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Circulating white blood cells and risk of tonsillar and base of tongue squamous cell carcinoma: A retrospective and mendelian randomization study.

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Keywords: Tonsillar Neoplasms; Leukocytes; Mendelian Randomization Analysis; Human Papillomavirus; Squamous Cell Carcinoma of Head and Neck.

Abstract. This study aimed to investigate the relationship between circulating white blood cells (cWBC) and the risk of tonsillar and base of tongue squamous cell carcinoma (TSCC/BOT SCC) using retrospective clinical data and Mendelian randomization (MR) analysis. A retrospective cohort of 239 TSCC/BOT SCC patients was analyzed for cWBC subtypes and their association with clinicopathological variables, stratified by human papillomavirus (HPV) status. Blood tests, tumor staging, and immunological markers were included. For causal inference, MR analysis was performed using genome-wide association study (GWAS) data on cWBC from the Blood Cell Consortium (UK Biobank) and TSCC/BOT SCC outcome data from the FinnGen consortium. Single-nucleotide polymorphisms (SNPs) were chosen based on genome-wide significance ($p<5 \times 10^{-8}$), low linkage disequilibrium ($r^2 < 0.001$), and F-statistic >10. The inverse-variance weighted (IVW) method was used as the primary MR approach, supplemented by MR-Egger, weighted median, and weighted mode analyses. The retrospective analysis showed significant differences in cWBC subtypes by gender, age, lifestyle factors, and HPV status. Notably, neutrophils (cNEU) and monocytes (cMON) were strongly associated with tumor stage and immune markers. MR analysis confirmed a causal link between total cWBC count and TSCC/BOT SCC risk (OR=1.516, p=0.005), with no evidence of heterogeneity or pleiotropy. No causal relationship was identified for cWBC subtypes or other head and neck squamous cell carcinoma (HNSCC) sites. This study provides the first comprehensive evidence supporting a causal role of elevated cWBC in the development of TSCC/BOT SCC. These findings indicate that cWBC may serve as a potential biomarker and therapeutic target in HPV-related or unrelated TSCC/BOT SCC.

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Leucocitos circulantes y riesgo de carcinoma de células escamosas de amígdalas y base de la lengua: un estudio retrospectivo y de aleatorización mendeliana.

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Palabras clave: Neoplasias Tonsilares; Leucocitos; Análisis de la Aleatorización Mendeliana; Virus del Papiloma Humano; Carcinoma de Células Escamosas de Cabeza y Cuello.

Resumen. Este estudio tuvo como objetivo explorar la relación entre los leucocitos circulantes (LC) y el riesgo de carcinoma de células escamosas de amígdala y de base de la lengua (CCEA/CCEB), mediante datos clínicos retrospectivos y análisis de aleatorización mendeliana (AM). Se analizó una cohorte retrospectiva de 239 pacientes con CCEA/CCEB para determinar los subtipos de LC y su asociación con variables clínico-patológicas, estratificadas según el estado del virus del papiloma humano (VPH). Se incluyeron análisis de sangre, estadificación tumoral y marcadores inmunológicos. Para la inferencia causal, se realizó un análisis de AM utilizando datos del estudio de asociación del genoma completo (GWAS) sobre LC del Consorcio de Células Sanguíneas (Biobanco del Reino Unido) y datos de resultados de CCEA/CCEB del consorcio FinnGen. Los polimorfismos de un nucleótido (SNP) se seleccionaron en función de su significancia a nivel genómico ($p<5 \times 10^{-8}$), bajo desequilibrio de ligamiento (r^2 <0,001) y un estadístico F >10. Se utilizó la ponderación por el inverso de la varianza (IVW: Inverse Variance Weighted) como método principal de AM, complementado con análisis de regresión MR-Egger, mediana ponderada y moda ponderada. El análisis retrospectivo reveló diferencias significativas en los subtipos de leucocitos totales (LCt) según el sexo, la edad, los factores del estilo de vida y el estado del VPH. Cabe destacar que los neutrófilos y monocitos se asociaron fuertemente con el estadio tumoral y los marcadores inmunitarios. El análisis de AM confirmó una asociación causal entre el recuento total de LC y el riesgo de carcinoma de células escamosas de la lengua/base de la vejiga (OR=1,516, p=0,005), sin evidencia de heterogeneidad ni pleiotropía. No se encontró ningún vínculo causal entre los subtipos de LC y otros sitios de carcinoma de células escamosas de cabeza y cuello. Este estudio proporciona la primera evidencia integrada que respalda un papel causal de los LC elevados en la patogénesis del CCEA/CCEB. Estos hallazgos sugieren que el recuento de leucocitos en sangre periférica (LC) podría servir como biomarcador y objetivo terapéutico en el carcinoma de células escamosas de la lengua/base de la lengua (CCEA/CCEB), relacionado o no con el VPH.

