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Circulating T Cell Activation and Exhaustion Markers Are Associated With Radiation Pneumonitis and Poor Survival in Non-Small-Cell Lung Cancer

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Introduction: Persistent inflammation and immune activation in the lungs are associated with adverse outcomes such as radiation pneumonitis (RP) and poor survival in non-small-cell lung cancer (NSCLC) patients. However, it is unknown how this is reflected by leukocyte activation markers in serum.

Objective: The aim was to evaluate the serum levels of activation of different leukocyte subsets and to examine those in relation to the pathogenesis of RP and survival in NSCLC.

Methods: We analyzed the serum levels of MPO, sCD25, sTIM-3, sPD-L1, sCD14, sCD163, CCL19 and CCL21 in 66 inoperable NSCLC patients with stage IA-IIIA disease. The patients were treated with stereotactic body radiation therapy (SBRT) or concurrent chemoradiation therapy (CCRT), followed by regular blood sampling for 12 months after treatment and for 5 years for survival.

Results: Nineteen (29%) patients developed RP, which occurred more frequently and earlier in patients receiving CCRT than in those receiving SBRT. Increases in sCD25, sTIM-3 and CCL21 levels were observed at the last 6 months of follow-up in patients who had RP after SBRT. Patients who had RP after CCRT had higher sTIM-3 levels during the first 3 months of follow-up. Baseline sCD25 was independently associated with both 2- and 5-year mortality outcomes, while baseline sTIM-3 was independently associated with 2-year mortality.

Conclusion: We showed that T cell activation and exhaustion markers such as sCD25 and sTIM-3 are enhanced in patients developing RP and are associated with poor survival in NSCLC.

Keywords: lung cancer, radiotherapy, stereotactic body radiation therapy, radiation pneumonitis, radiation-induced lung injury (RILI), blood biomarkers, t cell, leukocyte subsets

INTRODUCTION

In 2020, lung cancer was the second most diagnosed cancer in the world and was the leading cause of cancer death. It is estimated that 2.2 million new cases occurred in the world in 2020 (1).

Radiation therapy is the most commonly used treatment modality for non-small-cell lung cancer (NSCLC) patients. It is administered for curative purposes as a solo treatment or in combination with surgery and/or chemotherapy for patients with early-stage or locally advanced stage (stage I-III) NSCLC. It can also be used as palliative treatment to prolong life and improve quality of life for patients with distant metastases (stage IV). Curative radiotherapy options are stereotactic body radiation therapy (SBRT) and concurrent chemoradiation therapy (CCRT). During the recent years, however, a number of novel treatment modalities has been developed such as check-point inhibitors and several tyrosine kinase inhibitors targeting specific genetic alterations.

The lung is a complex organ consisting of at least 40 different types of cells with distinct functions. This complexity is associated with impaired regeneration potential, and consequently, the lung is the organ most exposed to damage following various forms of radiation therapy (2–4). The risk of developing radiation-induced lung injury limits effective high-dose thoracic radiation therapy for early-stage and locally advanced NSCLC patients (5) (5). The reported incidence of radiation pneumonitis (RP) after SBRT for NSCLC varies from 2% to 47% (6–11), and the incidence after CCRT varies from 5% to 40% (12–17).

The tumour microenvironment (TME) plays an important role in tumour growth (18) and in the outcome of treatment, including affecting resistance to cancer treatment (19). TME of a solid tumour consists of tumour cells, local cells, infiltrating nontumour cells, molecules present in the vicinity of these cells and cells comprising the blood and lymph vessels. Radiotherapy affects cancer cells and the TME, in particular the tumour blood vessels and cells of the immune system.

Radiation induces reactive oxygen species and reactive nitrogen species (ROS and NGS), which cause damage to mitochondrial DNA and to the alveolar–capillary barrier, both of which are sensitive to the effects of ionizing radiation (20–22). One of the many effects of radiation is increased vascular permeability and exudation of proteins into the alveolar space, causing the apoptosis of alveolar type-I pneumocytes. This triggers an influx of inflammatory cells (e.g., neutrophils, macrophages, and lymphocyte subsets) from the peripheral and pulmonary vasculature that infiltrate the damaged lung. These cells are further activated by ROS and NGS as well as danger-associated molecular patterns (DAMPs), leading to the release of various inflammatory molecules (23–26) and contributing to altered tissue remodeling, fibrogenesis and local and systemic inflammation associated with the development of complications to radiotherapy (27, 28) and poor survival (29, 30). The radiation-induced release of cytokines and related molecules the first 24 hours after radiation might be an important contributor to RP (31–34).

