



ORIGINAL ARTICLE

The association between inflammatory biomarkers and statin use among patients with head and neck squamous cell carcinoma

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Abstract

Background: Tumor-infiltrating lymphocytes (TILs) and cytokines are associated with prognosis among patients with head and neck squamous cell carcinoma (HNSCC). Statins (cholesterol-lowering drugs) may improve HNSCC prognosis, particularly in human papillomavirus (HPV)-positive cases, but the mechanism remains unclear.

Methods: Statin use was collected from medical records for HNSCC cases (2008–2014). TILs were counted in tumor tissue, and a total weighted score (TILws) was created. Cytokines were measured in blood. The associations between statins and biomarkers were estimated using logistic (biomarker categories: <median, ≥median) and linear regression models (log-transformed continuous biomarkers) adjusted for age, smoking, and comorbidities.

Results: We observed a positive association between statins and TILs among HPV-positive patients (TILws odds ratio [OR] = 2.80; 95% CI = 1.03–7.61), but no association among HPV-negative patients. We observed no association between statins and cytokines.

Conclusions: Statins may influence TILs in HPV-positive patients. This may be the mechanism through which they improve prognosis in HPV-positive HNSCC patients.

KEYWORDS

biomarkers, epidemiology, head and neck cancer, HPV, inflammation

1 | INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a debilitating cancer that can be found in the mucosal lining of

the aerodigestive tract with prominent sites including the nasopharynx, oral cavity, oropharynx, hypopharynx, and larynx.^{1–3} The main risk factors associated with HNSCC development can be delineated into two subgroups: patients

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with human papillomavirus (HPV)-positive tumors and those with HPV-negative tumors, whose risk for disease is often attributed to smoking and alcohol drinking.¹ Etiology of disease, underlying tumorigenesis, patient characteristics, site of disease, treatment, and prognosis often differ between patients with HPV-positive versus HPV-negative tumors.⁴

Statins are a class of cholesterol-lowering medications that are often utilized to prevent the development or progression of heart disease.⁵ In addition to their cholesterol lowering attributes, statins possess anti-inflammatory and immunomodulatory actions that may inhibit the development or progression of cancer.⁶ Research has established a protective association between the use of statin drugs and the incidence and mortality of cancer at numerous sites,^{7,8} including HNSCC.⁹⁻¹¹ Although the relationship between statin use, cancer development, and cancer-related outcomes has been investigated across various cancer sites with promising findings, the potential mechanisms by which statins may be exerting their protective effect remains unclear, particularly for HNSCC. However, their effects on inflammation and immunomodulation that may be responsible for factors that influence the progression and development of cancer are largely unknown.¹²

Recent research findings suggest that there is an inverse association between number of tumor infiltrating lymphocytes (TILs) and HNSCC death and recurrence.^{13,14} Another inflammatory marker that potentially influences HSNCC outcomes is the level of circulating cytokines. Previous research has identified that higher levels of the pro-inflammatory cytokine interleukin-6 (IL-6) have been found to be positively associated with recurrence and death among patients with HNSCC.¹⁵

Given the established relationship between HNSCC outcomes and both TILs and circulating cytokines, as well as the known effects of statins on inflammation and immune modulation, this study aimed to identify the association between statin use and these inflammatory biomarkers. Through this research, we determined whether statins may be influencing HNSCC outcomes through these inflammatory processes. Because patients with HPV-positive tumors may have a different immune response and are different etiologically than HPV-negative tumors, we explored whether HPV status may modify the association between statin use and the presence and quantity of TILs and circulating cytokines.

2 | MATERIALS AND METHODS

2.1 | Study population

Subjects in this study were recruited to participate in the University of Michigan Head and Neck Cancer

Specialized Program of Research Excellence II (SPORE II). The SPORE II cohort consists of incident HNSCC patients who were diagnosed and/or treated at the University of Michigan Rogel Cancer Center from 2008 to 2014. In order to be eligible to participate in the study patients had to be 18 years or older, their cancer could not have been previously treated, and their disease could not be a recurrence of disease (i.e. they had to be free of disease for 5 years prior to their current diagnosis). A more detailed description of the cohort and information about the percent of patients who agree to participate has been published previously.^{15,16} The SPORE II cohort consists of 1042 participants; complete TILs and statin use information was available for 475 participants and circulating cytokines were measured in 205 participants.