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INTRODUCTION

The global incidence of oropharyngeal squamous cell carcinoma (OPSCC) has been gradually rising, with new cases reaching 98, 412 in 2020 [1]. Smoking, alcohol consumption, and human papillomavirus (HPV) infection are three independent risk factors for its development [2]. HPV- positive OPSCC accounts for over 70% of cases in some re-

gions, with TSCC/BOT SCC being the most common subtypes 1 [1,3-5]. The survival rate for HPV- positive OPSCC is roughly twice as high as that for HPV- negative cases [6]. Although treatments such as surgery, chemotherapy, targeted therapy, and immune checkpoint inhibitors (ICI) have improved outcomes to some extent, these tumors exhibit high heterogeneity, increasing incidence, rapid progression, and high rates of recurrence and metastasis. This highlights the urgent need for better stratification tools and immune-based biomarkers to personalize therapy and predict prognosis. The tumor immune microenvironment (TIME) involves continuous interactions between tumor cells and various immune cells, playing a key role in cancer development, progression, and response to therapy [7]. These interactions influence immune response, tumor cell proliferation, angiogenesis, and tumor recurrence and spread. Additionally, complex regulation occurs among different immune cells and their cytokines within the TIME [8]. The heterogeneity of the tumor itself can also impact the TIME [9]. Currently, immune checkpoint inhibitor (ICI) therapy is increasingly used, utilizing immunotherapy agents to boost the immune system's ability to recognize and destroy malignant cells more effectively[10]. ICI therapy is considered one of the most promising approaches for Head and Neck Squamous Cell Carcinoma (HNSCC) [11]. It has significantly improved treatment outcomes for HNSCC and has become the standard first-line therapy for advanced cases. However, despite its success, many patients still experience disease progression, recurrence, and metastasis after treatment[12, 13]. Circulating white blood cells (cWBCs), which consist of various immune cell types, reflect systemic immune status and may offer insights into the TIME [14]. Previous studies suggest that certain cWBC subsets, such as lymphocytes and monocytes, are linked to tumor immune surveillance, immunotherapy outcomes, and disease prognosis [15]. Nonetheless, the relationship between cWBCs and TSCC/BOT SCC, especially in the context of HPV infection, remains poorly understood. In this study, we explore the association between cWBC subtypes and TSCC/BOT SCC through a dual approach: retrospective clinical analysis and Mendelian randomization (MR) to assess potential causality using genome- wide association study (GWAS) data. This combined analysis aims to identify reliable immune biomarkers and clarify the immunogenic mechanisms underlying TSCC/BOT SCC pathogenesis.

MATERIALS AND METHODS

Retrospective analysis

The clinical and pathological data of all patients with TSCC/BOT SCC in our hospital from July 2020 to January 2025 were collected, including gender, age, smoking and alcohol consumption status, blood routine tests (counts of circulating white blood cells (cWBC), neutrophils (cNEU), lymphocytes (cLYM), monocytes (cMON), eosinophils (cEOS), basophils (cBAS), and derived ratios such as neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) before biopsy or radical surgery. Tumor HPV-related status was also recorded, along with TNM staging: TI-TIV (indicating increasing size and/or local extent of the primary tumor), T stage (T1-4: tumor size and extent), and N stage (N0 to N3: spread to regional lymph nodes). The KI-67 value (a protein expressed in dividing cells and a marker for tumor cell proliferation), imaging stage or postoperative pathological stage, and Combined Positive Score (CPS) expression were included as well.

Approval from the Ethics Committee of Beijing Tongren Hospital, Capital Medical University, was obtained prior to data collection and analysis (Approval no. TREC2022-KY018.R1).

The retrospective data of patients aged ≥18 years of both genders were retrieved by passing the following criteria: pre-biopsy/radical surgery and routine blood tests con-

ducted at the host institute; pathological confirmation of TSCC/BOT SCC; available HPV and CPS data; no history of other malignancies or immunologic diseases, and no recent infection or anti-infective therapy.

The exclusion criteria included: patients under 18 years old; TSCC/BOT SCC treated at other institutions or hospitals; missing records of pre-biopsy or radical surgery; no routine blood tests performed at the host hospital; prior induction therapy before radical surgery elsewhere; recent infection or ongoing anti-infective therapy; history of other cancers or immunologic disorders; and use of hormonal drugs, anti-infective agents, or traditional medicine before blood collection.

The patients were divided into two groups based on the HPV status of TSCC/BOT SCC. Differences in cWBC were compared between the two groups, accounting for factors such as gender and age. Subsequently, subgroup analyses were performed within the HPV-positive and HPV-negative groups to assess differences in these indicators.

