Post–radiation neutrophil–to–lymphocyte ratio is a prognostic marker in patients with localized pancreatic adenocarcinoma treated with anti–PD-1 antibody and stereotactic body radiation therapy

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Purpose: To investigate the role of pre- and post-stereotactic body radiation therapy (SBRT) neutrophil-to-lymphocyte ratio (NLR) in patients with localized pancreatic cancer treated with anti-PD-1 (programmed cell death protein-1) antibody and SBRT.

Materials and Methods: This was a retrospective review of 68 patients with borderline resectable or locally advanced pancreatic cancer treated with anti-PD-1 antibody and SBRT after multi-agent chemotherapy. Immunotherapy was administered with 5-fraction SBRT in the neoadjuvant, concurrent, or adjuvant/maintenance setting. Clinical outcomes included overall survival (OS), local progression-free survival, distant metastasis-free survival, and progression-free survival. Median pre- and post-SBRT peripheral blood markers were compared with the Mann-Whitney U test. Univariate and multivariable analyses (UVA and MVA) were performed to identify variables associated with clinical outcomes. Linear regression was performed to determine correlations between variables and peripheral blood markers.

Results: A total of 68 patients were included in the study. The percent change between median pre- and post-SBRT absolute lymphocyte count (ALC), absolute neutrophil count, and NLR were -36.0% ($p <$ 0.001), -5.6% (p = 0.190), and +35.7% (p = 0.003), respectively. Median OS after SBRT was 22.4 months. On UVA, pre-SBRT CA19-9 (hazard ratio [HR] = 1.001; 95% confidence interval [CI], 1.000–1.001; p = 0.031), post-SBRT ALC (HR = 0.33; 95% CI, 0.11–0.91; p = 0.031), and post-SBRT NLR (HR = 1.13; 95% CI, 1.04–1.22; p = 0.009) were associated with OS. On MVA, induction chemotherapy duration (HR = 0.75; 95% CI, 0.57–0.99; p = 0.048) and post-SBRT NLR (HR = 1.14; 95% CI, 1.04–1.23; p = 0.002) predicted for OS. Patients with post-SBRT NLR ≥3.2 had a median OS of 15.6 months versus 27.6 months in patients with post-SBRT NLR <3.2 (p = 0.009). On MVA linear regression, $\log_{10}$CTV had a negative correlation with post-SBRT ALC (regression coefficient = -0.314; 95% CI, -0.626 to -0.003; p = 0.048).

Conclusion: Elevated NLR after SBRT is primarily due to depletion of lymphocytes and associated with worse survival outcomes in localized pancreatic cancer treated with anti-PD-1 antibody. Larger CTVs were associated with decreased post-SBRT ALC.

Keywords: Stereotactic body radiotherapy, Immunotherapy, Pancreatic cancer, PD-1 inhibitor, Immune checkpoint inhibitor, Neutrophil, Lymphocyte

Introduction

Pancreatic cancer is currently the third most common cause of cancer related deaths in the United States, responsible for over 48,000 deaths each year [1]. In fact, it is projected to be the second most common cause by the year 2030 [2]. Treatment of pancreatic cancer usually involves a combination of chemotherapy, radiation therapy, and surgery [3]. Even with aggressive therapy, prognosis is poor with 5-year overall survival (OS) rates of less than 15% for borderline resectable and locally advanced pancreatic adenocarcinoma (BRPC/LAPC) [4]. Novel therapies are needed to improve outcomes.

One area of investigation is the use of immune checkpoint inhibitors (ICIs), such as programmed cell death-1 (PD-1) inhibitors, which have shown great promise in a wide range of malignancies including non-small cell lung cancer, melanoma, and esophageal cancer [5-7]. However, the role of ICIs in pancreatic cancer is tenuous. The current literature is sparse, with some reports demonstrating that there is limited benefit of ICI monotherapy in pancreatic cancer [8,9]. This is thought to be due to the immunosuppressive and stroma-rich microenvironment of pancreatic cancer [10]. The combination of ICIs with other immunotherapeutic agents and stereotactic body radiation therapy (SBRT) may increase the immunogenicity of the pancreatic tumor microenvironment (TME) and is currently being investigated [11-16].