While clinical outcome like survival is the most important parameter when evaluating novel treatment options, biomarkers are of importance in order to predict treatment responses and risk categories and even more importantly, to select correct treatment options and to discover pathways that are not modulated by the current treatment modalities.

The inflammatory response in the lungs causing the development of RP and fibrosis is still not well understood. The regulation and importance of the different inflammatory and immune-related mediators in the TME are at present not clear. In the present study, we examined the serum parameters of the activation of different leukocyte subsets, including myeloperoxidase (MPO) as a marker of neutrophil activation; soluble CD25 (sCD25), soluble T cell immunoglobulin mucin domain-3 (sTIM-3) and soluble programmed cell death 1 (sPD-1) as markers of T cell activation and exhaustion; and sCD14 and sCD163 as markers of monocyte/macrophage activation. We also analyzed the levels of the homeostatic chemokines CCL21 and CCL19 as mediators of lymphocyte trafficking. These markers were evaluated in relation to RP and survival after curative radiotherapy for early-stage and locally advanced NSCLC.

MATERIALS AND METHODS

Trial Design

This is a prospective, longitudinal, clinical, single-institution (Vestfold Hospital Trust, Tønsberg, Norway) study for patients with early-stage and locally advanced stage (stage IA-IIIA) NSCLC (ClinicalTrials.gov NCT02428049).

Patients

Eligible patients were > 18 years old and had early-stage or locally advanced-stage (stage IA-IIIA) NSCLC. Tumours were staged in accordance with the Union for International Cancer Control, Tumor, Node, Metastasis staging system 8th edition (TNM 8). Patients were examined with CT scans of the chest and abdomen and PET-CT, and all patients in stage IIIA were examined with brain MRI. Patients were technically resectable but deemed medically inoperable by a multidisciplinary tumour board, and the assignment was independent of the study. Patients were recruited from Vestfold Hospital Trust, Tønsberg, Norway, received SBRT or concomitant chemoradiotherapy at Oslo University Hospital, Radiumhospitalet, and underwent clinical follow-up at Vestfold Hospital Trust. A total of 66 patients were included in the study. Changes in pulmonary function, symptoms, and radiological signs of RP after SBRT have previously been studied in 44 of these patients (10).

Ethics

All patients provided written informed consent. The study was conducted following legal and regulatory requirements as well as with the general principles outlined in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002) and the Declaration of Helsinki (World Medical

Association 1996 and 2008). Regional Ethical Committee, REK nr. 2013/169/REK sør-øst D. The trial is registered with ClinicalTrials.gov (NCT02428049).

Blood Sample Processing

Peripheral venous blood was collected with 4-mL Vacutainer tubes (BD Biosciences, San Diego, CA), kept in room temperature for coagulation for one hour and then spun at 1610 g for 10 minutes. Serum samples were stored immediately at –80°C in several aliquots in cryovials until analysis. Blood samples were collected before radiotherapy (baseline), on the last day of radiotherapy, at 1-1.5 months after treatment, and every 3 months thereafter until 12 months after radiotherapy. The samples were thawed only once.

Enzyme Immuno-Assays

Serum levels of sCD14, sCD163, sCD25, sTIM-3, MPO, sPD-1, CCL19 and CCL21 (**Table 1**) were measured in duplicate by EIA using commercially available antibodies (R&D Systems, Minneapolis, MN) in a 384-format using a combination of a SELMA pipetting robot (Analytik Jena AG, Jena, Germany) and a BioTek dispenser/washer (BioTek Instruments, Winooski, VT). Absorption was read at 450 nm by using an EIA plate reader (BioTek Instruments) with wavelength correction set to 540 nm. Samples from a patient were run on the same 384-well plate, with controls randomly distributed on all plates; the intra- and interassay coefficients of variation were <10%.

Radiotherapy

Of the 66 patients in this study, 44 were treated with SBRT, and 22 were treated with CCRT. SBRT was administered as a total dose of 45–56 Gy in 3–8 fractions. The tumour was given an inhomogeneous dose where the prescribed dose encompassed the periphery of the planning target volume (PTV) and the maximum dose in the tumour was approximately 150% of the prescribed dose. Treatment planning was performed on an ordinary CT series. Respiratory-dependent tumour movement was visualized radiologically, and if more than 10 mm, abdominal compression was used to reduce it. This was applied for nine patients.