Mendelian Randomization

Sources of GWAS data for cWBC and HNSCC

The cWBC exposure data were obtained from the Blood Cell Consortium (BCX) meta-analysis (UKBB cohort, N=562,243) [16]. The details of the GWAS data used in this study were obtained from the FinnGen database (https://www.finngen.fi/en) [17] and are presented in Table 1.

Fig. 1 shows the MR framework used to explore the causal link between cWBCs and specific head and neck cancers. GWAS summary statistics for cWBCs served as the exposure data, while outcome data were obtained from GWAS datasets for tonsillar and base-of-tongue cancers, along with other head and neck cancer subsites, including hypopharyngeal, nasal, oral, and nasopharyngeal cancers. This method allows for the assessment of potential causal effects while reducing confounding and reverse causality.

Selection of genetic instruments

We identified SNPs strongly associated with the exposure, applying a genome-wide significance threshold of $p < 5 \times 10^{-8}$. To control for linkage disequilibrium, we applied stringent clumping parameters ($r^2 < 0.001$ within a 10,000-kb window). Palindromic SNPs with ambiguous allele frequencies were excluded to prevent strand misalignment. Additionally, we filtered out weak instruments by calculating the F-statistic (β^2/SE^2) for each SNP-exposure association, retaining only those with $F > 10$ to ensure robust instrument strength [18].

Table 1. Information of summary level genome-wide association study (GWAS) used in this study.

Phenotype	GWAS ID / Name	Sample size*	
		Patients	Controls
Malignant cancer of the tonsil and base of the tongue	Finngen_R12_C3_Malignant cancer of tonsil and base of tongue	813	378,749
Hypopharyngeal cancer	Finngen_R12_C3_Malignant neoplasm of hypopharynx	124	378,749
Nasal cavity and sinus cancer	Finngen_R12_C3_Malignant neoplasm of the nasal cavity and sinuses	345	378,749
Oral cancer	Finngen_R12_C3_Malignant neoplasm of the oral cavity	1,614	378,749
Nasopharyngeal cancer	Finngen_R12_C3_Malignant neoplasm of nasopharynx	152	378,749

*All other cancers were excluded except the selected type. Controls against each type of cancer in the patients column.

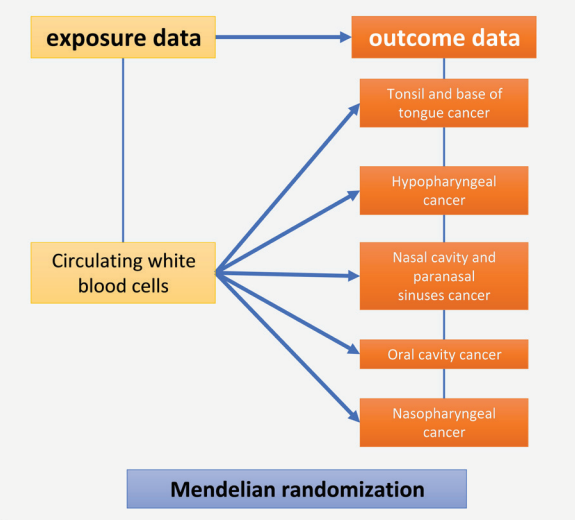

Fig. 1. Mendelian Randomization framework used to investigate the relationship between circulating white blood cell subtypes and site-specific head and neck cancers.

MR analysis

Four MR methods were used: inverse-variance weighted (IVW), MR-Egger, weighted median, and weighted mode. The IVW method served as the main approach to estimate the causal effect. By thoroughly considering the effects and precisions of multiple SNPs, the IVW method provided a solid estimate of causality. Additionally, other methods like MR-Egger, weighted median, and weighted mode were also employed in the analysis. If the statistically significant results from IVW did not agree with those from the other methods, such as MR-Egger, weighted median, and weighted mode, we compared the effect estimates (β and OR) across methods to evaluate the size and direction of the differences. Heterogeneity and horizontal pleiotropy were checked using the IVW and MR-Egger tests. A p-value above 0.05 for Cochran's Q-statistic (in MR-IVW) and Rucker's Q-statistic (in MR-Egger) suggested no heterogeneity in the MR analysis [19]. Additionally, MR-Egger could identify and assess potential pleiotropy through the MR-Egger intercept test [20]. If pleiotropy was detected, the MR results were considered invalid.