2.2 | Inflammatory marker measurements

2.2.1 | Tumor infiltrating lymphocytes (TILs)

Tumor tissue was collected from previously untreated patients who had tissue available from biopsies. Details of the TILs measurement procedure and creation of variables have been previously published.^{13,14} Briefly, triplicate 0.7-mm diameter cores for each patient sample were selectively punched/extracted and transferred to a recipient tissue array block. Immunohistochemical staining was completed on a DAKO autostainer using LSAB+ (liquid streptavidin biotin horseradish peroxidase) and DBA (DAKO labeled avidin-biotin-peroxidase kits) as chromogens. Deparaffinized sections were stained with six monoclonal antibodies at the following titrations: cluster of differentiation 4 (CD4)-1:250 (Abcam Ab846); cluster of differentiation 8 (CD8)-1:40 (Nova Castra VP-C320); forkhead box P3 (FoxP3) -1:200 (Abcam Ab20034); cluster of differentiation 104 (CD104) -1:50 (Beta-4 integrin, eBioscience 439-9b). Appropriate negative (without primary antibodies) and positive (tonsillar tissue and various carcinomas) controls were stained concurrently on the same slides. The stained TMA slides were assessed by a technician blinded to patient clinical status and treatment outcome. The whole TMA slides were digitally imaged, scanned, and retrieved with Aperio ImageScope v.12 software. Grid software (Meazure, C Thing Software 2.01) was used to overlay each tissue core image prior to counting cells. CD104 staining (beta-4 integrin) for each core was examined first to locate and confirm the extent and location of carcinoma within the tissue cores. TILs were manually counted within tumor parenchyma on tissue microarrays and

presented as number of cells per millimeters squared; in addition, a total weighted score (TILws) was created combining data across the three types of TILs.¹⁴ TILs were measured for CD4 ($N = 481$), CD8 ($N = 481$), FoxP3 ($N = 485$), and TILws ($N = 475$).

2.2.2 | Circulating cytokines

Circulating cytokines were measured from blood samples collected from participants at diagnosis, prior to treatment. A detailed explanation of the procedure to measure the circulating cytokines was previously published.^{15,17} Briefly, cytokines were measured at the University of Michigan Cancer Center Immune Monitoring Core using commercially available ELISA (Enzyme-Linked Immunosorbent Assay) kits. Blood samples were stored at 4°C for less than 2 h after collection when they were centrifuged, and serum was separated in 2 µl aliquots for storage at -80°C. When samples were selected for cytokine measurement, they were thawed and incubated overnight at 4°C on microtiter plates pre-coated with monoclonal antibodies specific to each cytokine. All samples were measured in duplicate and blinded duplicates of study samples for two participants were included as quality control samples. After washing away unbound substances, biotin-linked polyclonal antibodies for each cytokine were introduced. After incubation for 2 h at room temperature, the plates were washed and incubated with streptavidin-HRP for an hour. After a final wash, substrate solution was added; color development was stopped after 25 min. Colorimetric densities were measured for each sample from a standard curve using a microplate reader. The cytokines measured were interferon gamma (IFN-γ), interleukin-6 (IL-6), interleukin-8, (IL-8), interleukin-10 (IL-10), interleukin-17 (IL-17), growth related oncogene (GRO), hepatocyte growth factor (HGF), tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF). Samples that exceeded the limit of detection were assigned the maximum value.

2.3 | Confounding variables

Variables that may confound the association between statin use and biomarkers were collected in various ways. Clinical variables such as age (continuous) and comorbidities (none, mild, moderate, or severe) measured through the Adult Comorbidity Evaluation 27 score (ACE-27) were collected through medical record review, whereas potential confounders associated with behavior

such as smoking status (never, former, current) were collected through a baseline health survey. Individuals that were missing the listed potential confounders, specifically smoking status ($N = 20$) and ACE-27 ($N = 4$) were assigned to the most common category for the TILs analysis; no individuals were missing confounding variables for the cytokines analysis.

2.4 | Statin measurement

Statin use was collected through a retrospective medical record review by trained research personnel. Study personnel identified the patient's initial encounter at the University of Michigan hospital for the patient's HNSCC diagnosis and medications were recorded from that encounter. If the patient did not have medications recorded at the initial encounter, the next closest encounter was checked. Medications were recorded from the closest encounter to initial diagnosis prior to treatment initiation. If a participant was identified as using a statin at diagnosis, he/she was considered a statin user. Data were collected by two reviewers and achieved an interrater reliability coefficient, Kappa of 95%. Information that was not concordant across reviewers was reconciled after comparison. Data were stored in a Research Electronic Data Capture (REDCap) database.¹⁸

