REVIEW

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From Radiation to Immune Response: A Systematic Review of Systemic Immunomodulation in Squamous Cell Head and Neck Cancer

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is frequently treated with radiotherapy (RT), often combined with chemotherapy. Beyond local tumor control, RT induces systemic immunomodulatory effects. This systematic review evaluates alterations in peripheral immune cell subpopulations and circulating biomarkers in HNSCC patients undergoing RT with or without chemotherapy. **Methods:** A systematic search across five databases identified 12 eligible studies. Inclusion criteria encompassed adult patients with HNSCC treated with external beam RT and analysis of immune parameters in peripheral blood. The review protocol was registered in the Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42021283028). **Results:** RT induced lymphopenia, with consistent reductions in T, B, and dendritic cells, and increases in regulatory T cells (Tregs). Altered expression of immune activation markers (such as *CD69* and *HLA-DR*) and exhaustion markers (such as *PD-1* and *CTLA-4*) was noted. Gene expression of *FDXR, GADD45,* and others demonstrated sustained modulation post-RT. Changes in cytokines, adhesion molecules (*CX3CR1, CD11a*), and immune checkpoint proteins (*PD-L1*) were associated with treatment response and toxicity. Baseline immune profiles correlated with the risk of acute toxicity. **Conclusion:** RT, either alone or in combined with chemotherapy, significantly alters systemic immunity in HNSCC. Immunophenotyping and peripheral biomarkers show prognostic potential, supporting their integration into personalized treatment strategies. However, standardized, large-scale longitudinal studies are warranted.

Keywords: head and neck cancer- squamous cell carcinoma- radiotherapy- immune response cytokines- plasma proteins

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Introduction

Head and neck cancer represents a group of primary neoplasms affecting the lips, oral cavity, pharynx, larynx, nasal cavity, paranasal sinuses and salivary glands with a significant impact on morbidity and mortality. The annual global incidence is approximately 1.200.000 cases, with an estimated mortality rate around 50% [1].

About 95% of these tumors are squamous cell carcinomas (SCC), an aggressive neoplasm associated with changes in immune and inflammatory responses as well as angiogenesis [2, 3]. Currently, independent prognostic factors include not only tumor staging and differentiation degree but also the frequency of leukocyte cell populations and subpopulations [4, 5]. The literature has demonstrated that various immune system cell populations, along with circulating biomarkers, are involved in immune responses to tumors, such as natural killer (NK) cells, regulatory T (Treg) cells, CD4 and CD8 T lymphocytes, B lymphocytes, dendritic cells (DC), myeloid-derived suppressor cells (MDSC) and components of the complement system, especially the C3a fraction [2, 6-8].

Radiotherapy (RT) is a commonly used treatment

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for these types of neoplasms, generally involving the fractionated administration of ionizing radiation doses to induce cellular death in the tumor microenvironment. Traditionally, RT has been considered a local treatment modality, but various series have demonstrated that exposing areas to ionizing radiation can induce changes in both nearby tissues and systemically [9-12]. These non-target effects may result from the secretion of inflammatory mediators, induction and/or repression of specific genes, and radio-adaptive responses from immune system cells [9, 12].

We aim to describe the changes in components of the immune response in peripheral blood (cell subpopulations and cytokines) in individuals with head and neck squamous cell carcinoma (HNSCC) undergoing curative-intent treatment with radiotherapy whether definitive, postoperative (pRT), or concurrent with chemotherapy (CRT). This systematic review was guided by the PICO framework to address the following research question: What modifications in peripheral blood can be observed in patients undergoing (chemo)radiotherapy? The population of interest included adult patients aged 18 to 70 years with confirmed cancer diagnosis, undergoing external beam radiotherapy, either alone or in combination with chemotherapy. The intervention considered was the evaluation of cellular inflammatory markers in peripheral blood. Comparisons were made between values obtained before and after the therapeutic regimen, and in some studies, with a control group of healthy individuals. The primary outcome assessed was whether (chemo) radiotherapy induces measurable changes in immune status.

Materials and Methods

Search Strategy and Quality Assessment

We conducted a systematic review using the following databases: Scopus, PubMed, Embase, BVS and Web of Science. The research question was formulated according to the formula described in the PICO acronym, following the criteria established in the document Preferred Reporting Items for Systematic Reviews and Meta-Analyses, known as PRISMA [13]. The review protocol was included in the database Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42021283028).