Statistical analysis

For the retrospective analysis, R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria) was used. Non-parametric tests were employed to compare quantitative variables: the Wilcoxon rank-sum test for two independent samples and the Kruskal-Wallis test for multiple samples. The chi-square test was used to compare proportions (rates), and analysis of variance (ANOVA) was used to compare continuous variables across groups. Statistical significance was defined as a two-sided p-value<0.05. MR analyses were performed using the TwoSampleMR software (version 0.5.11) in R (version 4.3.1). To rigorously evaluate the causal relationship, false discovery rate (FDR) correction was applied to the final results. A corrected p-value<0.05 was considered to indicate a statistically significant result.

RESULTS

Patient Baseline Characteristics

A total of 239 patients with TSCC/BOT SCC were included. Demographic and clinical characteristics are summarized in Table 2. In terms of gender distribution, there were 203 male patients, accounting for 84.94%. Regarding age, 206 patients were under 50 years old, making up 86.19%. Concerning lifestyle habits, 149 patients had a history of smoking or drinking, representing 62.34%, while 77 patients had no such habits, accounting for 32.22%. For HPV infection status, 150 patients were HPV-positive, constituting 62.76%, and 89 patients were HPV-negative, representing 37.24%. In tumor staging, T2 was the most common (40.17%), while N2 (43.10%) and N3 (33.47%) were the predominant N stages. TNM stage IV accounted for 25.10%. Regarding the immunohistochemical index Ki-67 expression level, the largest group had values >70, with 116 cases, or 48.54%. The distribution of CPS scores was as follows: 48 patients scored 1-10 (20.08%), 44 patients

Table 2. Baseline characteristics of all included oropharyngeal squamous cell carcinoma patients.

Characteristics	Patients (N=239)	Percentage
Gender		
Male	203	84.94
Female	36	15.06
Age		
≤50	33	13.81
>50	206	86.19
Smoking or Drinking		
No	77	32.22
Yes	149	62.34
NA	13	5.44
HPV status		
Positive	150	62.76
Negative	89	37.24
T Stage		
1	43	17.99
2	96	40.17
3	54	22.59
4	46	19.25
N Stage		
1	54	22.59
2	103	43.1
3	80	33.47
4	2	0.84
TNM Stage		
I	84	35.15
II	57	23.85
III	38	15.9
IV	60	25.1
Ki-67		
10-39	27	11.3
40-69	71	29.71
>70	116	48.54
NA	25	10.46
CPS		
1-10	48	20.08
11-59	44	18.41
60-99	23	9.62
<1	6	2.51
NA	118	49.37

NA: Not available (data missing); HPV: human papilloma virus; TNM system stages cancer; T stage: tumor stage; N stage: Node stage; Ki-67: proliferation index; CPS: Combined Positive Score.

scored 11-59 (18.41%), 23 patients scored 60-99 (9.62%), and 6 patients scored less than 1 (2.51%).

Correlation between cWBC and clinicopathological characteristics

In the overall cohort, circulating white blood cell subtypes (cWBC, cNEU, cEOS, cMON, and cLMR) were significantly associated with demographic and clinical variables, as shown in Fig. 2. Gender differences were particularly notable in cMON and cLMR ($p<0.001$). Smoking and drinking habits were significantly linked to cWBC, cMON, and cEOS levels ($p<0.001$). Additionally, cNEU and cNLR were associated with T-stage ($p<0.05$ and $p<0.01$, respectively), whereas cMON differed across CPS score groups ($p<0.05$). In the HPV-positive subgroup (n=150), gender had a significant impact on cMON and cLMR ($p<0.001$), with significant differences also observed in cEOS levels ($p<0.01$). Smoking and drinking were associated with higher cWBC ($p<0.01$) and cMON ($p<0.001$), while cNEU varied significantly with TNM stage ($p<0.05$). For the HPV-negative subgroup (n=89), gender-related differences were found in cMON ($p<0.05$), and smoking/drinking were linked to cMON ($p<0.05$) and cEOS ($p<0.01$). T-stage was associated with variations in cLYM and cNLR ($p<0.05$), and cMON showed differences across CPS score categories ($p<0.05$).

MR analysis

IVW analysis revealed a significant positive association between cWBC and TSCC/BOT SCC risk ($\beta=0.416$, OR=1.516, 95% CI=1.189-1.935, P/FDR=0.005). Although cMON showed a positive trend ($\beta=0.254$, OR=1.289, P/FDR=0.018), results were excluded due to significant horizontal pleiotropy ($p<0.05$). No statistically significant associations were observed between other cWBC subtypes and TSCC/BOT SCC or other HNSCC subsites. These findings are shown in Fig. 3.