2.5 | Statistical analysis

Basic descriptive statistics were calculated to identify if demographic and clinical characteristics were different for statin users compared to non-statin users at diagnosis. TIL and cytokine variables were operationalized continuously and categorically. Due to the highly skewed distribution of the data toward zero, TILs and cytokine values were log transformed to achieve a more normal distribution. In addition to analyzing this association linearly, based on the highly skewed distribution, individual TIL measures and cytokine measures were categorized as low or high (i.e. < median or ≥ median). Since TILs and cytokines were examined continuously and dichotomously (< median vs. ≥ median), linear and logistic regression models were conducted. Logistic regression models calculated odds ratios (OR). Multivariable-adjusted (MV-adj) models included the following variables that may confound the association between statin use and TILs; age, smoking status, and ACE-27. Because statin use appeared to be associated with HPV status and disease site for participants who had cytokines measured, we conducted models adjusting for those variables as well.

TABLE 1 Demographic and clinical characteristics by statin use (TILs participants)

	Statin user (N = 156, 29.32%)	Non-statin user (N = 376, 70.68%)	p-value
Age at diagnosis, years	66.89	58.91	<0.0001
Sex (male)	67.95%	71.81%	0.37
Race			0.45
White	92.95%	91.49%	
Not white	3.21%	5.59%	
Missing	3.85%	2.93%	
Disease site			0.78
Larynx	14.10%	15.16%	
Oral cavity	47.44%	49.20%	
Oropharynx	30.13%	28.99%	
Hypopharynx	3.21%	3.72%	
Other	5.13%	2.93%	
Stage at diagnosis			0.997
0 and 1	13.46%	13.30%	
2	14.74%	15.16%	
3	14.74%	14.10%	
4	57.05%	57.45%	
HPV status			0.73
Negative	48.08%	48.94%	
Positive	26.28%	28.46%	
Invalid/missing	25.64%	22.61%	
ACE-27 score ^a			<0.0001
None	11.54%	29.26%	
Mild	50.00%	47.07%	
Moderate	21.15%	17.55%	
Severe	15.38%	5.85%	
Missing	1.92%	0.27%	
Highest education			0.56
Less than high school	3.21%	6.65%	
High school/GED	22.44%	24.20%	
Some college	25.64%	23.67%	
4-year degree	8.97%	9.04%	
More than 4-year degree	10.90%	7.71%	
Missing	28.85%	28.72%	
BMI			0.01
Underweight/normal weight	24.36%	39.36%	
Overweight/Obese 1	58.33%	47.07%	
Obese 2/Obese 3	12.82%	9.84%	
Missing	4.49%	3.72%	
Smoking status			<0.0001
Current	26.28%	48.67%	
Former	46.79%	25.80%	
Never	21.79%	22.34%	
Missing	5.13%	3.19%	

TABLE 1 (Continued)

	Statin user (N = 156, 29.32%)	Non-statin user (N = 376, 70.68%)	p-value
Drinking status			
Current	53.21%	64.10%	0.13
Former	28.85%	21.81%	
Never	12.82%	10.64%	
Missing	5.13%	3.46%	

Abbreviations: ACE-27, Adult Comorbidity Evaluation 27; BMI, body mass index; GED, general educational development; HPV, human papillomavirus; TILs, tumor infiltrating lymphocytes.

^aFisher's exact test p-value, for variables that had cell sizes smaller than 5.

Based on our previous research, we observed an association between statin use and HNSCC outcomes, but only among patients whose disease was HPV-positive.¹⁹ We therefore wanted to assess if effect modification by HPV status was present (HPV-positive, HPV-negative, HPV status invalid/missing) for the statin-inflammatory marker associations. Statistical interaction was evaluated using the likelihood ratio test.

HPV status was missing for 125 participants who had at least 1 of the TIL biomarkers measured and 30 participants who had the circulating cytokines measured. In order to identify if these missing values influenced the association of statin use and the TIL measures, we utilized various methods to analyze the HPV status as a potential effect modifier. Initially participants who had HPV status missing were excluded from the analysis. We also conducted the analysis by including the participants with HPV missing as a separate category. Lastly, we utilized inverse probability weighting (IPW) by generating weights to emulate a population where no participants are missing HPV status. We created the weights by calculating the probability of having HPV status missing by conducting a logistic regression model consisting of variables (year enrolled in the study, age at diagnosis, sex, smoking status, marital status, stage of disease at diagnosis, disease site, ACE-27, drinking status, and body mass index [BMI]); this is the denominator of the weight. Participants with missing values for the predictors in the logistic regression model were dropped from the analysis. The weights were then applied to a logit model with a binomial distribution and robust 95% confidence intervals, excluding participants who were missing HPV status. This approach was also utilized to assess the potential effect modification of HPV status on the association between statin use and the circulating cytokine measures.