Data were collected on November 13, 2024, and the controlled terms were used: "radiotherapy" OR "radiation therapy" AND "immunophenotyping" AND "Squamous Cell Carcinoma of Head and Neck" OR 'head and neck squamous cell carcinoma'. The research was limited to studies conducted in humans, in vivo. All selected publications had their references manually evaluated for relevant publications that were eventually lost in the primary search.

Inclusion and exclusion criteria

The timeframe for the selected studies spanned from 2014 to 2024. Studies involving patients with hematological diseases, those undergoing immunotherapy, and cases of reirradiation were excluded. Oral presentations at conferences or abstracts published in annals of scientific events were also excluded. The inclusion criteria focused on articles in English, with full texts accessible.

The inclusion criteria were: 1) studies involving adult individuals diagnosed with HNSCC and undergoing external beam radiotherapy; 2) assessment of immune response cells and molecular markers in peripheral blood samples.

Data Extraction, Collection, Analysis, and Review

Title and abstract screening were conducted independently by reviewers using Rayyan (Qatar Computing Research Institute), a web-based tool designed to facilitate systematic reviews by enabling blinded screening and efficient management of included and excluded studies [14]. The collected articles were evaluated by investigators in a blind and independent way and the publications were selected after consensus. Papers selected for retrieval were assessed by the reviewers for methodological quality.

Results

We identified 1095 studies (Figure 1). After removing duplicates and applying our specified criteria, 227 records were deemed potentially relevant. Subsequent record analysis and full-text screening excluded non-matching studies, leaving 12 articles.

Table 1 summarizes the main characteristics of the study populations, which included patients with a median age between 58 and 67 years, with primary site tumors in the oral cavity, pharynx, or larynx, TNM staged from I to IVb [15], who underwent definitive radiotherapy, with or without systemic treatment, or postoperative radiotherapy. The systemic treatments included chemotherapy with cisplatin and/or targeted therapy with cetuximab, a monoclonal antibody that acts by blocking epidermal growth factor receptor (EGFR). All patients underwent external beam radiotherapy in linear accelerators, with photon energy, three-dimensional planning and treatment dose range from 57,5Gy to 70Gy. In all the selected articles, the main limiting factor of the studies was the small number of patients included.

Circulating Immune Cell Populations

Donaubauer et al. conducted the largest study to date involving 1,000 patients with HNSCC and 100 healthy controls. Following chemoradiotherapy (CRT), a generalized reduction in peripheral immune cell counts was observed across all analyzed cell populations. Dendritic cells (DCs), B cells, and T cells showed statistically significant declines, whereas eosinophils and neutrophils returned to baseline levels. Although monocytic myeloid-derived suppressor cells (M-MDSCs) decreased post-CRT, they remained elevated compared to pre-surgical values. Granulocytic MDSCs (G-MDSCs) declined to below baseline levels, although this change was not statistically significant [16].

Similarly, a progressive decrease in B cells and an increase in regulatory T cells (Tregs) were observed throughout treatment [9, 17, 18], suggesting