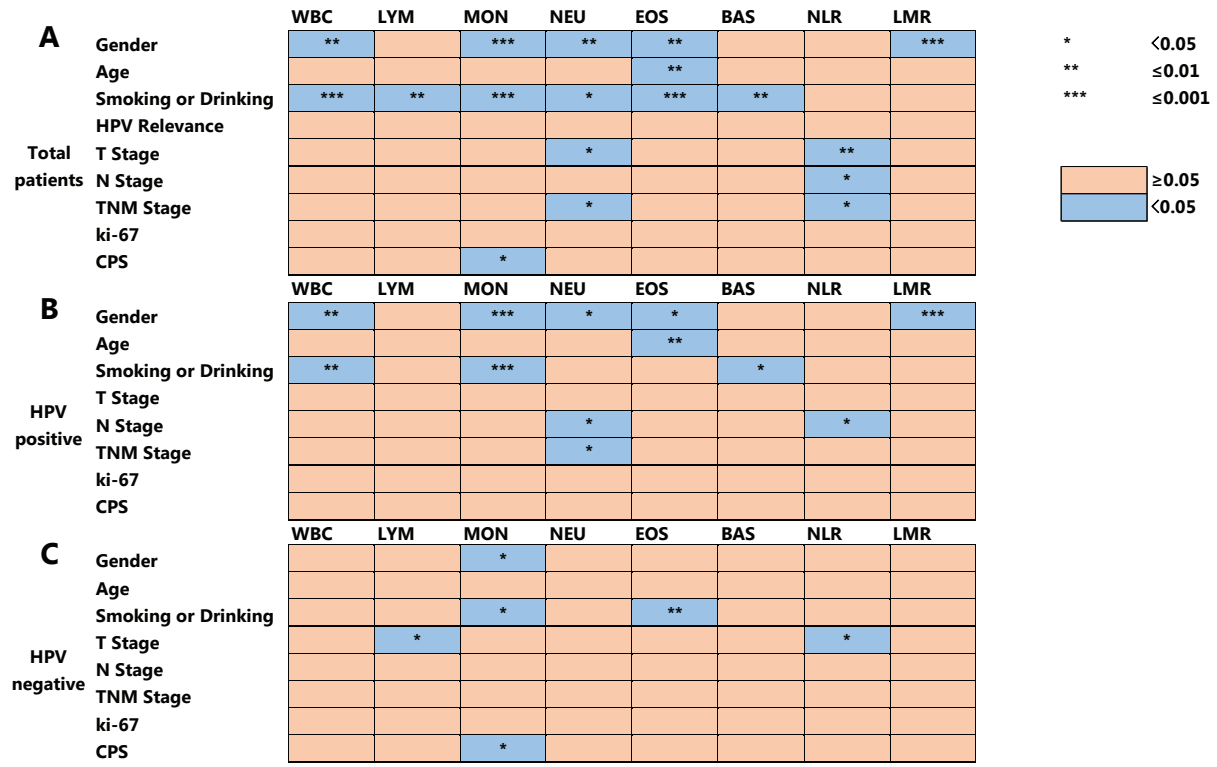

Fig. 2. Association between circulating white blood cell subtypes with demographic and clinical variables. cWBC: circulating white blood cell; cNEU: neutrophils; cLYM: lymphocytes; cMON: monocytes; cEOS: eosinophils; cBAS: basophils; NLR: neutrophils-to-lymphocyte ratio; LMR: lymphocyte-to-monocyte ratio; TNM: system stages cancer; T stage: tumor stage; N stage: Node stage; Ki-67: proliferation index; CPS: Combined Positive Score; HPV: Human Papilloma Virus.

Table 3 shows the results of heterogeneity and horizontal pleiotropy tests for the MR estimates. For the association between WBC and TSCC/BOT SCC, neither the heterogeneity nor the horizontal pleiotropy tests were statistically significant ($p>0.05$ for both). In contrast, the horizontal pleiotropy test for the association between MON and TSCC/BOT SCC was statistically significant ($p<0.05$).

DISCUSSION

Globally, the proportion of HPV-positive OPSCC has increased significantly. The cumulative risk of OPSCC is 0.21% in males and 0.05% in females, with a significantly higher proportion of male patients than female patients [1]. In this study, HPV-positive patients accounted for 62.76%, with males making up 84.94%, consistent with the global epidemiological trend in OPSCC. The biological behavior of HPV-positive OPSCC differs markedly from that of HPV-negative OPSCC. Systemic inflammatory markers have become reliable prognostic tools in HNSCC, reflecting the dynamic interaction between tumor biology and host immunity. HPV-positive OPSCC shows strong immune cell infiltration, better treatment response, and improved prognosis [21-22]. Notably, circulating leukocyte levels differ significantly between HPV-positive and HPV-negative cases, with variations in immune cell types potentially indicating differences in the tumor immune microenvironment (TIME) between these groups. These distinct immune states may influence the effectiveness of immuno-