One of the hallmarks of cancer is inflammation which is an immunologic response mediated by a variety of cells and signaling molecules [17]. The neutrophil-to-lymphocyte ratio (NLR) is a readily available marker of inflammation and immunogenicity, with elevated levels associated with poor outcomes in many solid tumors [18]. Although studies have demonstrated the prognostic and predictive value of NLR in various cancers treated with ICIs, there have been no studies investigating the role of NLR in localized pancreatic cancer treated with ICIs and SBRT [19,20]. Such information may inform decisions regarding radiation planning (dose, target volume, etc.) in the setting of immunotherapy for pancreatic cancer. At our institution, we have been exploring the combination of ICI therapy and SBRT in localized pancreatic cancer in the setting of several clinical trials. As such, the purpose of this study was to investigate the impact of pre- and post-SBRT NLR on clinical outcomes in a cohort of localized pancreatic cancer patients treated with anti-PD-1 antibody and SBRT as well as to identify factors associated with NLR dynamics.

Materials and Methods

1. Study design

This study was was a single institution retrospective review of patients with BRPC/LAPC who were treated with multi-agent induction chemotherapy followed by anti-PD-1 antibody and SBRT between August 2016 and May 2021. Patients were included in the study if they met the following criteria: (1) biopsy proven diagnosis of pancreatic adenocarcinoma, (2) locally advanced or borderline resectable disease per the National Comprehensive Cancer Network (NCCN) guidelines [3], (3) treatment with induction chemotherapy, anti-PD-1 antibody, and SBRT, (4) peripheral blood markers available for review pre- and post-SBRT, and (5) regular follow-up with post-treatment diagnostic imaging. All data including patient information, treatment details, clinical outcomes, and peripheral blood markers were retrospectively collected. Initial induction chemotherapy regimens consisted of FOLFIRINOX (FFX) and gemcitabine/nab-paclitaxel (GnP). Immunotherapy consisted of various experimental immunologic agents in combination with anti-PD-1 antibody. Immunotherapy was administered with SBRT in the neoadjuvant, concurrent, and/or adjuvant/maintenance setting. When given in the concurrent setting, anti-PD-1 antibody was administered a few hours after fraction one of SBRT. The study was conducted in accordance with the Declaration of Helsinki, and was approved by our institutional review board of Johns Hopkins University (No. 00285919). Given the retrospective nature of the study, written informed consent was waived.

2. SBRT details

Patients were treated with 5-fraction SBRT on 5 consecutive business days. Prior to simulation, endoscopic ultrasound guided placement of fiducials was performed to be used for daily image guidance. At time of simulation, thin sliced computed tomography (CT) scans with intravenous contrast were obtained, with patients positioned supine and arms above head in a Vac-lok device (CIVCO Medical Solutions, Coralville, IA, USA) for immobilization. Active breathing control (ABC; Elekta, Stockholm, Sweden) was utilized for respiratory motion management. In patients who could not tolerate ABC, a four-dimensional CT scan was acquired, and an internal target volume (ITV) was generated from the maximum inspiratory and expiratory phases. Pinnacle treatment planning system (Phillips Radiation Oncology Systems, Fitchburg, WI, USA) was used for target and organ-at-risk delineation. The clinical target volume (CTV) included the gross tumor plus regions at risk for microscopic disease, such as full circumference of involved and/or adjacent vasculature. The planning target volume was generated by adding a 2–5 mm isotropic margin to the CTV in breath-hold cases and to the ITV in free-breathing cases. Radiation prescription goals were as follows: (1) dose coverage—prescription dose to cover ≥98% of CTV and ≥90% of PTV, 25 Gy to cover 100% of the CTV and ≥99% of PTV, (2) gastrointestinal organs (stomach, duodenum, bowel)—V_{33} <1

mL, $V_{20} < 20$ mL, max dose to planning organ-at-risk volume (3 mm margin around organs) < 40 Gy. Pre-treatment and intrafractional cone-beam CTs were acquired to verify patient positioning. Patients were aligned to spine and then shifted to align to fiducials. All patients were treated on an Elekta linear accelerator unit.

3. Peripheral blood markers

Absolute lymphocyte counts (ALC) and absolute neutrophil count (ANC) were collected pre- and post-SBRT. Values were recorded within 4-weeks prior to SBRT and 1–6 weeks after completion of SBRT. If multiple values existed, the values closest to start of SBRT and closest to 4-weeks after SBRT were recorded. The NLR was calculated by dividing the ANC by ALC for each patient.