CCRT was administered with a radiation dose of 60-66 Gy and two courses of cisplatin and etoposide for 18 patients. Four patients were administered conventionally fractionated radiotherapy of 60-66 Gy alone.

Follow-Up Specifications

Follow-up included a physical examination by a pulmonologist and pulmonary function evaluation at baseline, at 1-1.5 months after treatment, and every 3 months thereafter until 12 months after radiotherapy. CT scans were performed at all follow-up visits except at 1-1.5 months and 9 months when chest X-rays were carried out. Patients with symptoms were also referred for CT scans at 1-1.5 months and 9 months. After the first year, CT scans of the chest, a physical examination by a pulmonologist, spirometry, and determination of the DLCO according to national guidelines were performed two times the second year and yearly for the next three years.

Grading of RP

The patients' symptoms were graded according to the Common Terminology Criteria for Adverse Events (CTCAE). Radiological changes were graded according to the European Organization for Research and Treatment of Cancer and Late Effects Normal Tissues-Subjective, Objective, Management, Analytic (EORTC/LENT-SOMA). Based on the CTCAE and EORTC/LENT-SOMA grading, the patients were divided into the following 2 groups:

1. The no radiation pneumonitis group included patients with mild symptoms equivalent to CTCAE grade 0-1 and with no, patchy or increased density on imaging equivalent to EORTC (LENT-SOMA) grade 2-3.

2. The radiation pneumonitis group included patients with symptoms equivalent to CTCAE grade 2-5 and with patchy or increased density on imaging equivalent to EORTC (LENT-SOMA) grade 2-3. CTCAE grade 2 represents the need for some medical intervention (e.g., steroids), and grade 3 indicates the use of supplemental oxygen (35). None of the patients in this study had CTCAE grade 4 (life-threatening respiratory dysfunction) or 5 (death).

All CT scans were evaluated by an experienced thoracic radiologist focusing on RP.

Statistics

Patient characteristics were compared by using Student's *t* test or the chi-square test for continuous and categorical variables, respectively (**Table 2**). We did not have samples from all patients at all time points to analyze the temporal profile of the markers; therefore, we used a univariate general linear model. Markers were categorized as 0: baseline, before radiotherapy; 1: last day of radiotherapy; 2: 1-1.5 months after radiotherapy; and

TABLE 1 | Markers included in the study.

Markers for	Protein short name	Protein full name
T cells activation and exhaustion	sTIM3	T cell immunoglobulin and mucin domain-containing protein 3
	sPD-1	Programmed cell death 1
	sCD25	Soluble interleukin-2 receptor alpha chain (IL-2Rα)
Chemokine (chemotactic cytokines motif) family	CCL19	Chemokine (C-C motif) ligand 19
	CCL21	Chemokine (C–C motif) ligand 21
Neutrophil activation	MPO	Myeloperoxidase
Macrophage/monocyte activation	sCD163	Cluster of differentiation 163
	sCD14	Cluster of differentiation 14

TABLE 2 | Patient characteristics in relation to study outcomes.

	Radiation Pneumonitis		2-year mortality		5-year mortality	
	No (n = 47)	Yes (n = 19)	No (n = 46)	Yes (n = 20)	No (n = 25)	Yes (n = 41)
Age, years	73.7 (6.8)	71.2 (9.4)	73.4 (7.3)	71.9 (8.4)	73.0 (6.7)	72.9 (8.2)
Male sex	25 (53%)	9 (47%)	20 (44%)	14 (70%)*	13 (52%)	21 (51%)
SBRT	36 (77%)**	8 (42%)	34 (74%)	10 (50%)	20 (80%)	24 (59%)
CCRT	11 (23%)	11 (58%)*	12 (26%)	10 (50%)	5 (20%)	17 (42%)
Previous smoker	36 (77%)**	8 (42%)	34 (74%)	10 (50%)	18 (72%)	26 (63%)
COPD	27 (57%)	9 (47%)	25 (54%)	11 (55%)	12 (48%)	24 (59%)
Morphology	15 (38%)	7 (41%)	12 (32%)	10 (53%)	8 (33%)	14 (42%)
Stage III	8 (17%)	8 (44%)*	9 (20%)	7 (35%)	4 (16%)	12 (30%)
Emphysema	22 (47%)	9 (47%)	23 (50%)	8 (40%)	11 (44%)	20 (49%)
Radiation Pneumonitis			10 (22%)	9 (45%)	6 (24%)	13 (32%)