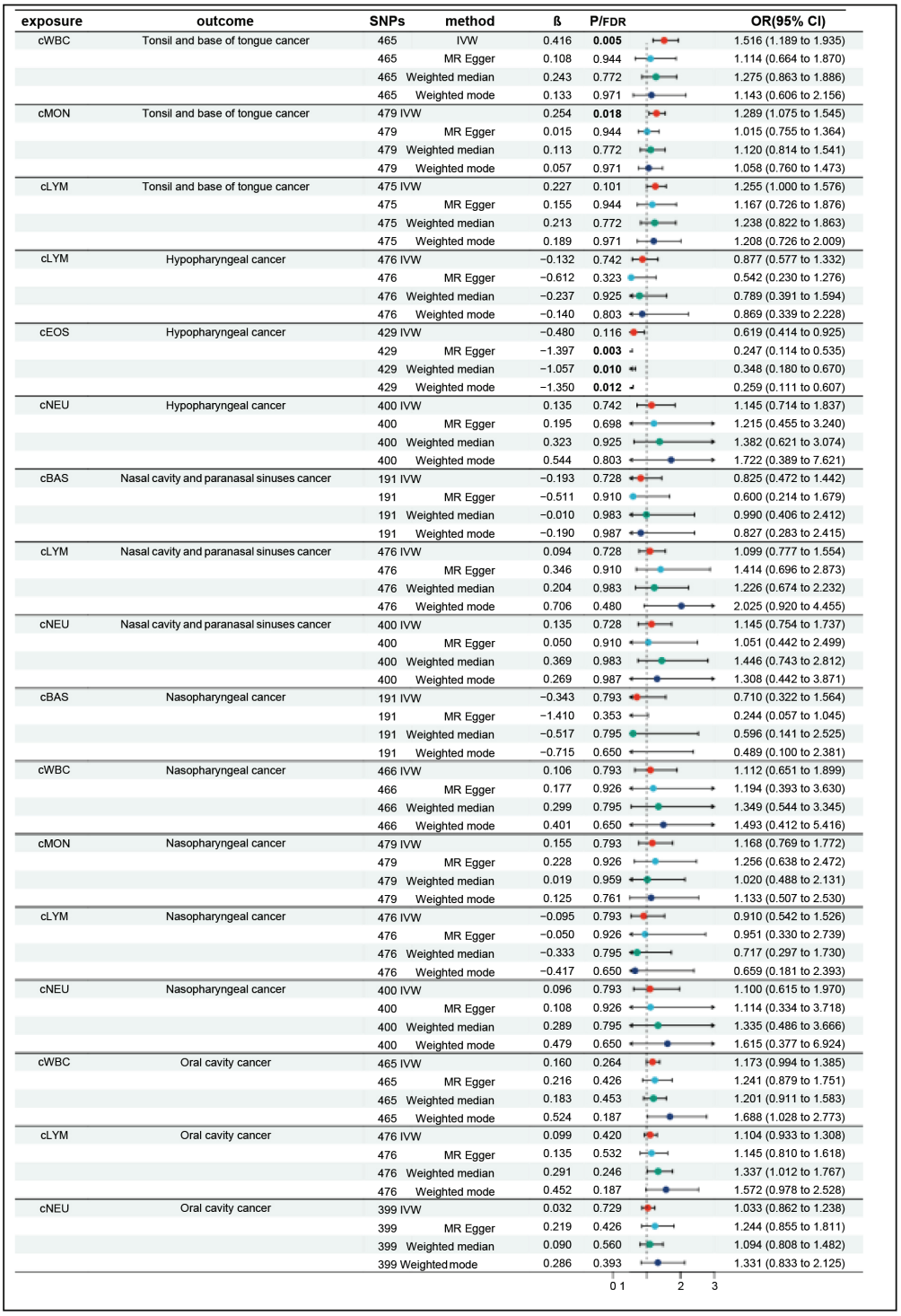

Fig. 3. Associations between circulating white blood cell (cWBC) subtypes and Tonsillar Squamous Cell Carcinoma and Base of Tongue Squamous Cell Carcinoma (TSCC/BOT SCC) or other Head and Neck Squamous Cell Carcinoma (HNSCC) subsites. cNEU: neutrophils; cLYM: lymphocytes; cMON: monocytes; cEOS: eosinophils; cBAS: basophils; LMR: lymphocyte-to-monocyte ratio; HPV: Human Papilloma Virus; IVW: Inverse variance weighted; MR-Egger: Mendelian randomization.

Table 3. Results of Mendelian Randomization heterogeneity and horizontal pleiotropy.