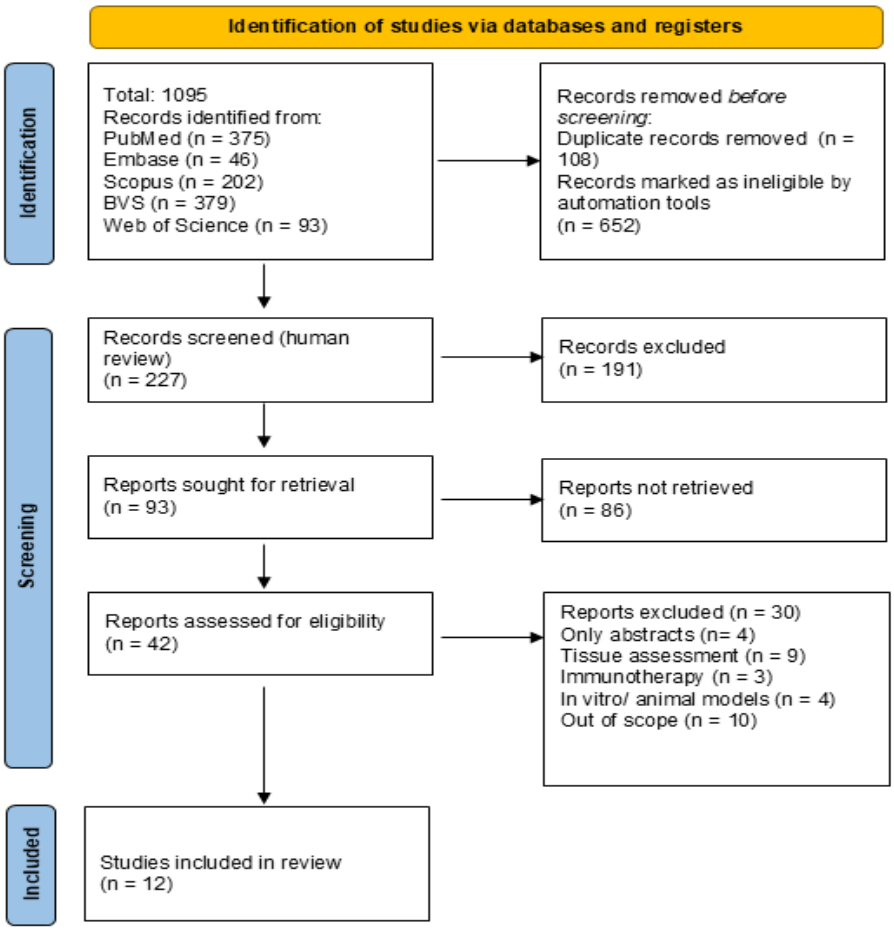

Figure 1. Prisma 2020 Flow Diagram for Systematic Reviews. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar 29;372:n71. doi: 10.1136/bmj.n71.

a compounding immunosuppressive shift driven by radiotherapy (RT). $CD8^+$ T cells and natural killer T (NKT) cells were elevated in patients compared to healthy controls, with further increases during treatment. Lack of B cell recovery and alterations in helper T cell (Th) and Treg populations post-CRT were associated with tumor recurrence [17]. Furthermore, pre-treatment $CD4^+/CD8^+$ ratios and the proportions of B cells expressing CD39 (an ectonucleotidase) and CD71 (a proliferation marker) were associated with improved relapse-free survival [18].

An increase in Tregs, more pronounced in patients treated with CRT, was also observed in another cohort of HNSCC patients [9, 19].

Impact of CRT on Lymphocyte Activation and Cytokine Expression

Beyond changes in immune cell counts, CRT significantly altered activation profiles. Post-treatment, upregulation of activation markers CD69 (an earlier activation marker) and *PD-1* (programed death-1) was noted in T helper and cytotoxic T cells. Basophils displayed elevated *CD25* expression. Importantly, human leukocyte antigen-DR (HLA-DR), a class II major histocompatibility complex molecule involved in antigen presentation, showed increased expression on T and natural killer (NK) cells, while the proportion of HLA-DR-positive monocytes significantly declined postoperatively and remained unchanged following CRT [16]. In contrast, $HLA\text{-}DR^+$ monocytes were significantly reduced postoperatively and remained low post-CRT [16].

In addition, enhanced polyfunctional cytokine expression in peripheral T cells was reported, indicating that the remaining T cells may retain antitumor potential and represent viable targets for immunotherapeutic expansion [19]. Expression of cytotoxic T-lymphocyte antigen-4 (*CTLA-4*) a marker of T cell inhibition and Treg activity was significantly elevated in HNSCC patients before treatment and increased further post-RT. *PD-1*, a marker of T cell exhaustion, followed a similar trajectory [9].

In a prospective study involving 51 patients with oropharyngeal carcinoma, CRT led to a significant reduction in $CD4^+$ responses to human papillomavirus type 16 (HPV16) proteins. A significant reduction in $CD4^+$ T cell responses to E6 and E7 peptides was observed, with a proportionally greater decline in $CD4^+$ than $CD8^+$ cells after treatment. In HPV-positive individuals, a significant increase in Tregs was also noted [20].

