed seroconversion was 6 years (IQR 4.7–8.8). The remaining 64 individuals were HPV16-E6 seronegative in all serial samples (Figure 2C).

HPV16-E6 seropositivity was observed across the spectrum of age at initial blood draw (Figure 3). In OPSCC cases who were HPV16-E6 seropositive throughout follow-up (Figure 3A), HPV16-E6 seropositivity was first observed in a participant who was 38 years old. HPV16-E6 seroconversion was observed

Table 2. HPV16-E6 seroprevalence in cases and controls overall, by anatomic subsite and cohort

E6 overall	Controls		OPSCC overall		Tonsil SCC		Base of tongue SCC		Other OPSCC subsites[b]	
	*N*neg/*N*total	%Seronegative[a] (95% CI)	*N*pos/*N*total	%Seropositive (95% CI)	*N*pos/*N*total	%Seropositive (95% CI)	*N*pos/*N*total	%Seropositive (95% CI)	*N*pos/*N*total	%Seropositive (95% CI)
Over all	5792/5814	99.6 (99.4–99.8)	195/743	26.2 (23.1–29.6)	124/345	35.9 (30.9–41.3)	56/227	24.6 (19.3–30.9)	15/171	8.8 (5.2–14.3)
Whites	5488/5509	99.6 (99.4–99.8)	191/701	27.2 (24.0–30.7)	121/328	36.9 (31.7–42.4)	55/218	25.2 (19.7–31.6)	15/155	9.7 (5.7–15.7)
Blacks	207/208	99.5 (96.9–100)	3/39	7.7 (2.0–21.7)	3/17	17.6 (4.7–44.2)	0/7	0 (0.0–43.9)	0/15	0 (0.0–25.3)
Others	97 / 97	100 (95.3–100)	1/3	–	0/0	–	1/2	–	0/1	–
JANUS	1318/1319	99.9 (99.5–100)	56/307	18.2 (14.2–23.1)	42/177	23.7 (17.8–30.8)	12/72	16.7 (9.3–27.7)	2/58	3.5 (0.6–13)
ATBC	223/223	100 (97.9–100)	1/38	2.6 (0.1–15.4)	0/2	–	0/31	0.0 (0.0–13.7)	1/5	20.0 (1.1–70.1)
HUNT	155/156	99.4 (95.9–100)	19/24	79.2 (57.3–92.1)	17/20	85.0 (61.1–96.0)	1/1	100 (5.5–100)	1/3	33.3 (1.8–87.5)
EPIC	1828/1831	99.8 (99.5–100)	43/124	34.7 (26.5–43.8)	29/68	42.6 (30.9–55.2)	7/14	50.0 (26.8–73.2)	7/42	16.7 (7.5–32.0)
CLUE										
Whites	168/168	100 (97.2–100)	6/34	17.6 (7.4–35.2)	0/6	0.0 (0.0–48.3)	3/19	15.8 (4.2–40.5)	3/9	33.3 (9.0–69.1)
Blacks	2/2	–	0/0	–	0/0	–	0/0	–	0/0	–
PLCO										
Whites	1378/1390	99.1 (98.5–99.5)	40/85	47.1 (36.3–58.1)	19/30	63.3 (43.9–79.5)	21/38	55.3 (38.5–71)	0/17	0.0 (0.0–22.9)
Blacks	84/84	100 (94.5–100)	0/1	–	0/0	–	0/1	–	0/0	–
Others	67/67	100 (93.2–100)	0/0	–	0/0	–	0/0	–	0/0	–
SCCS										
Whites	52/52	100 (91.4–100)	5/14	35.7 (14.0–64.4)	4/7	57.1 (20.2–88.2)	1/5	20.0 (1.1–70.1)	0/2	0.0 (0.0–80.2)
Blacks	107/108	99.1 (94.2–100)	3/37	8.1 (2.1–23.0)	3/16	18.8 (5.0–46.3)	0/6	0.0 (0.0–48.3)	0/15	0.0 (0.0–25.3)
Others	8/8	100 (59.8–100)	0/2	–	0/0	–	0/1	–	0/1	–
WHI										
Whites	210/212	99.1 (96.3–99.8)	9/42	21.4 (10.8–37.2)	4/9	44.4 (15.3–77.3)	4/23	17.4 (5.7–39.5)	1/10	10.0 (0.5–45.9)
Blacks	14/14	100 (73.2–100)	0/1	–	0/1	–	0/0	–	0/0	–
Others	22/22	100 (81.5–100)	1/1	–	0/0	–	1/1	–	0/0	–
MCCS	156/158	98.7 (95.0–99.8)	12/33	36.4 (21.0–54.9)	6/9	66.7 (30.9–91.0)	6/15	40.0 (17.5–67.1)	0/9	0 (0–37.1)

European and Australian studies included only subjects of white ethnicity.