Exposure	Outcome	Heterogeneity			Pleiotropy		
		Method	Q	Q_pval	Egger_intercept	se	*p*val
cWBC	Tonsil and base of tongue cancer	MR-Egger	491.095	0.177	0.008	0.006	0.187
		IVW	492.95	0.17			
cMON	Tonsil and base of tongue cancer	MR-Egger	468.766	0.597	0.009	0.005	0.045*
		IVW	472.802	0.558			
cLYM	Tonsil and base of tongue cancer	MR-Egger	457.389	0.688	0.002	0.006	0.732
		IVW	457.506	0.699			
cLYM	Hypopharyngeal cancer	MR-Egger	432.64	0.914	0.013	0.011	0.208
		IVW	434.231	0.91			
cEOS	Hypopharyngeal cancer	MR-Egger	427.628	0.482	0.03	0.011	0.007*
		IVW	435.049	0.397			
cNEU	Hypopharyngeal cancer	MR-Egger	382.328	0.705	-0.002	0.011	0.893
		IVW	382.346	0.717			
cBAS	Nasal cavity and paranasal sinuses cancer	MR-Egger	215.195	0.093	0.009	0.013	0.471
		IVW	215.789	0.097			
cLYM	Nasal cavity and paranasal sinuses cancer	MR-Egger	481.968	0.39	-0.007	0.009	0.425
		IVW	482.615	0.395			
cNEU	Nasal cavity and paranasal sinuses cancer	MR-Egger	462.155	0.014*	0.002	0.01	0.826
		IVW	462.211	0.016*			
cBAS	Nasopharyngeal cancer	MR-Egger	152.792	0.975	0.032	0.019	0.088
		IVW	155.731	0.967			
cWBC	Nasopharyngeal cancer	MR-Egger	452.706	0.638	-0.002	0.013	0.886
		IVW	452.727	0.65			
cMON	Nasopharyngeal cancer	MR-Egger	409.642	0.988	-0.003	0.01	0.789
		IVW	409.713	0.989			
cLYM	Nasopharyngeal cancer	MR-Egger	462.862	0.634	-0.001	0.013	0.925
		IVW	462.871	0.646			
cNEU	Nasopharyngeal cancer	MR-Egger	395.543	0.525	0	0.014	0.982
		IVW	395.544	0.539			
cWBC	Oral cavity cancer	MR-Egger	438.241	0.79	-0.001	0.004	0.716
		IVW	438.373	0.798			
cLYM	Oral cavity cancer	MR-Egger	532.671	0.032*	-0.001	0.004	0.815
		IVW	532.733	0.034*			
cNEU	Oral cavity cancer	MR-Egger	376.332	0.765	-0.005	0.004	0.267
		IVW	377.566	0.762			

cWBC: circulating white blood cell; cNEU: neutrophils; cLYM: lymphocytes; cMON: monocytes; cEOS: eosinophils; cBAS: basophils; IVW: Inverse variance weighted; MR-Egger: Mendelian randomization Egger Regression. *p<0.05.

therapy. This study found that cWBC, cNEU, and cEOS had significant differences across various genders, ages, smoking/drinking habits, and tumor stages. In particular, among HPV-positive patients, cMON and cLMR exhibited notable differences. Previous research has demonstrated that HPV can modulate the immune microenvironment to promote tumorigenesis and progression[23]. Lower lymphocyte counts and reduced LMR are associated with poorer outcomes. A systematic review involving 5,234 HNSCC patients revealed that higher LMR (≥4) was linked to better overall survival (HR = 1.36) and disease-free survival (HR = 0.94) [24].

This threshold effect likely reflects a balance between lymphocyte-driven immune surveillance and monocyte-derived tumor-associated macrophage recruitment, which fosters immune evasion.

Circulating leukocyte subtypes such as cMON and cNEU are closely linked to immune suppression and angiogenesis in the TIME [25], and they may work together to support tumor growth. Notably, cNEU are the most common circulating leukocytes [26]. However, their levels show significant variation across different tumor stages (T/TNM stage), which could indicate different functions during disease progression in TSCC/BOT SCC. Research shows that cNEU display high plasticity within the TIME, with their roles changing based on tumor stage and microenvironmental factors. Especially in advanced tumor stages, cNEU may encourage tumor growth through mechanisms like promoting new blood vessel formation and suppressing immune responses [27]. In colorectal cancer studies, increased neutrophil levels are linked to disruptions in the intestinal microbiota and facilitate peritoneal metastasis of colorectal cancer by interacting with tumor cells [28]. Additionally, higher pretreatment neutrophil counts and NLR have been repeatedly confirmed as independent indicators of poorer overall survival (OS) and disease-free survival (DFS) in meta-analyses involving over 10,000 patients [29]. The observed correlation between cNEU and tumor stage in this study likely reflects their dynamic contribution to tumor progression.