All analyses were conducted using SAS version 9.4 (Cary, NC). All tests were two-sided, and results were considered statistically significant if $p < 0.05$.

3 | RESULTS

3.1 | Descriptive statistics

Presented in Tables 1 and 2 are the distribution of baseline demographic characteristics by statin use for the participants included in the TILs and circulating cytokines analyses, respectively. For the TILs analysis, participants taking a statin at diagnosis (29.32%) appear to be older, more likely to have a higher BMI, higher ACE-27, and are less likely to be current smokers compared to non-statin users at baseline. The distribution of characteristics among statin users (36.10%) and non-users was slightly different for participants who had circulating cytokines measured at baseline. Statin users were older, less likely to have larynx or oral cavity as their primary site of disease, more likely to have HPV status missing, higher ACE-27, and less likely to be current smokers compared to participants who were not using a statin at diagnosis.

3.2 | Multivariable analysis

When assessing the relationship between statin use and TILs, operationalizing the outcome as high versus low TIL counts, there did not appear to be a clear association between statin use and TILs. After adjusting for confounders: age, smoking status and ACE-27, those who were taking a statin had a slightly higher odds of having higher TILs but this association was not statistically significant for any of the measured TIL values (TILws: [MV-adj OR = 1.15; 95% CI = 0.74–1.78]; FoxP3: [MV-adj OR = 1.13; 95% CI = 0.74–1.73]; CD4: [MV-adj OR = 1.23; 95% CI = 0.80–1.90]; CD8: [MV-adj OR = 1.10; 95% CI = 0.72–1.69]). Similar null findings were observed when TILs were log-transformed and examined as a continuous variable (Table 3). The association between statin use and circulating cytokines was also null (Table S1).

TABLE 2 Demographic and clinical characteristics by statin use (cytokines participants)

	Statin user (N = 74, 36.10%)	Non-statin user (N = 131, 63.90%)	p-value
Age at diagnosis, years	63.34	57.55	<0.0001
Sex (male)	78.38%	82.44%	0.48
Race			0.75 ^a
White	95.95%	93.89%	
Not white	4.05%	6.11%	
Disease site			0.05 ^a
Larynx	16.22%	22.90%	
Oral cavity	24.32%	31.30%	
Oropharynx	44.59%	40.46%	
Hypopharynx	1.35%	2.29%	
Other	13.51%	3.05%	
Stage at diagnosis			0.97
0 and 1	8.11%	6.87%	
2	8.11%	6.87%	
3	16.22%	16.03%	
4	67.57%	70.23%	
HPV status			0.0015
Negative	35.14%	53.44%	
Positive	39.19%	38.17%	
Invalid/missing	25.68%	8.40%	
ACE-27 score			0.0005 ^a
None	13.51%	34.35%	
Mild	51.35%	41.98%	
Moderate	20.27%	20.61%	
Severe	14.86%	3.05%	
Highest education			0.53 ^a
Less than high school	4.05%	6.87%	
High school/GED	25.68%	32.06%	
Some college	27.03%	28.24%	
4-year degree	14.86%	7.63%	
More than 4-year degree	14.86%	15.27%	
Missing	13.51%	9.92%	
BMI			0.77
Underweight/normal weight	29.73%	33.59%	
Overweight/Obese 1	59.46%	58.02%	
Obese 2/Obese 3	10.81%	8.40%	
Smoking status			0.08
Current	29.73%	45.80%	
Former	43.24%	32.82%	
Never	27.03%	21.37%	
Drinking status			0.63
Current	64.86%	70.99%	
Former	27.03%	21.37%	
Never	8.11%	7.63%	

Abbreviations: ACE-27, Adult Comorbidity Evaluation 27; BMI, body mass index; GED, general educational development; HPV, human papillomavirus.

^aFisher's exact test p-value, for variables that had cell sizes smaller than 5.

