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Histological tumor necrosis predicts decreased survival after neoadjuvant chemotherapy in head and neck squamous cell carcinoma

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ABSTRACT

Objective: Despite growing interest in neoadjuvant therapies, there are no methods to predict radio- (RT) or chemoradiotherapy (CRT) response in head and neck squamous cell carcinoma (HNSCC). The aim of this research was to study the effect of neoadjuvant RT or CRT on the tumor immune landscape and patient survival in HNSCC.

Methods: All HNSCC patients treated with neoadjuvant RT or CRT (n = 53) were identified from a retrospective cohort of 1033 patients. Pre- and post-neoadjuvant cancer samples from the same patient were analyzed with biomarkers related to cancer immunology: tumor-infiltrating lymphocytes (CD8), tumor-associated macrophages (CD68, CD206, Clever-1), immune response regulator (PD-L1) and histologic tumor necrosis. Outcomes of interest were individual immune landscape profiling and its impact on 5-year overall survival (OS) in HNSCC patients treated with neoadjuvant RT/CRT.

Results: Results from 588 whole-section stainings revealed multiple statistically significant alterations in immune landscape in response to RT/CRT. Pretreatment tumor necrosis was the most useful biomarker in predicting poor outcome, as the OS was 14.3% with necrosis and 48.5% without necrosis (HR 2.87; 95% CI: 1.23 to 6.66, p=0.014). In addition, an artificial intelligence-based (AI) deep learning method for identifying tumor necrosis from histopathological specimens was successfully developed. The predictive role of histological necrosis in neoadjuvant RT/CRT was validated in additional samples from 171 HNSCC patients untreated with neoadjuvant therapy.

Conclusions: Detection of tumor necrosis and AI-driven deep learning effectively predict neoadjuvant RT/CRT responses in HNSCC.

Introduction

Head and neck cancers are the seventh most common cancer group worldwide [1]. By far, the most common histological type is squamous cell carcinoma (HNSCC), accounting for 90 % of all head and neck cancers. Despite available modern diagnostic and treatment methods, HNSCC-associated mortality is still approximately 50 % [2]. Particularly challenging in terms of choosing the most optimal cancer treatment options are HNSCCs that already contain local or distant metastases. A promising approach for managing locally advanced HNSCC is the possible use of neoadjuvant therapies before planned surgery [3–8] However, the lack of reliable and easy-to-use predictive biomarkers remains a major obstacle to improving patient survival and integrating neoadjuvant therapies into clinical practice, despite the recent identification of a promising prognostic biomarker for HNSCC [9,10]. In addition, very poorly is understood how neoadjuvant treatment, including RT and CRT, changes the tumor microenvironment, immune landscape and how these changes affect the survival of HNSCC patients.

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Recent studies have demonstrated the importance of tumor microenvironment and in particular different immune cells such as tumor-infiltrating lymphocytes (TILs) in predicting the aggressiveness and treatment response of HNSCC [11]. Most TILs are known to be CD8-expressing cytotoxic T cells, and thus, CD8 is widely accepted as a specific marker for TILs [12]. Tumor-associated macrophages (TAMs) are also known to influence the cancer immune landscape, cancer aggressiveness and patient survival in HNSCC [13–15]. Furthermore, in a recent study, TAMs were reported to reduce the radiosensitivity of HNSCC, supporting the importance of analyzing RT-induced TIL and TAM changes in HNSCC [16,17]. While CD68 is expressed by all macrophages, of particular interest in cancer research are TAMs expressing CD206, CD163, and Clever-1 due to their immunosuppressive and tumor-promoting roles [18,19]. Interestingly, a widely used biomarker for predicting immunotherapy response, PD-L1 (programmed death-ligand 1), is detected not only in cancer cells but also in TILs and TAMs, indicating the need to better understand the role of these immune cells in predicting treatment responses in HNSCC [20]. In addition to cancer immune cells, the immunogenicity caused by dying cancer cells has recently been the subject of growing research interest, and the new term 'immunogenic cell death' has been introduced [21,22]. Immunogenic cell death can involve diverse molecular pathways, and morphologically it often manifests as tumor necrosis [21]. Although tumor necrosis is one of the most fundamental histological findings in cancer, the prognostic or predictive role of necrosis as part of the immune landscape in HNSCC has not been thoroughly investigated so far.

In this study, to investigate the effect of neoadjuvant RT/CRT on the immunological microenvironment, all patients who received neoadjuvant RT or CRT were identified from a retrospective population-based cohort of 1033 newly diagnosed HNSCC [23–25]. Thereafter, our aim was to analyze the occurrence and possible changes in expression levels of CD8, CD68, CD206, Clever-1, PD-L1 and histological tumor necrosis in HNSCC samples before and after RT/CRT and also combine these findings with patient-specific follow-up data. In addition, the possibilities of developing novel AI-based deep learning method in the best performing predictive model were investigated. The overall aim of this study was to investigate at the individual level how tumor immune landscape is altered by neoadjuvant RT/CRT and whether immune landscape profiling or alteration have a prognostic or predictive significance in HNSCC.