Following adjuvant RT, NK cells demonstrated increased expression of NKG2D (natural killer group 2 member D), a key marker of activation and cytotoxicity [21]. RT was also associated with modulation of

Table 1. Characteristics of Studies Population

Author (ref)	Publication year	N	Age (years)	Tumor Stage	Treatment protocol	Treatment time	Radiation technique	Radiation dose (median Gy)	Primary tumor location
Balázs et al. [9]	2019	23	64	I-IVa	RT	Definitive	IMRT	57.5	Oral cavity, oropharynx, larynx, parotid.
Beschel et al. [23]	2016	48	63	II- IVa	CRT	Post-operative	IMRT	62.4	Oral cavity, oropharynx, hypopharynx larynx.
Doescher et al. [19]	2017	9	58	I-IVa	CRT ± cetuximab	Definitive or post-operative	IMRT	66	NA
Donaubauer et al. [16]	2024	150	66	I-IVb	CRT	Definitive or post-operative.	3DRT	NA	Oral cavity, oropharynx, nasopharynx, hypopharynx, larynx.
Gehrmann et al [21]	2014	21	66	II-IVa	RT	Post-operative	3DRT	64	Oral cavity, oropharynx, hypopharynx, larynx, paranasal sinuses
Idel et al. [25]	2024	17	67	I-IVa	CRT	Definitive	3DRT	NA	NA
Masterson et al. [20]	2016	51	58	I-IVa	RT or CRT	Definitive	3DRT	65	Oropharynx
Mytileneos et al. [24]	2020	22	62.4	I-IV	RT or CRT	Definitive or post-operative	3DRT	NA	Oral cavity, oropharynx, hypopharynx, larynx,
Niu et al. [17]	2021	32	59	III-IVa	CRT	Definitive	3DRT	70	NA
Paya et al. [22]	2023	16	NA	I-IVa	RT	Definitive	3DRT	NA	oropharynx, larynx
Turner et al. [18]	2023	25	62.5	II-IVb	RT or CRT ± cetuximab	Definitive or post-operative	3DRT	NA	NA
von Witzleben et al[28]	2021	22	62.4	III-IVa	RT or CRT	Definitive or post-operative	3DRT	NA	Oral cavity, oropharynx, hypopharynx, larynx.

Legend: N, number of individuals; NA, not available; RT, radiotherapy; CRT, chemoradiotherapy; ± cetuximab - with or without addition of cetuximab; 3DRT, tridimensional radiotherapy planning; IMRT, intense modulated radiotherapy.

immunoregulatory gene expression. In a study involving 16 patients, a significant decrease in *CD44* expression - a cell adhesion molecule involved in tumor progression and metastasis - was observed postoperatively and post-RT, correlating with altered gene expression profiles [22].

Another investigation assessed the expression of five radiation-responsive genes ferredoxin reductase (FDXR), sestrin 1 (SESN1), growth arrest and DNA damage-inducible alpha (GADD45), damage-specific DNA-binding protein 2 (DDB2), and mouse double minute 2 (MDM2) at three time points: pre-RT, immediately post-RT, and one month post-treatment. FDXR, GADD45, DDB2, and MDM2 were upregulated immediately after RT, while SESN1 was downregulated. The most notable increase was a 1.9-fold rise in GADD45. At one month, FDXR and DDB2 returned to baseline, whereas SESN1, GADD45, and MDM2 remained altered, suggesting persistent transcriptional deregulation [9].

Correlation Between Immune Profiles and Acute Toxicity

An association between pre-treatment immune profiles and the development of acute toxicity was reported. Patients who experienced high-grade toxicity presented with higher percentages of T, B, and NK cells, and lower monocyte counts and monocyte-to-T cell ratios. These findings suggest that baseline peripheral immune composition may serve as a predictive marker for treatment-related adverse effects [23].

Serum Cytokines and Immune Mediators

An analysis of 15 cytokines in the serum of 22 patients before, during, and after treatment revealed no major changes in most markers. However, alterations in T cell-associated mediators specifically Perforin and soluble Fas (sFas) were noted during radiotherapy, potentially impairing immune cell function. In HPV-positive patients, increased levels of interleukin-4 (*IL-4*) and interleukin-10 (*IL-10*) were detected during treatment, reflecting a heightened pro-inflammatory state [24].