TABLE 3 Multivariable models-TILs

	Crude model Median TILs OR (95% CI)	Adjusted model ^a Median TILs OR (95% CI)	Crude model (log transformed TILs)	<i>p</i>	Adjusted model ^a (log transformed TILs)	<i>p</i>
TILws (<i>N</i> = 475)			0.06 (0.14)	0.67	0.09 (0.14)	0.54
Non-statin user	1	1				
Statin user	1.20 (0.80, 1.78)	1.15 (0.74, 1.78)				
FoxP3 (<i>N</i> = 485)			0.01 (0.16)	0.95	0.09 (0.17)	0.59
Non-statin user	1	1				
Statin user	1.01 (0.69, 1.50)	1.13 (0.74, 1.73)				
CD4 (<i>N</i> = 481)			0.06 (0.19)	0.76	0.14 (0.20)	0.50
Non-statin user	1	1				
Statin user	1.08 (0.73, 1.59)	1.23 (0.80, 1.90)				
CD8 (<i>N</i> = 481)			0.19 (0.18)	0.31	0.12 (0.19)	0.55
Non-statin user	1	1				
Statin user	1.19 (0.80, 1.77)	1.10 (0.72, 1.69)				

Abbreviations: CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CI, confidence interval; FoxP3, forkhead box P3; OR, odds ratio; TILs, tumor infiltrating lymphocytes; TILws, total weighted score.

^aAdjusted for age at diagnosis, smoking status, and ACE-27.

3.3 | Effect modification

We observed a statistically significant interaction with HPV status such that there was a statistically significant positive association between statin use and having a high number of FoxP3 (HPV-positive: [MV-adj OR = 4.15; 95% CI = 1.55–11.14]; HPV-negative: [MV-adj HR = 0.81; 95% CI = 0.43–1.52], *p* for interaction = 0.003; Table 4). There was a marginally significant association observed for TILws (above the median) among statin users whose tumors were HPV-positive (TILws: HPV-positive [MV-adj OR = 2.80; 95% CI = 1.03–7.61]; HPV-negative [MV-adj HR = 1.07; 95% CI = 0.57–2.02]), *p* for interaction = 0.1; Table 4), which may be driven by FoxP3. Interactions were suggestive, but not statistically significant for the association between statin use and CD4 and CD8.

When we examined the association between statin use and circulating cytokines, we observed no statistically significant interaction between statin use at diagnosis and HPV status for any of the individual circulating cytokines except HGF. Patients who were HPV-positive and on a statin at diagnosis had higher odds of having a high level of HGF compared to those who had HPV-positive tumors and were not taking a statin, whereas those who were HPV-negative and were taking a statin appear to have an inverse relationship with HGF. However, neither of the stratum-specific associations were statistically significant (HPV-positive: [MV-adj OR = 2.27; 95% CI = 0.82–6.27]; HPV-negative: [MV-adj HR = 0.47; 95% CI = 0.17–1.31], *p* for interaction = 0.03; Table S2).

Similar findings were observed when the HPV status missing category was included and when using the IPW method, although the associations observed using the IPW method were slightly stronger they did not appear to be meaningfully different for both the individual TIL and circulating cytokine measures (Tables S3 and S4).

4 | DISCUSSION

In this study, we observed that HNSCC patients taking a statin at the time of diagnosis had higher lymphocyte infiltration in their tumors than non-users, but only for HPV-positive patients. The strongest association was observed for FoxP3. Our recently published study found that statins were protective for HNSCC outcomes, but this protective association was observed only among patients whose tumors were HPV-positive.¹⁹ The findings from this manuscript support that the inverse association between statin use and outcomes among HPV-positive HNSCC previously reported by our group may be due to an effect of statins on TILs in patients with HPV-positive tumors.¹⁹

The inflammatory and immunomodulatory effects of cancer are not clearly directional by cell type. This effect often depends on the type of cancer, whether there is a presence of inflammatory markers and what combination of these markers are beneficial or harmful to cancer prognosis.²⁰ Research has established that FoxP3 influences cancer prognosis but the directionality of this association differs by cancer type, with certain cancers such as breast, cervical, pancreatic, and melanoma observing a positive

TABLE 4 HPV-stratified TILs models (dropping missing HPV status)

	# of events	Non-statin user OR (95% CI) ^a	# of events	Statin user OR (95% CI) ^a
TILws				
HPV status w/o missing				
HPV-positive	64	1 (REF)	29	2.80 (1.03, 7.61)
HPV-negative	72	1 (REF)	31	1.07 (0.57, 2.02)
<i>P for interaction</i>	0.1			
FoxP3				
HPV status w/o missing				
HPV-positive	59	1 (REF)	32	4.15 (1.55, 11.14)
HPV-negative	81	1 (REF)	27	0.81 (0.43, 1.52)
<i>P for interaction</i>	0.003			
CD4				
HPV status w/o missing				
HPV-positive	62	1 (REF)	29	2.42 (1.00, 5.86)
HPV-negative	73	1 (REF)	32	1.42 (0.75, 2.69)
<i>P for interaction</i>	0.32			
CD8				
HPV status w/o missing				
HPV-positive	63	1 (REF)	29	1.84 (0.75, 4.55)
HPV-negative	64	1 (REF)	30	1.10 (0.58, 2.07)
<i>P for interaction</i>	0.34			

Abbreviations: CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CI, confidence interval; FoxP3, forkhead box P3; HPV, human papillomavirus; OR, odds ratio; TILs, tumor infiltrating lymphocytes; TILws, total weighted score.