Patients and methods

Data collection

Study cohort was formed by detecting all patients diagnosed with new HNSCC in Southwest Finland area between 2005 and 2015 (n = 1033 patients) [10,23,24]. Patients who received neoadjuvant RT/CRT and had available tumor samples both before and less than six months after RT/CRT were identified and included to the study (Fig. 1). Complete clinical data before neoadjuvant RT/CRT was collected and is shown in Table 1. The treatment protocols were decided by a Multidisciplinary Tumor Board for head and neck cancer and tumor staging was re-evaluated based on the eighth edition of the UICC/ AJCC TNM classification. A total of 53 HNSCC patients met inclusion criteria for the study aiming to investigate the effects of oncological neoadjuvant treatments on the cancer immune landscape at the individual level. A validation cohort, of representative primary tumor samples from HNSCC patients who had received either surgery alone (n = 49) or definitive RT/CRT (n = 122), was also identified from the 1033 HNSCC cohort described above.

Tumor specimens and histology review

The histological assessment was performed retrospectively using diagnostic FFPE tumor samples from each patient. The diagnostic tumor samples from baseline HNSCC and tumor samples within six months after RT/CRT were evaluated. 3.5–5-µm thick whole tissue sections were

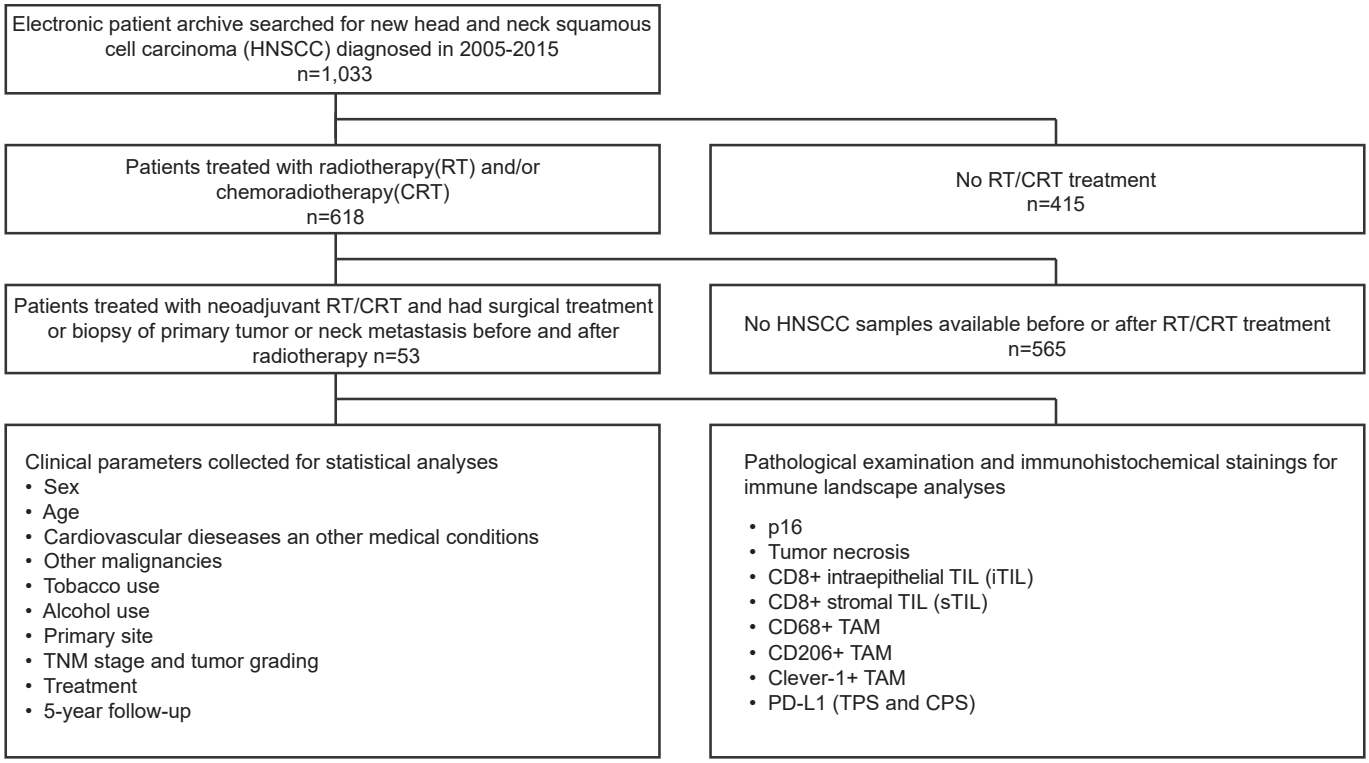

Fig. 1. The study scheme for retrospective neoadjuvant RT/CRT HNSCC patient selection. Electronic database screen was performed to include all patients diagnosed and treated for new HNSCC in Southwest Finland region during years 2005–2015. Patients who received RT/CRT treatment and had paired cancer samples from the tumor before and after (within six months of treatment) oncological treatment (RT/CRT) were selected for further analyses.