Years of enrollment: ATBC (1985–1988), CLUE (1974–1989), EPIC (1991–1999), HUNT (1995–2008), JANUS (1972–2012), MCCS (1990–1994), PLCO (1993–2001), SCCS (2002–2009), WHI (1994–1998).

[a]% Seronegative is displayed as it is the marker specificity; 100% seronegativity = 0% seropositivity.

[b]HPV-E6 seroprevalence in cancers of 'other anatomic sites': 3.8% of 26 soft palate cancers; 11.1% of 9 posterior oropharyngeal wall cancers; 0.0% of 7 anterior oropharyngeal surface of epiglottis; 18.2% of 11 lateral oropharyngeal wall cancers; 14.3% of 14 oropharyngeal vallecular cancers; 0.0% of 13 oropharyngeal uvula cancers; 0.0% of 1 branchial cleft cancer; and 10.0% of 90 overlapping OPSCC cancers.

Janus, Janus Serum bank; MCCS, Melbourne Collaborative Cohort Study; PLCO, Prostate, Lung, Colorectal and Ovarian study; SCCS, Southern Community Cohort Study; WHI, Women's Health Initiative; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CLUE I and II; EPIC, European Prospective Investigation into Cancer and nutrition; HUNT, Nord-Trøndelag Health Study; OPSCC, oropharyngeal squamous cell carcinoma; HPV16, human papillomavirus type 16.

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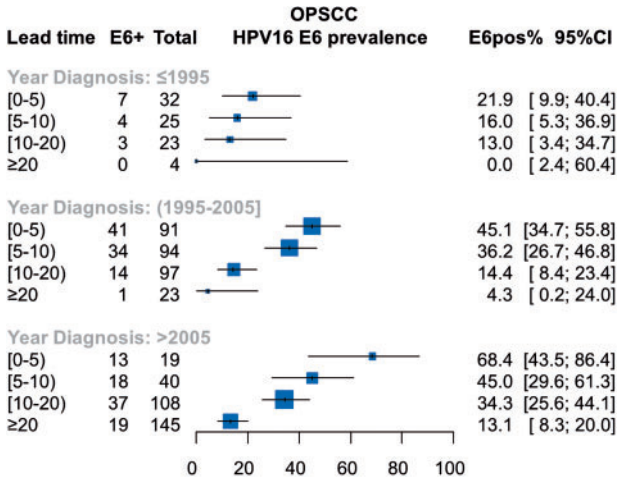

Figure 1. Human papillomavirus type 16 (HPV16)-E6 seroprevalence in oropharyngeal squamous cell carcinoma (OPSCC) white cases, by lead and calendar time. This figure shows the impact of lead time in 5-year categories on HPV16-E6 seroprevalence in OPSCC cases, stratified by year of cancer diagnosis. HPV16-E6 seroprevalence increases with decreasing lead time and in the most recent calendar year of diagnosis.

as early as age 38 years and as late and 79 years, median age at seroconversion was 52 (IQR 44–64; Figure 3B and supplementary Table S4, available at *Annals of Oncology* online).

Discussion

We evaluated circulating HPV16-E6 antibodies before diagnosis of OPSCC in collaboration with nine prospective cohort studies from the United States, Europe, and Australia. This enabled us to describe temporal patterns of pre-diagnostic HPV16-E6 seropositivity and seroconversion for cases up to 40 years before diagnosis. Based on data from 743 incident OPSCC cases, 111 of whom had multiple pre-diagnostic blood samples available, we demonstrated that HPV16-E6 seroconversion can occur several decades before OPSCC diagnosis. HPV16-E6 seropositivity was associated with a nearly 100-fold risk increase of OPSCC in whites and 20-fold risk increase in blacks. Shorter time from blood draw to diagnosis and more recent calendar years of diagnosis were also associated with higher HPV16-E6 seroprevalence. We finally demonstrated, based on data from 5814 controls, that HPV16-E6 seropositivity is exceptionally rare (0.4%) in individuals who are OPSCC free.