^aAdjusted for age at diagnosis, smoking status, and ACE-27.

association between FoxP3 infiltration and death whereas other cancer sites such as HNSCC, colorectal, and esophageal cancers observe an inverse association.^{13,14,21} FoxP3 infiltration appears to have varying impacts on cell development and proliferation but the explanation behind why its influences differ by cancer sites is not clearly established.²²

Although to our knowledge this is the first study to investigate an association between statin use and TILs among HNSCC patients, a study investigating this association among patients with colorectal cancer identified similar results. Al-Husein et al. identified a positive association between statin use and FoxP3 among patients with colorectal cancer and determined this association was modified by stage of disease.²³ Another study by Lee et al. found that statins were associated with the increased production of T-cells ("FoxP3 transcription factor") in mice and lung tumor cell lines.²⁴

Our finding that the statin-TIL association may be limited to patients with HPV-positive tumors is plausible given that patients with HPV-positive tumors often have

a different immune response and may be less immunosuppressed than HNSCC patients with HPV-negative disease.^{25,26} HPV-positive HNSCC have been shown to have a stronger immune response, particularly stronger T-cell infiltration, than patients with HPV-negative disease.^{27,28} One possible explanation is that HPV-positive HNSCC is usually found in the oropharynx, (specifically the tonsils).^{28,29} Tonsils are made of lymphatic tissue which is rich in various immunological processes.³⁰ Therefore, one possible explanation for our finding of a statin-TIL association only among HPV-positive patients is that the stronger immune response of HNSCC patients with HPV-positive disease works synergistically with the anti-inflammatory and immunomodulatory actions of statins. Statins may, therefore, improve cancer-related outcomes specifically in HPV-positive patients.

Although our findings were relatively null for the association between statin use and circulating cytokines, other studies have reported statin-cytokine associations, but the specific cytokines reported to be associated with statin use were not consistent across studies.

One previous study identified an inverse association between pro-inflammatory cytokines in tissue and serum among patients with colorectal cancer.³¹ Two studies investigated this association in participants with hypercholesterolemia finding a reduction in the pro-inflammatory cytokines IL-6 and TNF for participants on a statin.^{32,33} In a study that investigated the association among a random sample of Swiss adults, the authors reported lower C-reactive protein (CRP) concentrations among participants using a statin.³⁴ To our knowledge, our study is the first to investigate this association in HNSCC patients. It is possible that our findings differ from those reported previously because cytokine levels may not be as affected by statin use in HNSCC patients. It is also possible that the results are different due to the design of the studies. The studies that found an association between statin use and circulating cytokine levels utilized a more experimental design in which they administered statins to participants and then measured circulating cytokine levels pre- and post-medication administration. Our study uses an observational approach, which may make it more difficult to identify the true effect of statins on circulating cytokines, particularly if the effect is modest.

4.1 | Strengths and limitations

To our knowledge, this study is the first to assess the association between inflammatory biomarkers and statin use among patients with HNSCC. A strength of this study is the amount of data we have for each patient and the opportunity we have to integrate biomarker data with behavioral, epidemiological, and clinical data. This allowed us to test and identify confounders and should mitigate bias that may arise due to lack of information from participants.

Although this study has many strengths, there are some notable limitations that should be addressed in future studies. The sample size for this study is quite small. Not all participants within the SPORE II cohort had tumor tissue from biopsy available to measure TILs or provided a blood sample at baseline to measure circulating cytokines. This may lead to a reduction in power especially when investigating the interaction between statin use and HPV status. As the effect size of the association particularly for FoxP3 was relatively large, this may not be an issue but as noted by the wide confidence interval the point estimate may not be precise. Future studies investigating this association among a larger study population are necessary. This may also help to explain why there did not appear to be a clear relationship between circulating cytokines and statin use among HPV-positive patients.