Table 1
Clinical data of HNSCC patients (n = 53) before neoadjuvant RT/CRT.

	Total	
	n	%
Sex		
Male	37	70
Female	16	30
Age at diagnosis		
< 65	31	58
> 65	22	42
Smoking status		
Current smoker	26	49
Former smoker	7	13
Non-smoker	19	36
Alcohol consumption		
Yes	22	42
No	31	58
Primary tumor site		
Oral cavity	31	58
Oropharynx	11	21
p16 positive	6	
p16 negative	5	
Larynx	5	10
Other	6	11
T Class		
T1-2	21	40
T3-4	32	60
N class		
N0	10	19
N+	43	81
Stage		
I-II	6	11
III-IV	47	89
Recidive in 5 years		
Yes	25	47
No	28	53
Living at 5 years		
Yes	16	30
No, died of HNSCC	31	58
No, died of other cause	6	11
Surgical treatment		
No curative surgery	4	8
Local operation	40	75
Neck dissection	43	81
Treatment type		
RT only	2	4
CRT only	2	4
RT + surgery	9	17
CRT + surgery	40	75

cut and stained with hematoxylin and eosin (H&E) using Ventana HE 600 (Roche Diagnostics, Basel, Switzerland). Evaluation of all tumor samples was conducted independently by two pathologists (ARK, PV) blinded for the patient outcome. Discrepancies were resolved by consensus. The presence or absence of histologic coagulative necrosis was analyzed according to established histologic criteria [26]. Tumor necrosis was defined as the presence of microscopic coagulative necrosis, characterized by degenerating and dead cells. The proportion of necrosis in the tumor was not evaluated but any amount of necrosis was sufficient to score the necrosis present. As RT/CRT affects tumor histology in many ways, histologic tumor necrosis was not analyzed on posttreatment samples. Histological images used in the development of the AI-based necrosis detection program were uploaded to the Aiforia Create deep learning cloud-based platform (Aiforia Technologies, Helsinki, Finland), a commercially available platform specifically designed for histologic images.

Immunohistochemistry

Representative pre- and post-RT/CRT FFPE tissue blocks from the same HNSCC patients were selected for immunohistochemical staining and for further evaluation of immune landscape. Serial whole sections were immunohistochemically stained for CD8, CD68, CD206, Clever-1, PD-L1 and p16 using antibodies and dilutions presented in Supplemental Table 1. Staining was performed using The BenchMark ULTRA (Ventana Medical Systems, Tucson, AZ) and proper control tissues (tonsil, placenta and ovarian adenocarcinoma) were used. The staining was assessed semi-quantitatively by two pathologists independently (ARK and PV). Discrepancies were resolved by consensus.

Immunohistochemistry scoring

CD8 staining was assessed separately for intraepithelial tumor infiltrating lymphocytes (iTIL) and stromal tumor infiltrating lymphocytes (sTIL) presenting in stroma within ½ field of 20X view from the tumor cell islands. CD68, CD206 and Clever-1 expressing macrophages were scored by the average amount of positive cells close to the tumor or regressed tumor areas. We performed a semi-quantitative scoring of TILs and TAMs by measuring the density of CD8 +, CD68 +, CD206 + and Clever-1 + cells in each sample as follows: no or sporadic positive cells (pattern 0), low number of positive cells (pattern 1); high number of positive cells (pattern 2). In final analyses staining patterns, 0 and 1 were considered as low expression and pattern 2 as high expression in multivariable analysis for all the above-mentioned markers. PD-L1 staining was assessed for tumor proportion score (TPS) and combined positive score (CPS) according to the FDA-approved method [27]. Cut-offs TPS 0–9 %, 10–49 %, ≥ 50 % and CPS < 1, 1–19 and ≥ 20 were used. In multivariable analysis CPS cut-offs < 20 and ≥ 20 were used due to low number of samples with CPS < 1. p16 was used as a surrogate marker for HPV-association and was scored according to the WHO guidelines [28].

Statistical analyses

Patient characteristics, histopathological variables, and results from immunohistochemical analyses were entered into the Statistical Package for Social Sciences (SPSS, version 25.0, IBM, Armonk, USA). For all statistical analyses, the significance level was set at 0.05. For evaluation of changes in the immune landscape, a Wilcoxon signed rank test was used. Z values and p values are reported. For survival analysis, 5-year overall survival (OS) was analyzed using Cox proportional hazards method. For all multivariable analyses, based on our previous prognostic model, the patient's age, high T class, nodal positivity, and alcohol history were entered as covariates [25]. Results of the regression analyses were used to plot survival curves for individual biomarkers. Hazard ratios (HR) with 95 % CI and p value are reported.