CCRT Concurrent chemoradiation therapy; SBRT Stereotactic body radiation therapy.
**p<0.05, **p<0.01.*

3, 6, 9 and 12 months after radiotherapy. Markers were log-transformed due to a skewed distribution. Markers were used as dependent variables, RP (yes/no) and time were used as fixed factors, and their interaction (RP*time) and patient number were used as random factors. To limit multiple comparisons, *post hoc* testing was performed only on variables where RP (between groups) or the RP*time interaction (paired comparisons) was significant. This model was also used within the two radiotherapy groups. When comparing levels between RP groups, MANCOVA was used for each time point with smoking, radiotherapy, and stage as covariates. Paired differences were assessed with paired t tests.

The discriminatory properties of baseline levels of serum markers in relation to 2- and 5-year mortality were assessed by receiver operating characteristic (ROC) analysis. The association between significant markers and mortality was further assessed with Cox regression analysis. Log levels of serum markers were standardized, and hazard ratios (HRs) were expressed as the risk per SD of the marker. As the sample size was limited, the most important baseline characteristics were tested as covariates one by one as well as with a propensity score composed of all of them. The temporal profile of the selected serum markers in relation to mortality was also assessed in the univariate general linear model replacing RP with 2- or 5-year mortality. When comparing levels between survivors and nonsurvivors, MANCOVA was used for each time point with sex as a covariate.

RESULTS

Patients

The CONSORT study flow diagram is presented in **Figure 1**. The study population and baseline characteristics in relation to the study outcomes are presented in **Table 2**. From February 2014 until December 2017, 66 eligible patients were included with a mean age of 73 years (range 51-90), of which 34 (52%) were male. There were no EGFR-positive patients in our study cohort. ALK fusion testing did not start until 2018 in Norway, so we have no information about this. Nineteen (29%) patients developed RP, which occurred more frequently and earlier in patients receiving CCRT (n=22) than in those receiving SBRT (n=44) and was less frequent in previous smokers and more frequent with advanced stage. RP on CT occurred after a median of 4.9 months after SBRT and 3 months after CCRP. The NSCLC recurrence percentage was similar among patients with and without RP (42% and 40%), the mean observation time was 30 months. Of the patients with RP, 75% had recurrence within 12 months versus 47% in patients without RP. Mortality was assessed at 2 and 5 years, in which 20 (30%) and 41 (62%) patients died, respectively. The only significant difference with regard to these mortality groups was a higher proportion of male patients who died before the 2-year follow-up.

Serum Markers and RP

The levels of serum markers in relation to RP, measured from baseline until 12 months after treatment, are presented in **Supplementary Table 1**. When evaluating the markers in relation to RP in the group as a whole, no significant difference in levels or temporal profile during the 12-month blood sampling was detected (**Figure 2A** and **Supplementary Figure 1A**). Further evaluation within the radiotherapy groups revealed that in patients receiving SBRT, an increase in sCD25, sTIM-3 and CCL21 levels was observed at the last 6 months of observation time in patients who developed RP (**Figure 2B**). In patients receiving CCRT, those who developed RP were characterized by higher sTIM-3 levels during the first 3 months of follow-up (**Figure 2**). Serum levels of MPO, sPD-1, sCD14, sCD163 and CCL19 showed no significant differences in relation to RP in the two radiation groups (SBRT and CCRT) (**Supplementary Figure 1**).

No difference in the level or course of the markers were found between patients receiving CCRT or conventionally fractionated radiotherapy alone, last group included only 4 patients.

Serum Markers and Mortality

We next evaluated whether baseline levels of serum markers could predict 2- and 5-year mortality by discriminatory analysis. As shown in **Figures 3A, B**, sCD25 was associated with both mortality outcomes, while sTIM-3 was associated with 2-year mortality. The areas under the curve (AUCs) and 95% confidence intervals (CIs) for all markers are shown in **Supplementary Table 2**.