There is the potential for selection bias as well. As not all participants provided specimen for biomarker measurement, it is possible that the patients who provided specimen were different from those who did not, with regard to the relationship between statin use and these biomarker measures. This does not appear to be an issue for the analytic sample who have TILs measured. The frequency of participants who were using a statin at diagnosis and distribution of the demographic and clinical characteristics between statin users and non-users was very similar to what was observed in the entire study population. There did appear to be some differences between the sample of participants who had baseline circulating cytokines measured compared to the complete study population. There appeared to be slightly more males and patients with higher stages of disease, but other factors that may bias the associations observed are similarly distributed in the total study population and the analytic sample who have baseline circulating cytokines measured. We additionally used IPW to emulate a population had no SPORE participants had TILs or circulating cytokines missing. This would provide participants who are similar to those who are missing to have larger weights. After including these weights, the association between TILs and statin use at diagnosis does not meaningfully change (Table S5). The point estimates for the association between circulating cytokines and statin use do slightly change but the findings still remain relatively null (Table S6). It is possible that selection bias may be an issue for the circulating cytokine measures.

Another limitation of this study is that the data are cross-sectional. Both the medication information and the inflammatory markers were measured at diagnosis prior to cancer treatment. This can possibly lead to reverse causation specifically with the cytokine measures. If inflammation and high cholesterol are associated, we may observe a positive association between inflammation and statin use if those who have higher levels of pro-inflammatory cytokines were taking a statin because of risk factors associated with high cholesterol such as coronary heart disease and obesity, but this association is not clearly defined.^{35–37} It would be very difficult to identify this bias because we do not have information on when statins were initiated. We also do not have biomarker information from patients (blood and tumor tissue samples) prior to their HNSCC diagnosis. As we did not appear to observe an association between statin use and circulating cytokines or an interaction between HPV status and statin use for the majority of the studied cytokines, it is possible that this limitation did not affect our study. This limitation should not be an issue with TILs because this measurement is based on inflammatory markers that are found within the tumor tissue. We would assume that TILs would not be influenced by other comorbidities that the patient may have at diagnosis.

5 | CONCLUSION

Our findings suggest that one mechanism by which statins may influence prognosis in HNSCC patients is through an effect on TILs, particularly FoxP3. This association appears to be restricted to HPV-positive patients. Future research investigating this association may shed light on the role of type, dose of statin, and duration of use, with TILs in HNSCC tumors. Additional studies are needed to examine other immune and inflammatory markers that predict HNSCC outcomes to further elucidate this potential mechanism by which statins may be protecting against poor outcomes in HPV-positive HNSCC patients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Patients recruited through SPORE II provided written informed consent, and the medical record review and SPORE study were approved through the University of Michigan Medical School's Institutional Review Board.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001
- Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. *Lancet*. 2008;371(9625):1695-1709. doi:10.1016/S0140-6736(08)60728-X
- Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med*. 2001;345(26):1890-1900. doi:10.1056/NEJMra001375
- Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789. doi:10.1016/S1470-2045(10)70017-6
- Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*. 2000;101(2):207-213. doi:10.1161/01.CIR.101.2.207
- Demierre M-F, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer*. 2005;5(12):930-942. doi:10.1038/nrc1751
- Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treat Rev*. 2015;41(6):554-567. doi:10.1016/j.ctrv.2015.04.005
- Mei Z, Liang M, Li L, Zhang Y, Wang Q, Yang W. Effects of statins on cancer mortality and progression: a systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals. *Int J Cancer*. 2017;140(5):1068-1081. doi:10.1002/ijc.30526
- Kao L-T, Hung S-H, Kao P-F, Liu J-C, Lin H-C. Inverse association between statin use and head and neck cancer: population-based case-control study in Han population. *Head Neck*. 2019;41(5):1193-1198. doi:10.1002/hed.25501
- Gupta A, Stokes W, Eguchi M, et al. Statin use associated with improved overall and cancer specific survival in patients with head and neck cancer. *Oral Oncol*. 2019;90:54-66. doi:10.1016/j.oraloncology.2019.01.019
- Lebo NL, Griffiths R, Hall S, Dimitroulakos J, Johnson-Obaseki S. Effect of statin use on oncologic outcomes in head and neck squamous cell carcinoma. *Head Neck*. 2018;40(8):1697-1706. doi:10.1002/hed.25152
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013
- Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma (HNSCC). *Head Neck*. 2016;38(7):1074-1084. doi:10.1002/hed.24406
- Spector ME, Bellile E, Amlani L, et al. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. *JAMA Otolaryngol Head Neck Surg*. 2019;145(11):1012-1019. doi:10.1001/jamaoto.2019.2427
- Duffy SA, Taylor JMG, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*. 2008;113(4):750-757. doi:10.1002/cncr.23615
- Peterson LA, Bellile EL, Wolf GT, et al. Cigarette use, comorbidities and prognosis in a prospective head and neck squamous cell carcinoma population. *Head Neck*. 2016;38(12):1810-1820. doi:10.1002/hed.24515
- Arthur AE, Peterson KE, Shen J, et al. Diet and proinflammatory cytokine levels in head and neck squamous cell carcinoma. *Cancer*. 2014;120(17):2704-2712. doi:10.1002/cncr.28778
- Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010
- Getz KR, Bellile E, Zarins KR, et al. Statin use and head and neck squamous cell carcinoma outcomes. *Int J Cancer*. 2021;148(10):2440-2448. doi:10.1002/ijc.33441