Monocyte Subsets, Adhesion Molecules, and Immune Checkpoints

A study involving 29 HNSCC patients treated with RT or CRT examined monocyte subpopulations and expression profiles of adhesion molecules and immune checkpoints [25]. No significant changes in total monocyte counts were observed post-treatment. However, classical and non-classical monocytes exhibited significantly elevated pre-treatment expressions of chemokine *CX3CR1* – which plays roles in leukocyte adhesion, migration, inflammation, and immune surveillance - and CD11a, which decreased in response to therapy.

Chemokine *CXCL11* implicated in tumor-lymphatic interactions and (programed death-ligand 1 (*PD-L1*) regulation also showed dynamic changes: RT increased *CXCL11* levels in some patients, while CRT generally reduced its expression. A significant positive correlation between plasma *CXCL11* and *PD-L1* expression on non-classical monocytes was observed post-treatment, suggesting a possible immune evasion mechanism and a potential therapeutic target [24, 26, 27].

Temporal changes in immune checkpoint molecule (ICM) expression were primarily associated with RT [28]. *PD-1* expression increased in $CD4^+$, $CD19^+$, and $CD4^+/CD39^+$ cells during therapy, but declined in $CD8^+$ and $CD4^+/CD39^+$ T cells at 3–6 months post-RT. B- and T-lymphocyte attenuator (BTLA) expression decreased progressively in T cells but remained high in B cells. $CD27^+$ cell populations declined in $CD4^+$, $CD4^+/CD39^+$ T cells, and B cells post-RT and stayed below baseline. Non-significant trends toward increased expression of co-stimulatory markers such as CD137, CD134, and glucocorticoid-induced TNFR-related protein (GITR) were also observed.

Table 2 summarizes the main findings of the studies included in this systematic review.

Discussion

Radiotherapy, with or without systemic treatment, has been shown across various tumor types to significantly influence the immune system, both locally and systemically. This systematic review aimed to consolidate recent evidence regarding peripheral immune alterations in patients with HNSCC, providing a more analytical perspective on patterns, divergences, and clinical relevance.

One of the most consistent findings is RT-associated lymphopenia, especially under conventional fractionation schedules, with or without concomitant chemotherapy [29-35]. Although expected, its depth, duration, and prognostic implications vary, likely influenced by factors such as radiation volume, dose intensity, and the addition of systemic agents.

CD8+ T cell responses appear highly context dependent. For example, in patients with oligometastatic prostate cancer undergoing stereotactic ablative radiotherapy (SART), no overall difference in CD8+ T cell frequency was observed pre- and post-treatment (36). However, subpopulation analysis revealed that higher proportions of central memory T cells (TCM) were associated with poor prognosis, while increased tumor-reactive T cells (TTR) correlated with better outcomes [36]. Additionally, SART has been linked to increased pro-inflammatory T cell profiles [37-39], which may reflect immunogenic modulation and local abscopal effects.

In nasopharyngeal carcinoma, RT was associated with an increase in CD8+ T cells compared to baseline, despite lower CD4+ and CD8+ levels relative to healthy controls [40]. Similarly, CRT in HNSCC patients led to a rise in CD8+ effector T cells, particularly when combined with immunotherapy [41], supporting the idea that immune activation may be potentiated by combination regimens. Treg cells were consistently more abundant in cancer patients compared to healthy individuals [9, 41].

However, their behavior in post-RT varies by modality. High-dose RT (e.g., SABR, intraoperative RT) generally did not alter Treg frequency [11, 12, 38, 39, 42, 43], while conventional fractionation RT significantly increased Treg proportions in HNSCC [9, 44, 45] and prostate cancer [46, 47]. Notably, this increase appeared to persist even after 3 years of follow-up, accompanied by higher expression

Table 2. Summary of Immunological Findings in HNSCC Patients undergoing (Chemo)Radiotherapy.