We assessed these associations further using survival analysis. As shown in **Figure 3C**, a one SD increase in baseline sCD25 was

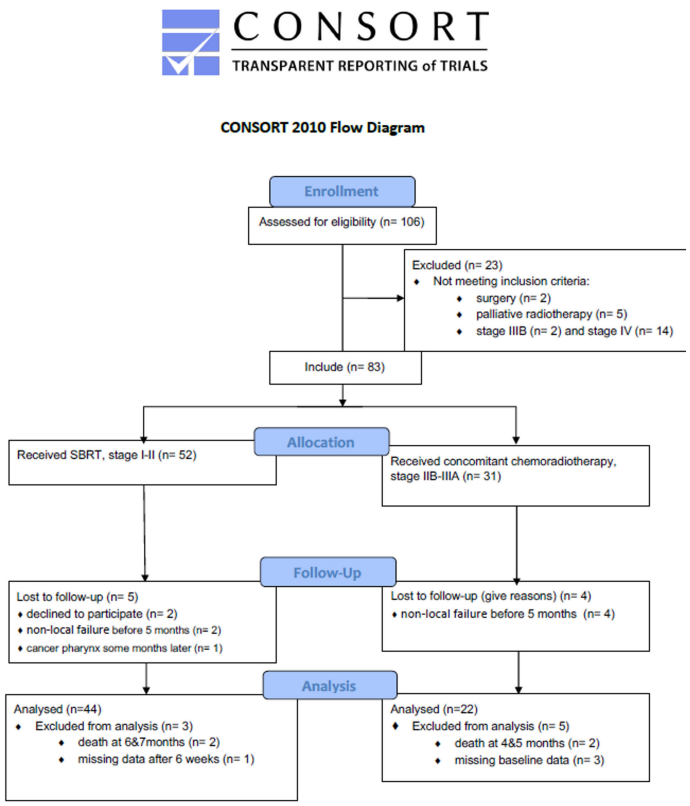

FIGURE 1 | The CONSORT study flow diagram.

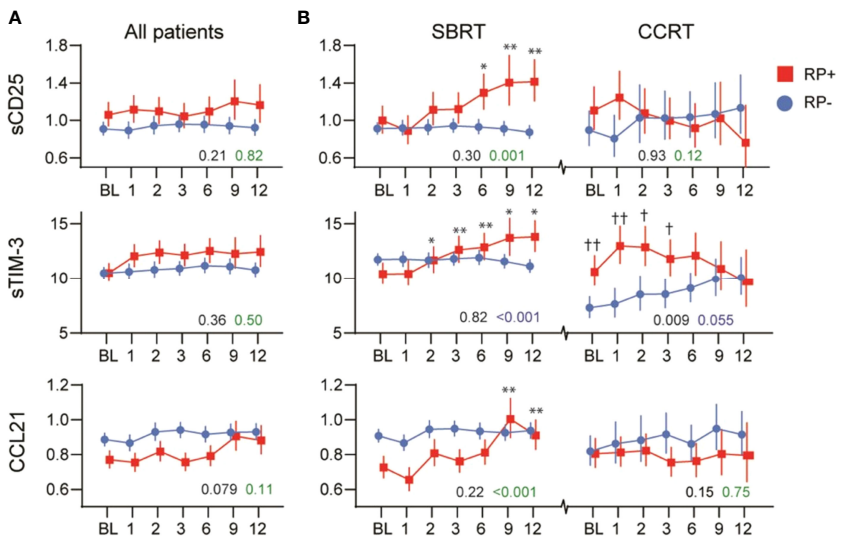

FIGURE 2 | Temporal profile of sCD25, sTIM-3 and CCL21 in relation to radiation pneumonitis (RP) in **(A)** all patients and **(B)** within patients receiving stereotactic body radiation therapy (SBRT) or concurrent chemoradiation therapy (CCRT). The black p-value represents the effect of RP from the univariate general linear model, while the green p-value represents the interaction with time (RP*time). *$p < 0.05$, **$p < 0.01$ *vs.* baseline. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. RP- same time-point.