Whether cWBC is causally associated with the occurrence of HNSCC, particularly with TSCC/BOT SCC, we conducted an MR analysis. The IVW method demonstrated a robust positive association between cWBC and TSCC/BOT SCC risk ($\beta=0.416$, OR=1.516, *P*/FDR=0.005), with consistent results across MR-Egger and weighted median methods (no evidence of heterogeneity or pleiotropy, $p>0.05$). In contrast, cMON associations were confounded by horizontal pleiotropy ($p<0.05$), limiting causal inference. This finding is consistent with previous studies, and cWBC is captured by tumour tissues to become infiltrating leukocytes that promote tumour progression [30]. The absence of significant associations between cWBC and other HNSCC subsites in our MR analysis may reflect the anatomical and biological heterogeneity of HNSCC, which could differentially influence immune cell recruitment and function. ICI therapy involves the binding of anti-programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) antibodies to induce autologous immune cells to kill tumors [31]. It has demonstrated significant efficacy across multiple advanced tumor types, with some patients achieving durable responses [32]. The role of cWBC in immunotherapy has also been supported by several studies. The PD-L1 status of cWBC is associated with PD-L1 expression in immune cells within the TIME [33, 34]. In melanoma patients, the efficacy of ICI therapy correlates with an adequate number of cLYM [35]. Studies have also found that the efficacy of ICI therapy is significantly associated with baseline cWBC and their subtypes (such as LYM and MON), where higher baseline cLYM is associated with better treatment responses and longer progression-free survival, while higher cNEU is associated with poorer prognosis [36, 37]. Incidental immune-related adverse events (irAEs) during im-

munotherapy are also associated with cWBC and their subtypes. Baseline cNEU, cLYM, cMON, and cEOS, baseline platelet counts, and increases in cWBC, cLYM, and cEOS during follow-up are all associated with an increased risk of irAEs [38-40]. Collectively, our data support a dual role for cWBC in TSCC/BOT SCC: they may contribute to tumor initiation and progression while concurrently modulating responses to immunotherapy. Clinically, CPS levels are typically used as a surrogate for PD-1 expression. Therefore, this study analyzed the differences between CPS expression and cWBC. Unfortunately, CPS was not detected in some patients. To exclude differences in detection results between our institution and other institutions, only patients with CPS expression detected in our hospital were included, resulting in many patients being recorded as having no CPS expression values. The results showed that different CPS expression levels were only associated with cMON in all patients and HPV-negative patients, but no differences were observed in cWBC or their subtypes in HPV-positive patients, further indicating the heterogeneity of tumor cells under different HPV infection statuses. Additionally, studies have found that retinoic acid secreted by tumor tissues can induce MON to differentiate into immunosuppressive tumor-associated macrophages (TAMs), thereby inhibiting the efficacy of immunotherapy [41]. TAMs and dendritic cells, a monocyte subset, may also indirectly promote tumor progression, which may explain the different prognoses of HPV-negative and HPV-positive tumors due to differences in immune cells [42]. This study has some limitations. First, its focus on TSCC/BOT SCC may limit the generalizability of findings to other OPSCC subsites. The retrospective design introduces potential confounding due to unmeasured clinical variables, such as treatment history. Additionally, the relatively small sample size in the HPV-negative subgroup may reduce the statistical power of subgroup analyses. Furthermore, lifestyle factors such as diet and environmental exposures, which were not assessed, could independently influence the observed associations between circulating white blood cells and tumor characteristics. Future research should further expand the sample size and incorporate more potential confounding factors to comprehensively evaluate the associations between cWBC and TSCC/BOT SCC. In-depth investigations using single-cell sequencing technology may help reveal the specific mechanisms of action of different WBC subtypes in the TIME. Finally, exploring the potential of cWBC as biomarkers and therapeutic targets for TSCC/BOT SCC will provide new insights for clinical diagnosis and treatment.

This study demonstrates a causal link between elevated cWBC and the risk of TSCC/BOT SCC through combined retrospective and Mendelian randomization analyses. Significant associations between cWBC subtypes and tumor characteristics, especially in HPV-positive cases, underscore their potential role in tumor progression and immune regulation. The findings indicate that cWBC may serve as non-invasive biomarkers for risk assessment and treatment stratification. Further research is necessary to validate these results and investigate underlying mechanisms in larger, prospective cohorts.

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Conflict of interest

The authors state that they have no conflicts of interest.

Ethical approval and informed consent

The retrospective analysis of patients at our hospital has been approved by the Ethics Committee under approval number TREC2022-KY018.R1, dated 21st April 2022. This study was performed in line with the principles of the Declaration of Helsinki.

Data availability statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Declaration of generative AI and AI-assisted technologies in the writing process

No generative AI or AI-assisted technology was used in the writing process.

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