Results

Patient characteristics

The overall goal of this research was to study the effect of neoadjuvant RT/CRT on the immunologic microenvironment of HNSCC by comparing representative sample pairs from the same patient before and after RT/CRT. Patients meeting inclusion criteria were identified from a robustly validated retrospective and population-based patient cohort including all newly diagnosed HNSCC (n = 1033) from a population of 697,000 people [23–25,29]. Among patients treated with RT/CRT (n = 618), all cases with neoadjuvant therapy and cancer samples available before and after RT/CRT were identified for further analysis (n = 53, Fig. 1). The main reasons for study exclusion were the absence of neoadjuvant RT/CRT treatment or lack of representative pre- or posttreatment cancer samples. The primary tumor site in the neoadjuvant RT/CRT cohort from most to least common was the oral cavity (31; 58 %), the oropharynx (11; 21 %, p16-positive n = 6 and p16-negative n = 5), the larynx (5; 10 %) and other (6; 11 %, Table 1). Most patients had advanced HNSCC disease with one or more neck metastases (n = 43; 81 %) and were therefore classified as stage III-IV disease (n = 47; 89 %). Of

the 53 patients, 42 were treated with CRT, and 11 patients received RT. Almost half of the patients (n = 25) had one or more recurrent tumors during the five-year follow-up. After five years, 16 (30 %) patients were alive; 31 (58 %) had died of HNSCC and 6 (11 %) of other causes. In conclusion, the selection of patients for the study cohort was successful in terms of investigating immune landscape alterations in HNSCC patients receiving curative intended neoadjuvant RT/CRT treatment.

Immune landscape characteristics in pre and post RT/CRT HNSCC specimens

For a reliable perspective on how RT/CRT influences individual HNSCC tumor immune properties and how the immune landscape reflects RT/CRT outcome, all analyses were conducted using whole-tissue sections. A set of five immunologic markers (CD8, CD68, CD206, Clever-1, PD-L1) were selected for immunohistochemical analysis (Supplemental Table 1 and Supplemental Fig. 1 and 2). CD8 expression was scored for both intraepithelial (iTIL) and stromal (sTIL) lymphocytes. Samples that were not available or the staining was not reliably successful were not included in the analyses. The most characteristic immune landscape profile in the pretreatment samples was low CD8 + iTILs (77 % n = 36/47), high CD8 + sTILs (61 % n = 28/46) and low CD206 + TAMs (79 % n = 37/47, Table 2). PD-L1 TPS ≥ 10 % was found in 61 % of patients (n = 28/46) and TPS ≥ 50 % in 24 % (n = 11/46). PD-L1 expression as CPS was ≥ 20 in 59 % of patients (n = 27/46). Histological tumor necrosis (scored as present or absent) existed in 32 % (n = 15/47) and was absent in 68 % (n = 32/47) of the pre-RT/CRT tumors. Among posttreatment samples, most of the tumors had low CD8 + sTILs (86 % n = 44/51), low CD206 + TAMs (62 % n = 31/50) and low Clever1 + TAMs (72 % n = 36/50). The distribution of the tumor properties examined is presented in Table 2.

Immune landscape alterations and association with survival after neoadjuvant RT/CRT

Neoadjuvant RT/CRT induced several quantifiable changes in the HNSCC immune landscape, when specimens before and after RT/CRT were examined. Expression of immunological markers in the pre- and posttreatment samples of the same patient was compared pairwise and determined as decreased, unvaried or increased in response to RT/CRT (Fig. 2A). The pairwise comparison of immune landscape (sTILs, CD68, CD206 and Clever-1) was statistically applicable for 46 patients (Fig. 2B). Changes in CD8 + iTILs and PD-L1 expression could not be reliably statistically analyzed, as tumor cells were in many cases (n = 21/51; 41 %) favorably eliminated by RT/CRT in posttreatment samples. Observed changes were surprisingly similar regardless of primary tumor locations and HPV status (Fig. 2B). In most cases, the presence of CD8 + TILs in the tumor stroma decreased (52 % of cases) in response to treatment, yielding a statistically significant result. For TAMs, the amount of CD68 was statistically unchanged, but significant decrease in Clever-1 expression (36 % of cases) and increase in CD206 expression (36 % of cases) in response to RT/CRT was observed. Surprisingly, although detectable and statistically significant changes in the immune landscape after neoadjuvant RT/CRT were observed, none of these changes were associated with OS (data not shown).

Immune landscape characteristics and association with survival before and after neoadjuvant RT/CRT

To further analyze the potential association of TILs, TAMs and PD-L1 with prognosis in neoadjuvant RT/CRT-treated patients, the expression levels of each immune marker was studied independently in pre- and posttreatment samples. High amount of CD8 + sTILs in baseline HNSCC tumors (61 % of samples) was significantly associated with improved prognosis in multivariable survival analysis (Table 2 and Fig. 2C). In addition, high amount of CD206 + TAMs in posttreatment samples (38 % of samples) was significantly associated with worse survival in multivariable survival analysis (Table 2 and Fig. 2D). Interestingly, for patients with residual tumors after RT/CRT, high PD-L1 expression in the tumor cells (TPS ≥ 10 % or ≥ 50 %) of post-RT/CRT samples (60 % and 32 % of samples respectively) was associated with better survival (Table 2).