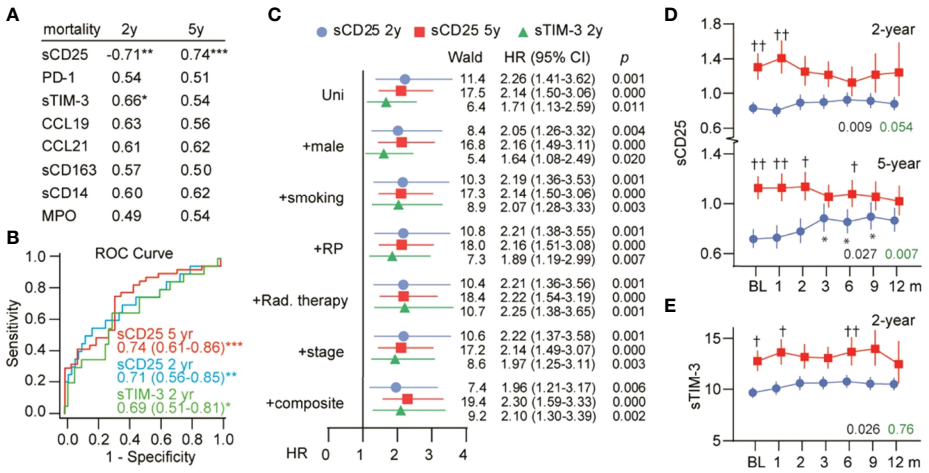

FIGURE 3 | Serum markers and mortality. **(A)** ROC analysis showing the AUC for baseline serum markers in relation to 2- and 5-year mortality. *p < 0.05, **p < 0.01, ***p < 0.001. **(B)** ROC curves for sCD25 and sTIM-3 with AUC (95% CI) in text. **(C)** Cox-regression analysis of baseline sCD25 and sTIM-3 in relation to mortality. HRs represent risk per 1 SD (log) change in serum markers and are shown with inclusion of the strongest baseline predictors as well as a composite score of these. Temporal profile of **(D)** sCD25 and **(E)** sTIM-3 in survivors (blue) and nonsurvivors (red). The black p-value represents the effect of mortality from the univariate general linear model, while the green p-value represents the interaction with time (mortality*time). *p < 0.05 vs. baseline. †p < 0.05, ††p < 0.01 *vs.* survivors at the same time point.

associated with a 2.3- and 2.1-fold higher risk of death at the 2- and 5-year follow-up, respectively. Furthermore, inclusion of the strongest baseline predictors of mortality (**Supplementary Table 3**) or a composite of these predictors had no influence on the association between sCD25 and mortality. A one SD increase in baseline sTIM-3 was associated with a 1.7-fold higher risk of 2-year mortality and was not influenced by covariates.

Evaluation of the temporal profile of sCD25 and sTIM-3 in relation to these outcomes is shown in **Figures 3D, E**. For sCD25, high levels were observed during the first two months in those who died. sTIM-3 remained mildly elevated in those who died during the 12-month blood sampling.

DISCUSSION

The present study evaluated serum leukocyte activation markers in NSCLC cancer patients in relation to RP and mortality. Whereas we found no associations with neutrophil and monocyte activation markers, which are traditionally thought to reflect acute and, to some degree, chronic (monocyte) inflammation, we found increased levels and different temporal profiles according to radiation treatment of the T cell activation marker sCD25, T cell exhaustion marker sTIM-3 and chemotactic T cell signal CCL21 in patients who developed RP. Moreover, sTIM-3 and, in particular, sCD25 predicted mortality independent of other demographics, including RP and the mode of radiation treatment. Our study suggests that the development of adverse outcomes in non-small-cell lung cancer patients is linked to T cell activation and exhaustion.

T cell activation is typical in both cancer progression (36), among others, in NSCLC (37, 38) and pneumonitis (39) due to persistent T cell receptor stimulation and is accompanied by the expression of inhibitory receptors such as PD-1 and TIM-3 (40–42) and T cell dysfunction (40). At the same time, radiotherapy modulates several immunological processes: revelation of antigens, activation of T lymphocytes, recruitment and accumulation of T cells in the tumour, and acknowledgement and killing of tumour cells by T lymphocytes. Our finding of increased levels of T cell activation and exhaustion markers in patients who developed RP indicates an active role in RP by likely overstimulation of T cells.

We could not, however, find any previous studies evaluating circulating levels of T cell activation and exhaustion markers in NSCLC or in response to RP. Herein, we found that changes in the levels of T cell markers in relation to RP occurred at different times according to radiation treatment. SBRT delivers the radiation dose to the pulmonary tumour more precisely than CCRT, allowing an escalation of SBRT treatment doses far beyond traditional conventional radiotherapy, damaging healthy lung tissue to a lesser extent and triggering RP more rarely and later than CCRT (11). While T cell exhaustion is mostly used in relation to chronic infections, we speculate that the enhanced early levels of sTIM-3 (i.e., within 3 months) in the CCRT group, without enhanced sCD25 levels, could reflect the effects of more acute RP, which is seen 1-3 months after CCRT (43–46), as a mechanism to prevent persistent and overshooting T cell activation. The more gradual increases in sTIM-3, sCD25 and CCL21 within the SBRT group correlate with the later onset of RP in this group occurring after 5-10 months (9–11, 47–49). Although PD-1, another T cell exhaustion marker, was not significantly associated with RP, the temporal trajectory was similar to sTIM-3 in the SBRT group but not in the CCRT group, possibly reflecting some different effects on T cell subsets of these radiation modalities. The concurrent increase in the T cell chemoattractant CCL21 in the SBRT group could potentially be linked to T cell migration or effects in regional lymph nodes (50).