Study (Author, Ref)	Key Immunological Findings
Balázs et al. [9]	Progressive decline in $CD4^+$ T cells; sustained increase in circulating Tregs; increased $CD4^+$ T cell proliferation (Ki67); elevated CTLA-4 and PD-1 expression on $CD4^+$ Tregs and effector T cells; reduced CD39 on Tregs; no change in NK cells; markedly elevated dendritic cells ($CD123^+$, $CD11c^+$) at baseline, only modestly decreased post-RT; reduced total MDSCs with increased proportion of $CD14^+$ MDSCs in patients; significant decrease in B cells one month post-RT.
Beschel et al. [23]	Higher T, B, NK cells and lower monocytes in patients with high-grade toxicity; low monocyte:T cell ratio
Doescher et al. [19]	CD4+ T cells reduced; Treg cells increased (more with CRT); polyfunctional cytokine expression increased in T cells
Donaubauer et al. [16]	CRT decreased dendritic cells, MDSCs, B and T lymphocytes; increased HLA-DR and activation markers
Gehrmann et al. [21]	NK cells upregulated activation marker NKG2D before and after RT
Idel et al. [25]	No major monocyte count changes; pre-RT increase in CX3CR1 and CD11a; CXCL11 levels decreased post-CRT
Masterson et al. [20]	CD4+ and CD8+ reduced post-CRT (CD4+ > CD8+); HPV+ patients showed increased Treg
Mytilineos et al. [24]	Minimal overall cytokine change; Perforin and sFas altered during RT; higher inflammatory cytokines in HPV+ patients
Niu et al. [17]	B cells decreased, Treg increased during CRT; CD8+ and NKT increased; Th decreased; NK increased
Paya et al. [22]	CD44 expression significantly reduced after treatment
Turner et al. [18]	Similar T and B cell dynamics; lower CD4+/CD8+ and CD39+CD73+CD19+ B cells pre-treatment
von Witzleben et al [28]	Significant changes were noted for PD1, BTLA and CD27 on multiple immune cell types during or after radiotherapy.

HNSCC, head and neck squamous cell carcinoma. Ki67, nuclear antigen. CTLA-4,Cytotoxic T-lymphocyte antigen 4. PD-1,Programmed cell death protein 1. Tregs, Regulatory T lymphocytes. NK cells, natural killer cells. RT, radiotherapy. MDSCs, myeloid-derived suppressor cells. CRT, chemoradiotherapy. HLA-DR, Human leukocyte antigen – DR isotype. HPV, Human papillomavirus. sFas, – Soluble Fas (CD95). Th,T helper cells. NKT, Natural killer T cells. BTLA, B and T lymphocyte attenuator.

of functional markers (44). Conversely, a reduction in Treg cells was observed in protocols involving induction chemotherapy [48], and this decrease was paradoxically associated with worse prognosis in some studies [49].

B lymphocytes tended to decrease following RT, both with conventional schedules [30, 31, 40] and with SABR [37]. In HNSCC, persistent B cell suppression post-treatment correlated with disease relapse [18], suggesting a possible role as a prognostic biomarker. Dendritic cells, although more prevalent in cancer patients overall [9, 41], did not exhibit consistent variation following RT, indicating relative stability or methodological limitations in detection.

The behavior of NK cells varied notably across studies. In HNSCC patients, RT was associated with increased NK cell proportions [9, 18, 40], a trend also seen in high-dose RT for breast cancer [37-43]. However, reduced NK levels were linked to tumor recurrence in both HNSCC [18] and breast cancer [50], while other studies reported NK depletion in seminoma [28], high-grade gliomas [30], and esophageal cancer [51]. These discrepancies may result from differences in timing of sampling (immediate vs delayed), immune status at baseline, tumor biology, and NK cell subset distribution.

Importantly, a recent study highlighted the prognostic role of immune cell subsets and humoral markers during CRT in HNSCC patients [51]. Elevated T cell levels (CD3+, CD4+, CD8+) before or after treatment were associated with better outcomes, while higher baseline NK cell percentages predicted poorer prognosis.

Similarly, elevated complement factors (C3, C4) correlated with favorable prognosis, whereas higher pre-treatment levels of IgA, IgE, IgG, and IgM were linked to unfavorable outcomes [51]. These findings reinforce the value of a comprehensive immunological assessment, integrating both cellular and humoral markers.

Peripheral blood biomarkers such as baseline CD4/CD8 ratio, B cell levels, and checkpoint expression (e.g., *PD-1*, *CTLA-4*) have potential prognostic value [52]. Persistent post-treatment B cell suppression or elevated Tregs may indicate risk for recurrence. These profiles could help guide RT fractionation intensity, indicate suitability for immunotherapy, or prompt earlier combination strategies. Additionally, immune status may assist in predicting treatment-related toxicities, supporting individualized supportive care interventions.