Histologic tumor necrosis predicted poor outcome in HNSCC treated with neoadjuvant RT/CRT

In addition to immunohistochemical analyses, predictive power of histologic tumor necrosis and its putative association with TILs, TAMs and PD-L1 in baseline tumor samples were studied. The histologic pattern of necrosis often presented in small foci inside tumor cell islands

Table 2
Distribution of immune cells, PD-L1 and histologic tumor necrosis, and their significance in multivariable analysis, in the HNSCC tissue samples before and after neoadjuvant RT/CRT.

		PRE				POST			
		N	**%**	**HR**	**P**	**N**	**%**	**HR**	**P**
CD8 + iTIL	Low	36	77 %	1	–	24	100 %	1	–
	High	11	23 %	0.74 (0.28 to 1.90)	0.53	0	0 %	0.64 (0.23 to 1.75)	0.39
CD8 + sTIL	Low	18	39 %	1	–	44	86 %	1	–
	High	28	61 %	**0.35 (0.16 to 0.79)**	**0.011**	7	14 %	0.31 (0.07 to 1.35)	0.12
CD68 + TAM	Low	26	55 %	1	–	24	48 %	1	–
	High	21	45 %	0.70 (0.32 to 1.53)	0.38	26	52 %	0.92 (0.43 to 1.94)	0.83
CD206 + TAM	Low	37	79 %	1	–	31	62 %	1	–
	High	10	21 %	0.91 (0.35 to 2.37)	0.86	19	38 %	**2.63 (1.22 to 5.65)**	**0.013**
Clever-1 + TAM	Low	22	48 %	1	–	36	72 %	1	–
	High	24	52 %	0.73 (0.32 to 1.63)	0.45	14	28 %	0.95 (0.36 to 2.49)	0.92
PD-L1 TPS	<10 %	18	39 %	1	–	10	40 %	1	–
	10–49 %	17	37 %	1.25 (0.51 to 3.07)	0.62	7	28 %	0.36 (0.11 to 1.16)	0.089
	≥50 %	11	24 %	1.66 (0.59 to 4.60)	0.33	8	32 %	0.36 (0.10 to 1.22)	0.10
	Low (<10 %)	18	39 %	1	–	10	40 %	1	–
	High (≥10 %)	28	61 %	1.39 (0.62 to 3.09)	0.42	15	60 %	**0.35 (0.12 to 0.97)**	**0.046**
	Low (<50 %)	35	76 %	1	–	17	68 %	1	–
	High (≥50 %)	11	24 %	1.49 (0.59 to 3.78)	0.39	8	32 %	**0.37 (0.14 to 0.94)**	**0.038**
PD-L1 CPS	Low (<20)	19	41 %	1	–	10	40 %	1	–
	High (≥20)	27	59 %	0.94 (0.42 to 2.08)	0.89	15	60 %	0.51 (0.20 to 1.30)	0.159
Tumor necrosis	Absent	32	68 %	1	–			NA	
	Present	15	32 %	**2.87 (1.23 to 6.66)**	**0.014**			NA	

For multivariable analysis, Hazard ratios (HR) with 95 % CI and p value are reported. The results yielding statistical significance (p < 0.05) are in bold.