This analysis allowed us to make several novel observations. Most notably, based on OPSCC cases with multiple pre-diagnostic samples, we observed wide variation in the timing of HPV16-E6 seroconversion before diagnosis. Seroconversion was estimated to occur just 6 years preceding OPSCC diagnosis, but also up to 28 years prior, conservatively basing the timepoint for seroconversion on the HPV16-E6 seropositive sample observed proximal to diagnosis. Seroconversion also occurred broadly across the age scale—the first observed seroconversion occurred in individuals in their mid-30s but was also observed into the mid-60s. Furthermore, HPV16-E6 seropositivity was substantially higher in whites compared with blacks. Despite the small sample size for black OPSCC cases ($n = 39$), this finding suggests that

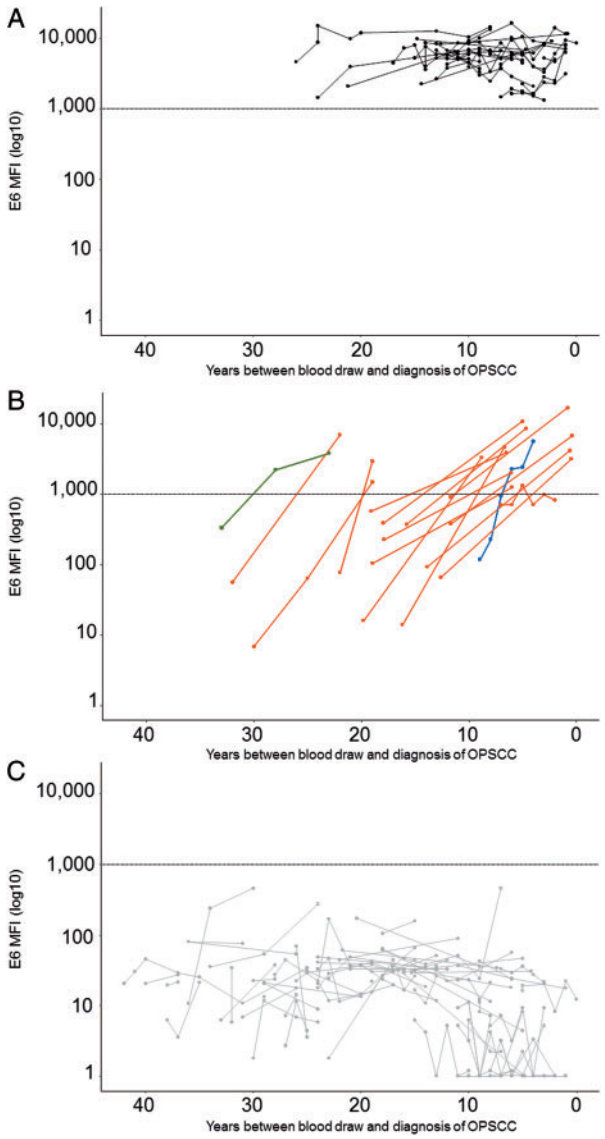

Figure 2. Human papillomavirus type 16 (HPV16)-E6 antibody median fluorescence intensity (MFI) value in serial samples from OPSCC white cases over the time period leading up to the cancer diagnosis (time 0), for cases who were HPV16-E6 seropositive throughout the entire follow-up period (A), cases who seroconverted during follow-up (B), and cases who were seronegative throughout follow-up (C).The dashed line represents the assay cut-off for HPV16-E6 seropositivity, MFI > 1000. In (B), the case who seroconverted furthest from cancer diagnosis is highlighted in green and the case with multiple measurements who stably seroconverted closest to diagnosis is highlighted in blue. One case fluctuated around the assay threshold for positivity and is HPV16-E6 seropositive in one of their six available serial samples.

OPSCC is substantially more often driven by HPV16 in United States white patients than in black patients. Because HPV-related OPSCC generally have better survival than HPV-negative OPSCC, this observation may at least partly explain the survival disparity in OPSCC by race [22].

Etiologic insights about the natural history of oropharyngeal HPV infection and its progression to OPSCC can now be