Recent evidence reinforces the relevance of immune modulation in HNSCC. A pivotal phase 3 trial evaluating neoadjuvant and adjuvant pembrolizumab in patients with locally advanced, resectable disease demonstrated a significant improvement in event-free survival compared to standard therapy alone [53]. The neoadjuvant use of immunotherapy with an anti–*PD-1* drug, followed by postoperative radiotherapy with or without concurrent systemic therapy and maintenance immunotherapy, was associated with sustained anti-tumor immune activity. Peripheral blood analyses revealed dynamic changes in immune cell populations, including increased T cell activation and reduction in immunosuppressive myeloid populations during treatment. These findings reinforce the concept that immune checkpoint blockade exerts systemic immunomodulatory effects and may mitigate the negative immune consequences of both tumor-induced dysfunction and treatment-related immunosuppression.

Despite offering a structured overview of current evidence, this review presents several limitations that must be acknowledged. A particularly relevant confounding factor is the concomitant use of CT, which is present in a significant proportion of the included studies. CT is known to have its own immunosuppressive and immunomodulatory effects, making it difficult to attribute observed immune alterations only to RT. Importantly, CRT remains the standard of care for most patients with locally advanced HNSCC, and evaluating the isolated impact of radiotherapy is challenging in clinical practice. This therapeutic overlap limits the possibility of distinguishing the specific contributions of each modality to immune changes. Additionally, many studies lacked functional analysis of immune cells, focusing solely on surface markers, which restricts conclusions about the actual immune competence of the identified subsets.

Another limitation is the relative scarcity of high-quality longitudinal studies. Most included research consisted of small, single-center cohorts without long-term immune follow-up or clinical outcome correlation, which hinders robust conclusions regarding prognostic value. Finally, potential publication bias must be considered, as studies with positive or significant immunological findings may be more likely to be reported and published.

The present review highlights a significant gap in literature: while immune modulation by radiotherapy is suggested, systematic investigations into peripheral immune changes in patients with head and neck cancer remain scarce. To our knowledge, this is one of the few systematic reviews that specifically addresses dynamic changes in circulating immune cells and soluble immune markers in this patient population. Given the increasing relevance of immunotherapy and the known interplay between radiotherapy and immune activation or suppression, this topic holds growing translational value [54]. Our findings suggest that monitoring peripheral blood may offer a feasible and non-invasive method to assess treatment response, stratify patient risk, and eventually tailor combined treatment strategies. Furthermore, by consolidating scattered evidence, this review provides a foundation for future prospective studies with standardized methodologies. It also offers guidance on which immune components may serve as candidate biomarkers for clinical trials involving immune checkpoint inhibitors or adaptive immunotherapy protocols in HNSCC.

In conclusion, this systematic review demonstrates that radiotherapy, either alone or combined with chemotherapy, induces significant alterations in the peripheral immune landscape of patients with head and neck squamous cell carcinoma. Observed changes in immune cell subpopulations, humoral immune markers show meaningful associations with clinical outcomes including treatment response, recurrence, and therapy-related toxicity. Given the current evidence, it remains challenging to delineate the specific immunomodulatory effects of radiotherapy alone, as the standard-of-care

treatment frequently involves concurrent chemotherapy. Immunophenotypic profiling and quantification of peripheral immune biomarkers emerge as promising tools for prognostic stratification and therapeutic personalization in HNSCC.

Future clinical trials with larger cohorts, standardized methodologies, and longitudinal immune monitoring are essential to validate these findings and facilitate the clinical integration of immune surveillance into the multidisciplinary management of head and neck cancer.

Author Contribution Statement

All authors contributed to the study conception and design. Data collection and analysis were performed by DAVF, DGM, JMPF, ARZ, CFV, EPC, HYL, LGR and RMAN. The first draft of the manuscript was written by DAVF and MMRC wrote some of the content and revised the paper. MMRC and MHFOS approved the final version and contributed to the conception of the paper. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Scientific body approved/part of thesis

This manuscript is not part of an approved student thesis, nor has it been formally endorsed by any institutional scientific body. However, it was developed independently by the authors following rigorous scientific methodology and ethical standards.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Registration dataset

The review protocol was included in the database Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42021283028).

Competing interests

The authors declare that they have no competing interests.

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