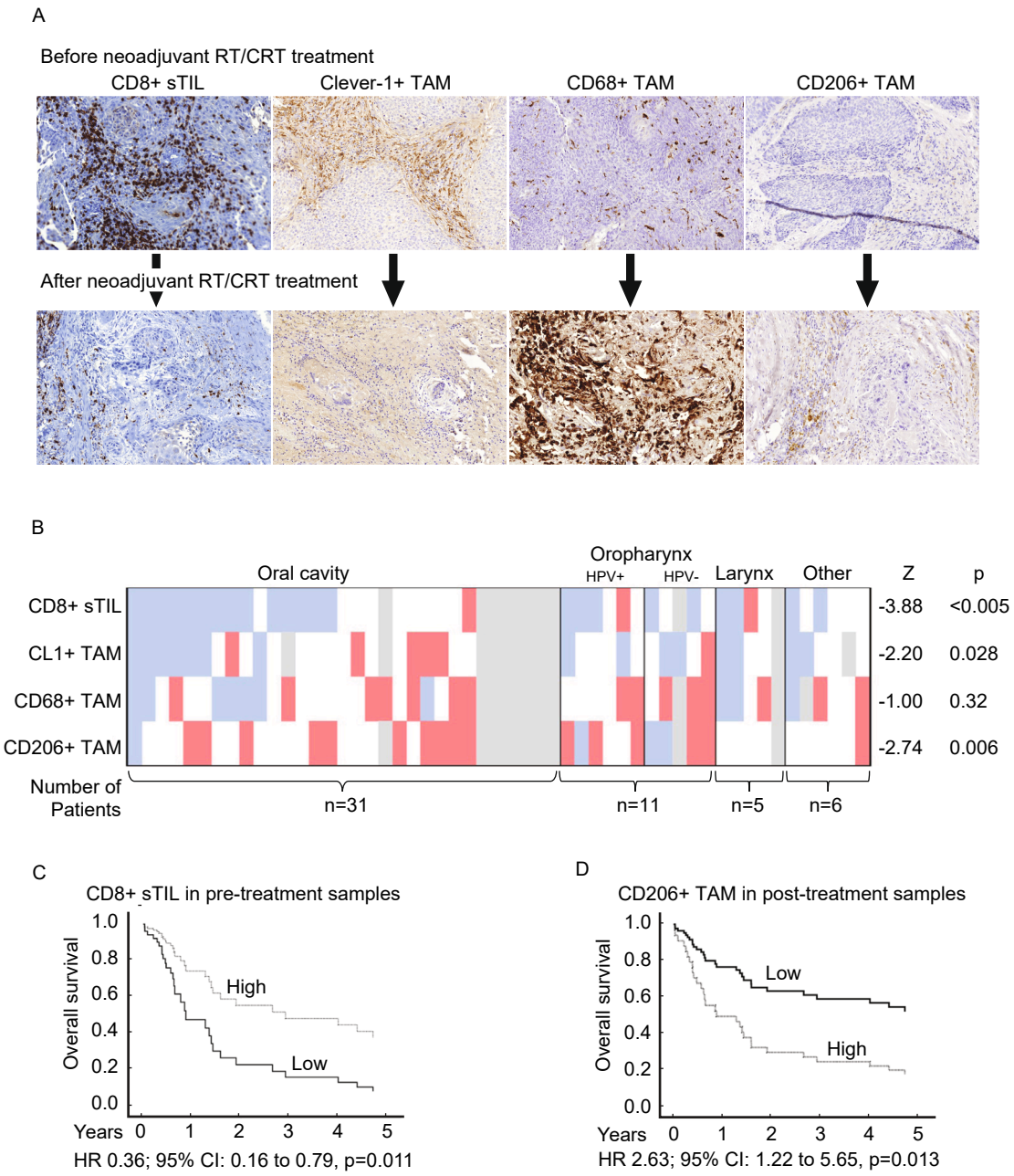

Fig. 2. Immune landscape characteristics and association with survival before and after neoadjuvant RT/CRT. A: Representative samples of pretreatment (upper row) and post-treatment (lower row) sample pairs. (All images 20x). B: Heatmap of the immune landscape alterations for each patient (n = 53). Blue, expression decreased by RT/CRT; Red, expression increased by RT/CRT. C: Association of pretreatment CD8 + sTILs with 5-year OS. D: Association of posttreatment CD206 + TAMs with 5-year OS. The significance of the changes was evaluated using the Wilcoxon signed rank test.

rather than as large and extensive areas. Surprisingly, the presence of tumor necrosis in pre-neoadjuvant HNSCC samples was a markedly strong predictor of worse treatment response, with a 5-year OS of 14.3 % with necrosis and 48.5 % without necrosis (Table 2 and Fig. 3A–C). However, tumor necrosis was not associated with clinical prognostic variables, nor with TILs, TAMs or PD-L1 expression (data not shown). Relatively straightforward detection of histological necrosis from H&E-stained sections raised the question of the applicability of histological necrosis in an automated AI-based deep learning. For this, a total of 20 HNSCC H&E stained slides with or without necrotic areas were uploaded to a commercial deep learning cloud-based platform (Fig. 3D). After AI-training, −testing and −validation, the detection of necrotic areas in H&E-stained samples proved to be almost as efficient and reliable as AI-based detection of tissue outlines alone; for tissue and necrosis, the prevalence of false positive regions was 0.02 % and 0.32 %, respectively, while the detection rate of false negative regions was 0.07 % and 0.18 %. In addition, to investigate the predictive utility of histological tumor necrosis in non-neoadjuvant patients, we collected primary specimens from three different HNSCC cancer cohorts for histological analyses. The HNSCC groups in which histological necrosis grading was performed were oral cavity SCC patients who underwent primary surgery alone (n = 49, Supplemental Table 2), patients who underwent definitive RT/CRT for OPSCC (n = 51 Supplemental Table 3) or oral cavity SCC (n = 71, Supplemental Table 4). However, tumor necrosis was not associated with reduced survival in any of these primary cancer groups (data not shown).