4. Clinical outcomes

Clinical outcomes included OS, local progression-free survival (LPFS), distant metastasis-free survival (DMFS), and progression-free survival (PFS). OS was defined as time from SBRT to death. LPFS and DMFS were defined as time from SBRT to radiographic evidence of locoregional progression and distant progression, respectively. PFS was defined as time from SBRT to any radiographic evidence of progression or death.

5. Statistical analysis

Descriptive statistics was used to record patient demographic, treatment, and disease characteristics, including age, sex, Eastern Cooperative Oncology Group status, disease extent, disease grade, chemotherapy duration and regimen, immunotherapy, radiation therapy, resection status, and peripheral blood values. Differences in median pre- and post-SBRT ALC, ANC, and NLR values were assessed using the Mann-Whitney U test. To account for multicollinearity, continuous variables were selected with backwards elimination such that variables with a variance inflation factor (VIF) > 2.5 were eliminated. Univariate Cox analysis was performed to identify associations between the aforementioned variables with OS, LPFS, DMFS, and PFS. Variables with $p < 0.1$ on univariate Cox analysis were entered in multivariable Cox analysis and subsequently removed if significance rose to $p > 0.1$. Receiver operative characteristics analysis was performed to identify the optimal post-SBRT NLR cut-off value in predicting OS. Kaplan-Meier method was used for survival analysis and log-rank test was performed to assess significance between groups. Correlations between peripheral blood markers and CTV were assessed with Spearman coefficient. Univariate and multivariable linear regression were performed to determine the association between variables and post-SBRT lymphocyte counts. The CTV values were log transformed

($\log_{10}$CTV) to generate a normal distribution. Variables with $p < 0.1$ on univariate linear regression were entered into multivariable linear regression and subsequently removed if significance rose to $p > 0.1$. All p-values were two-sided and statistical significance was considered $p < 0.05$. All statistical analysis was performed with JMP version 15.0 (SAS Institute, Cary, NC, USA).

Table 1. Patient, treatment, and disease characteristics (n = 68)

Characteristic	Value
Age (yr)	64.5 (41.7–84.1)
Sex	
Male	39 (57.3)
Female	29 (42.7)
ECOG performance status	
0	24 (35.3)
1	44 (64.7)
Location of primary tumor	
Head	37 (54.4)
Other	31 (45.6)
Disease extent	
Borderline resectable	9 (13.2)
Locally advanced	59 (87.8)
Baseline CA19-9 (U/mL)	224.5 (< 1.0–8094.0)
Pre-SBRT CA19-9 (U/mL)	40.0 (< 1.0–3264.4)
Induction chemotherapy duration (mo)	5 (1–8)
Initial induction chemotherapy	
FFX	61 (89.7)
GnP	7 (10.3)
Anti-PD-1 antibody duration (cycles)	2 (1–18)
Anti-PD-1 antibody timing with SBRT	
Neoadjuvant/concurrent	30 (44.1)
Neoadjuvant/concurrent/adjuvant/maintenance	20 (29.4)
Adjuvant/maintenance	15 (22.1)
Neoadjuvant	2 (2.9)
Concurrent	1 (1.5)
SBRT dose and fractionation	
33 Gy in 5 fractions	67 (98.5)
30.5 Gy in 5 fractions	1 (1.5)
CTV (cm^3)	88.2 (19.2–288.5)
Surgically resected	42 (61.8)
Whipple	26 (61.9)
Distal	13 (30.9)
Total pancreatectomy	3 (7.2)

Values are presented as median (range) or number of patients (%).
ECOG, Eastern Cooperative Oncology Group; CA19-9, cancer antigen 19-9; FFX, FOLFIRINOX; GnP, gemcitabine/nab-paclitaxel; PD-1, programmed cell death protein 1; SBRT, stereotactic body radiation therapy; CTV, clinical target volume.