As T cell exhaustion and dysfunction seem to be hallmarks of cancer progression (51) and are the targets of current immunotherapy (52), it is tempting to speculate that increased sTIM-3 and sCD25 could link RP and poor prognosis in lung cancer. However, while severe RP is associated with poor short-term outcome, prognosis in milder cases seems to be more dependent on underlying factors (53–55). Furthermore, we found that the association between sCD25, sTIM-3 and mortality was independent of both RP and the mode of radiotherapy. In patients with glioblastoma (56) and melanoma (57), only tumour-infiltrating, but not peripheral, T cells showed enhanced levels of exhaustion-associated inhibitory receptors.

Thus, our findings could potentially reflect immunological abnormalities in the TME, which has been associated with poor prognosis in lung cancer (58). This may also support double immunotherapy targeting both TIM-3 and PD-1, which has indeed been shown to improve antitumour T cell responses in preclinical models (59–61). Moreover, inflammation is a recognized hallmark in carcinogenesis (62). Macrophages are associated with chronic inflammation in cancer initiation and promotion and are also involved in tumour progression and metastasis (63). Tumour-associated macrophages correlate with poor prognosis (64). Radiotherapy changes the TME by attacking cancer cells, blood vessels and immune-related cells in the TME. Immunological changes occur in the affected tissue first and later become measurable in peripheral blood. The absence of neutrophil and monocyte activation markers after radiotherapy in our study suggests that those markers do not play a crucial role in the pathogenesis of RP.

We were unable to find studies evaluating serum levels of the monocyte/macrophage activation markers sCD14 or sCD163 or the neutrophil activation marker MPO in relation to survival in lung cancer patients. Enhanced tumour-associated CD163 expression by immunohistochemistry (IHC) was associated with poor survival in a small study (65), while a larger study (n=335) found no association between CD66b(+) neutrophils and CD163(+) macrophages and survival in lung cancer patients (66). Nevertheless, our data suggest that any potential activation of these cells in RP or in relation to disease progression is not reflected by circulating levels of activation markers.

Treatment with immune checkpoint inhibitors (ICIs) is a relatively new class of therapeutic agents that have shown impressive anticancer effects for a number of solid cancer types. ICI-induced pneumonitis is a rare but severe side effect and is occasionally fatal. Life-threatening pneumonitis has been reported in up to 2% of cases (67–69). The incidence of ICI-induced pneumonitis is higher in NSCLC than in other cancer types and in combined therapy with radiotherapy or chemotherapy (6.5%–10%) than in monotherapy (3%–4%) (70, 71). These findings can be useful in the study of ICI-induced pneumonitis pathogenesis.

The occurrence of RP after SBRT is lower than after CCRT (13, 14, 16, 72) and severe RP is quite uncommon (7, 8, 73). SBRT techniques allow minimizes the size of the planning target volume (PTV) and meaning the normal lung tissue in the target volume. The larger irradiation fields increase values of inflammation markers. In the present study, however, too few patients underwent conventionally fractioned radiotherapy alone in the CCRT group to make any reliable comparison.

In conclusion, our findings showing increased sCD25 and sTIM-3 in relation to RP and survival suggest that persistent T cell activation and exhaustion may contribute to progression and adverse outcomes in locally advanced NSCLC and support T cell-targeted treatment in this disorder.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Ethical Committee, REK nr. 2013/169/REK sør-øst D. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design: JB, TU, PA, ÅH. Provision of study materials or patients: JB, M-BB, ÅH. Collection and assembly of data: JB, TU. Data analysis and interpretation: JB, TU, PA, ÅH, ARH, ML. Manuscript writing: JB, TU, PA, ÅH. Final approval of manuscript: All authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.875152/full#supplementary-material

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