20. Fridman WH, Pagès F, Sauès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12(4):298-306. doi:10.1038/nrc3245
21. Shang B, Liu Y, Jiang S, Liu Y. Prognostic value of tumor-infiltrating foxp3+ regulatory t cells in cancers: a systematic review and meta-analysis. *Sci Rep*. 2015;5(1):1-9. doi:10.1038/srep15179
22. Jia H, Qi H, Gong Z, et al. The expression of foxp3 and its role in human cancers. *Biochim Biophys Acta (BBA) – Rev Cancer*. 2019;1871(1):170-178. doi:10.1016/j.bbcan.2018.12.004
23. Al-Husein BA, Dawah B, Bani-Hani S, Al Bashir SM, Al-Sawalmeh KM, Ayoub NM. Immunomodulatory effect of statins on regulatory t lymphocytes in human colorectal cancer is determined by the stage of disease. *Oncotarget*. 2018;9(87):35752-35761. doi:10.18632/oncotarget.26293
24. Lee KJ, Moon JY, Choi HK, et al. Immune regulatory effects of simvastatin on regulatory t cell-mediated tumour immune tolerance. *Clin Exp Immunol*. 2010;161(2):298-305. doi:10.1111/j.1365-2249.2010.04170.x
25. Heusinkveld M, Goedemans R, Briet RJP, et al. Systemic and local human papillomavirus 16-specific t-cell immunity in patients with head and neck cancer. *Int J Cancer*. 2012;131(2):E74-E85. doi:10.1002/ijc.26497
26. Lechien JR, Seminerio I, Descamps G, et al. Impact of HPV infection on the immune system in oropharyngeal and non-oropharyngeal squamous cell carcinoma: a systematic review. *Cell*. 2019;8(9):1061. doi:10.3390/cells8091061
27. Mandal R, Şenbabaoglu Y, Desrichard A, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight*. 2016;1(17):1-18. doi:10.1172/jci.insight.89829
28. Andersen AS, Solling ASK, Ovesen T, Rusan M. The interplay between HPV and host immunity in head and neck squamous cell carcinoma. *Int J Cancer*. 2014;134(12):2755-2763. doi:10.1002/ijc.28411
29. Chakravarthy A, Henderson S, Thirdborough SM, et al. Human papillomavirus drives tumor development throughout the head and neck: improved prognosis is associated with an immune response largely restricted to the oropharynx. *J Clin Oncol*. 2016;34(34):4132-4141. doi:10.1200/JCO.2016.68.2955
30. Perry M, Whyte A. Immunology of the tonsils. *Immunol Today*. 1998;19(9):414-421. doi:10.1016/S0167-5699(98)01307-3
31. Malicki S, Winiarski M, Matlok M, Kostarczyk W, Guzdek A, Konturek P. Il-6 and il-8 responses of colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin. *J Physiol Pharmacol*. 2009;60(4):141-146.
32. Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1194-1199. doi:10.1161/01.ATV.0000022694.16328.CC
33. Ascer E, Bertolami MC, Venturini ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177(1):161-166. doi:10.1016/j.atherosclerosis.2004.07.003
34. Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: a population-based Colaus study. *Atherosclerosis*. 2011;219(1):253-258. doi:10.1016/j.atherosclerosis.2011.07.117
35. Danesh J, Kaptoge S, Mann AG, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med*. 2008;5(4):e78. doi:10.1371/journal.pmed.0050078
36. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000;148(2):209-214. doi:10.1016/S0021-9150(99)00463-3
37. Lowe GDO. Circulating inflammatory markers and risks of cardiovascular and non-cardiovascular disease. *J Thromb Haemost*. 2005;3(8):1618-1627. doi:10.1111/j.1538-7836.2005.01416.x

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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