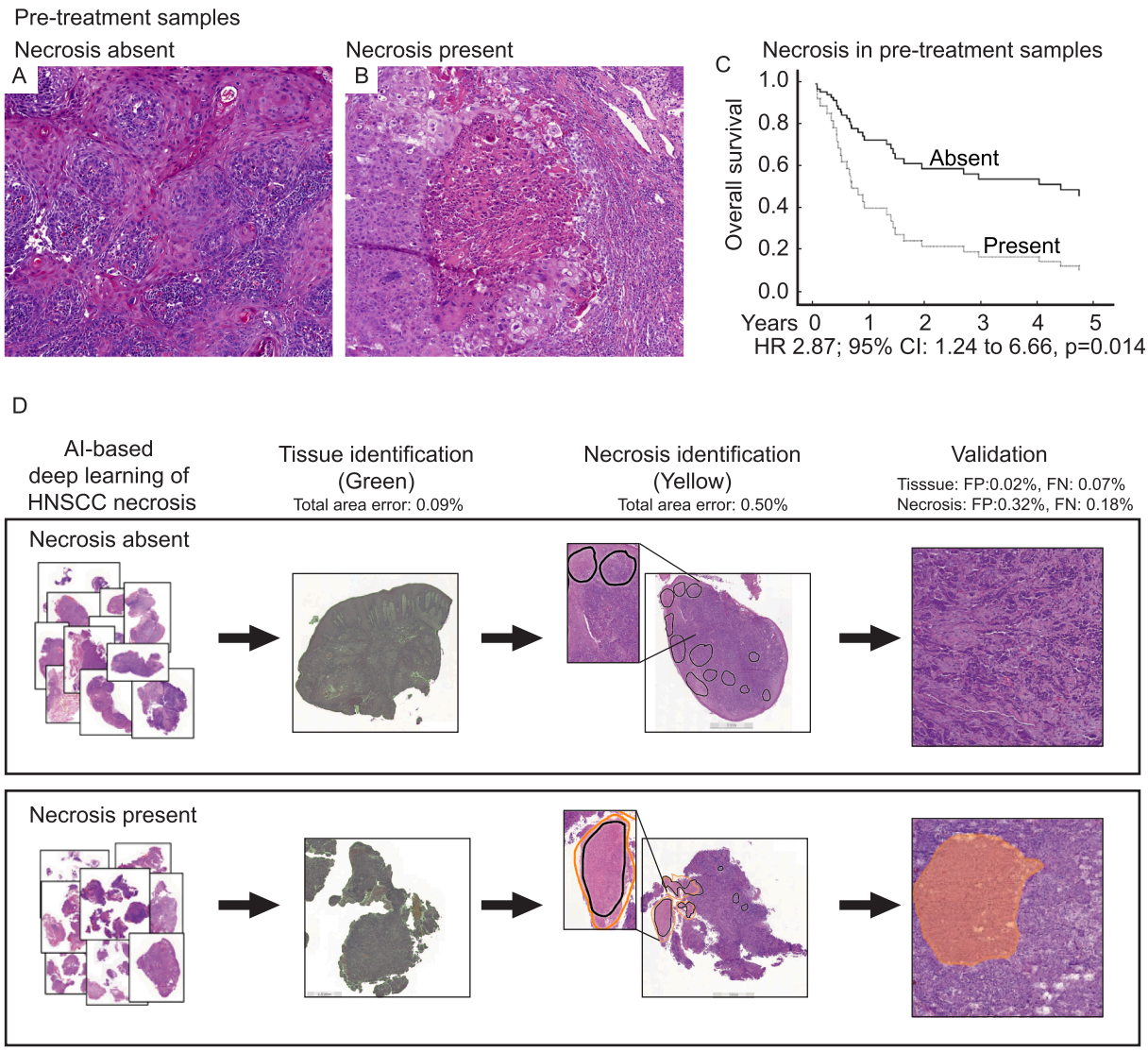

Fig. 3. Association of tumor necrosis with survival. A–C: Representative pretreatment samples with histologic necrosis absent (A), necrosis present (B), and association of necrosis with 5-year OS (C). (Histologic images 10x). D: Cohort digitization and algorithm development for deep learning and artificial intelligence-based histological necrosis detection. A total of 20 digitized H&E HNSCC samples were uploaded to the Aiforia Create deep learning cloud-based platform (Aiforia Technologies, Helsinki, Finland). In the first step, the identification of the tissue boundaries of the histological samples (green color) was successfully implemented (total area error: 0.09%). In the next step, necrosis was identified and marked on the histological sections by pathologist as either background tissue with no necrosis (black circles) and true necrosis (black circles with yellow color; total area error: 0.50%). At the end, AI functionality was tested and validated with 20 histological samples with or without histological necrosis. The occurrence of necrosis was visualized by Aiforia Create software in yellow color and specimens without detectable necrosis showed no yellow color at all. In the final results, for tissue and necrosis, the prevalence of false positive regions was 0.02% and 0.32%, while the detection rate of false negative regions was 0.07% and 0.18%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Discussion

The aim of this study was to clarify how neoadjuvant RT/CRT influence tumor immune landscape and how these changes affect HNSCC survival and treatment response. Evaluation of the immune landscape in HNSCC is a particularly topical issue, but the challenge has been to identify the most important immunological phenomena in terms of prognosis and treatment response [30–32]. To our knowledge, this is the most extensive study cohort in which the head and neck cancer immune landscape has been evaluated in whole sections of both baseline tumors and samples of the same patients collected shortly after neoadjuvant RT/CRT. The study cohort represented patients with advanced HNSCC who were still under curative treatment at the time of diagnosis but had a 5-year survival rate of only 30 %. Hence, this cohort represents patients with HNSCC for whom effective novel methods are needed to implement targeted cancer therapies.