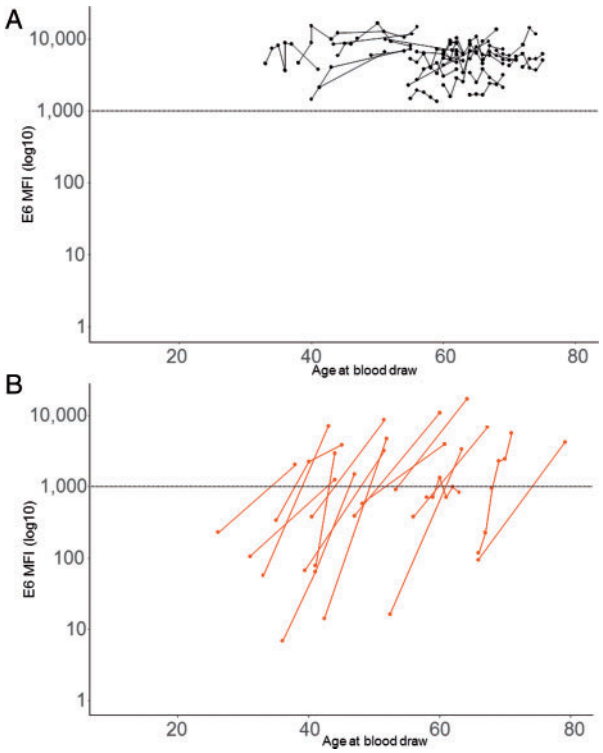

Figure 3. Human papillomavirus type 16 (HPV16)-E6 antibody median fluorescence intensity (MFI) value in serial samples collected from oropharyngeal squamous cell carcinoma (OPSCC) white cases by age at blood draw, for cases who were HPV16-E6 seropositive throughout the entire follow-up period (A) and cases who seroconverted during follow-up (B).

superimposed on what is known for HPV infections at other anatomic sites where HPV causes cancer. For cervical HPV infections, it is well established that incident cervical HPV infection most commonly occurs shortly after sexual debut and peaks in their 20s [23]. In contrast, incidence of penile HPV infection appears constant across the lifespan [24]. The patterns of HPV16-E6 seroconversion that we observed in OPSCC cases, with a median age of seroconversion at 52 years, suggest that acquisition of oral HPV16 infection may happen later in life (20s through 40s). This interpretation is bolstered by analyses of prevalent oral HPV infection, where peak prevalence estimates were observed at these older ages [25].

This work informs the potential for translating the HPV16-E6 biomarker into use for early detection of OPSCC. Given the increasing incidence of OPSCC among men, along with the increasing fraction of HPV-related OPSCC [4, 9], it is conceivable that a screening program for OPSCC could eventually be considered for selected high-risk groups. This is increasingly relevant since prophylactic HPV vaccination, despite being highly effective against oral HPV16 infections [26, 27], will not affect rates of OPSCC for decades, when vaccinated cohorts of adolescent age move into the cancer-susceptible age range of 50–70 years. Already, HPV16-E6 seropositivity is established as a highly sensitive marker for HPV-driven OPSCC [28–30], and continued studies document its exceptionally high specificity [20]—in this analysis, 99.6%. Here, we additionally show that individual OPSCC cases can be HPV16-E6 seropositive several decades before clinical presentation of the disease, which has major implications for potential use of the biomarker in clinical practice. Specifically, if an HPV16-E6 screening program were in place, one would expect screen-positive individuals to require years of continued evaluation before clinically-actionable disease may occur, leading to potential psychosocial harms including anxiety. Importantly, an additional major challenge in an early detection program is that we lack evidence-based actionable interventions for screen-positive individuals, and translating the E6 biomarker will likely require identification and description of an OPC precursor. Advances in diagnostics and treatment continue to be researched in preparation for this future [31] and we assert that HPV16-E6 should be an important tool in this research, as HPV16-E6 seropositive men have a notable cumulative incidence of future OPSCC [7].

The main limitation of this work is the inherent heterogeneity between the participating cohorts. For example, most cases with very long lead times came from the Norwegian JANUS cohort. It is, therefore, difficult to determine whether the observed HPV16-E6 seroprevalence in the longer lead times is attributed to the lead time specifically, or to being in the JANUS cohort. Unfortunately, there are few large cohort studies with both serial blood draws and very long follow-up, which are both required to understand HPV16-E6 seroconversion many years before OPSCC diagnosis.

The etiological landscape for OPSCC is shifting [32], in particular in western populations where the majority of OPSCC are now caused by HPV. Further, birth cohorts who experienced the increases in incidence at younger ages are now experiencing increased incidence at older ages—these changes will continue to influence the burden of this disease in younger and older males, and possibly females [33]. While HPV vaccination holds promise in preventing HPV-related cancer in general, a resulting diminution in OPSCC will not be observed for decades. Thus, we must continue to carefully assess opportunities for the early detection of OPSCC, including the use of HPV16-E6 antibody detection. Together with other efforts, such as improved diagnostic techniques and less-invasive treatments, there may be a great opportunity to reduce the morbidity and mortality from OPSCC that is forecasted to occur in the coming years [31].

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Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf. The Nord-Trøndelag Health Study (The HUNT Study) is collaboration between HUNT Research Centre (Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology), Nord-Trøndelag County Council, Central Norway Regional Health Authority, and the Norwegian Institute of Public Health. The study has used data from the Cancer Registry of Norway. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Cancer Registry of Norway is intended nor should be inferred.

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Disclosure

The authors have declared no conflicts of interest.

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