The main finding of this work was that high amount of CD8 + sTILs and, especially, the absence of necrosis in pretreatment cancer samples significantly predicted a better response to RT/CRT with better OS. In accordance, CD8 + cytotoxic T cells have been shown in previous studies to play an important role in HNSCC and to predict good overall survival in CRT-treated HNSCC patients [33–37]. Pretreatment PD-L1 expression did not predict OS in our study, nor have such results been obtained in previous reviews or *meta*-analyses [38,39]. An increased number of protumorigenic CD206 + TAMs in posttreatment samples was associated with poor survival; thus, it seems that in some cases, immune landscape is altered in an unfavorable way by RT/CRT. In conclusion, although the clinical effect and molecular mechanism of regulation are

unknown, these in-depth analyses of the changes in the immune landscape enhance our knowledge of the effect of RT/CRT on the HNSCC immune landscape and may provide new opportunities for designing more personalized cancer therapies in the future.

Our most surprising finding was that although several statistically significant immunological changes after RT/CRT treatment was observed, the strongest prognostic factor in neoadjuvant cohort was the presence of necrosis in baseline HNSCC tumors. The association between necrosis and reduced survival was not seen in the surgical or definitive RT/CRT groups, strongly suggesting that the presence of necrosis in HNSCC could be specifically used to identify patients who would benefit from neoadjuvant cancer surgery, such as TORS surgery [3]. The presence and impact of necrosis for decreased OS was superior to all tested immunologic markers independently of TNM classification, which is currently the most commonly used clinical parameter in HNSCC prognostication [40]. Evaluation of tumor necrosis for prognostic purposes is already in clinical use in a wide variety of cancers, such as sarcoma, renal cell carcinoma, malignant pleural mesothelioma and mucoepidermoid carcinoma of the head and neck [40–43]. The negative prognostic significance of histologic tumor necrosis is also very well characterized in several other cancers, such as lung, thyroid, colorectal, and bladder carcinomas [44–46]. For HNSCC, there are only a few publications evaluating histological tumor necrosis, and to date, the prognostic or predictive value of necrosis in HNSCC remains poorly characterized [47]. Although the prognostic or predictive significance of histologic tumor necrosis in HNSCC has been marginally studied, pretreatment necrosis detected in MRI or CT imaging has shown to have negative impact on CRT response in HNSCC [48,49].

AI-based deep learning digital pathology is expected to bring new updates to routine pathology reporting. However, the challenge has been the wide range of AI platforms and the challenges of clinical validation of AI-based histological analyses. Based on our results, AI-aided identification of histological tumor necrosis was very promising biomarker and method also in terms of predicting cancer treatment outcomes. In addition, in terms of future validations, the clear advantage in this study is that the automatic AI platform used is already clinically validated in colorectal carcinoma [50]. Based on these very promising results, a follow-up study has already been started, in which the functionality of the artificial intelligence-based necrosis detection developed in this work is tested in a larger prospective study.

The limitation of this study is the small number of patients who met the inclusion criteria in the neoadjuvant cohort and the heterogeneity of the primary tumor locations. However, it is noteworthy that the final number of patients was reached by examining the clinical and tumor sample data of more than a thousand HNSCC patients indicating that these types of patient-specific pre- and posttreatment sample pairs is particularly challenging to discover. Despite the limited number of patients, the strengths of this work are the thorough evaluation of the sample material and the use of whole sections instead of tissue microarrays, which allowed a more detailed and reliable analysis and also the development of AI-based deep learning methods. Furthermore, despite the limited number of patients and the heterogeneity of the primary tumor locations, the observed changes in the immune landscape were consistent and statistically significant also in multivariable analyses and the predictive role of histological necrosis in neoadjuvant RT/CRT group was validated in additional samples from 171 HNSCC patients untreated with neoadjuvant therapy.

Conclusion

Recent studies have shown that conventional oncological therapies can be utilized as neoadjuvant treatments for HNSCC; however, the absence of effective and reliable biomarkers limits the ability to predict treatment response [3]. Tumor necrosis in cancer is well known but clinical use of histologic necrosis in HNSCC is surprisingly poorly studied. Tumor necrosis is noted neither in the College of American Pathologists' Protocol for examination of HNSCC tumor specimens, the UICC Manual of Clinical Oncology nor the WHO Classification of Head and Neck Tumors as a predictive marker in HNSCC [51–53]. Based on our results, the superior predictability of necrosis in neoadjuvant cohort compared to other analyzed biomarkers and the possibility of using deep learning artificial intelligence in the identification of histological tumor necrosis increase the interest in a more thorough investigation of necrosis in larger HNSCC datasets. As evaluated using routine H&E-stained sections of FFPE tumor tissue, necrosis is accessible and easy to incorporate worldwide both into routine histopathological examinations and novel AI-based deep learning platforms.

CRediT authorship contribution statement

A.R. Koskenniemi: Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **T. Huusko:** Writing – original draft, Investigation, Data curation, Conceptualization. **J. Routila:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Jalkanen:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **M. Hollmén:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **P. Vainio:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Ventelä:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All authors contributed to the study conception and design. ARK, PV, JR, TH and SV performed study concept and design and performed development of methodology, original drafting, review, and revision of the paper; SV provided acquisition of data; ARK, PV, JR, and SV provided analysis and interpretation of data; JR performed statistical analysis of data; SJ and MH participated in the design of the study and commented on the results and its interpretation; ARK, JR and SV obtained funding supports; SV and PV supervised the study. ARK had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The first draft of the manuscript was written by ARK and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

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