Results

1. Cohort characteristics

Patient, treatment, and disease characteristics are shown in Table 1. From August 2016 to May 2021, 68 patients were treated with multi-agent induction chemotherapy followed by anti–PD-1 antibody and SBRT. The median age was 64.5 years (range, 41.7 to 84.1 years) and 57.3% were male. Borderline resectable and locally advanced disease was present in nine patients (13.2%) and 59 patients (87.8%), respectively. Baseline and pre-SBRT CA19-9 values were 224.5 U/mL (range, <1 to 8094.0 U/mL) and 40.0 U/mL (range, <1 to 3264.4 U/mL), respectively. Initial induction chemotherapy consisted of FFX (61/68; 89.7%) and GnP (7/68; 10.3%). Of note, five patients were transitioned from FFX to GnP, one patient from FFX to capecitabine, and one patient from GnP to gemcitabine due to intolerance of initial chemotherapy. Anti–PD-1 antibody therapy was delivered with SBRT in the neoadjuvant/concurrent (30/68; 44.1%), neoadjuvant/concurrent/adjuvant/ maintenance (20/68; 29.4%), adjuvant alone (15/68; 22.1%), neoadjuvant alone (2/68; 2.9%), and concurrent alone (1/68; 1.5%) setting. Nearly all patients received SBRT to 33 Gy in 5 fractions (67/68; 98.5%), with one patient receiving 30.5 Gy in 5 fractions. Surgical resection was performed in 61.8% of patients (42/68), with the specific types of surgical procedure including the Whipple procedure (26/42; 61.9%), distal pancreatectomy (13/42; 30.9%), and total pancreatectomy (3/42; 7.2%).

2. Increase in NLR is a result of depletion of lymphocytes

Table 2 shows pre- and post-SBRT ALC, ANC, and NLR values for the entire cohort. The median pre-SBRT ALC, ANC, and NLR values were 1.50 $\times 10^3$/μL (range, 0.33 to 3.73 $\times 10^3$/μL), 3.19 $\times 10^3$/μL (range, 1.36 to 17.9 $\times 10^3$/μL), and 2.38 (range, 0.52 to 44.1), respectively. The median post-SBRT ALC, ANC, and NLR values were 0.84 $\times 10^3$/μL (range, 0.32 to 1.93 $\times 10^3$/μL), 2.87 $\times 10^3$/μL (range, 1.24 to 9.64 $\times 10^3$/μL), and 3.41 (range, 1.32 to 16.9), with a change of -36.0% (p < 0.001), -5.6% (p = 0.190), and +35.7% (p = 0.003), respectively. To determine if timing of blood draws after SBRT influenced NLR, we performed Mann-Whitney U test between patients who had labs drawn 1–4 weeks versus 4–6 weeks

after SBRT, with no significant difference in NLR between the two groups (p = 0.994). We did the same analysis to determine if chemotherapy regimen (FFX vs. GnP) influenced NLR, with no significant difference detected (p = 0.703).

3. Clinical outcomes: post-SBRT NLR is associated with OS

The median follow-up time for the entire cohort was 10.7 months (range, 1.9 to 53.3 months). Of the 68 patients, 33 (48.5%) had died and 35 (51.5%) were alive. Among patients who were alive, median follow-up time was 8.3 months (range, 1.9 to 53.3 months). Median OS from time of SBRT was 22.4 months, with 1-year, 2-year, and 3-year OS rates of 66.9%, 47.3%, and 28.2%, respectively. On UVA, pre-SBRT CA19-9 (hazard ratio [HR] = 1.00; 95% confidence interval [CI], 1.000–1.001; p = 0.031), post-SBRT ALC (HR = 0.33; 95% CI, 0.11–0.91; p = 0.031), and post-SBRT NLR (HR = 1.13; 95% CI, 1.04–1.22; p = 0.009) were associated with OS, with a trend towards significance for induction chemotherapy duration and CTV volume (Table 3). On stepwise MVA, only induction chemotherapy duration (HR = 0.75; 95% CI, 0.57–0.99; p = 0.048) and post-SBRT NLR (HR = 1.14; 95% CI, 1.04–1.23; p = 0.002) were associated with OS (Table 3). Receiver operator characteristic analysis identified the optimal post-SBRT NLR cutoff value of 3.2 in predicting OS (area under the curve = 0.639, sensitivity = 74.3%, specificity = 56.3%) (Fig. 1A). Patients with post-SBRT NLR ≥3.2 had a median OS of 15.6 months versus 27.6 months in patients with post-SBRT NLR <3.2 (p = 0.009) (Fig. 1B).

Supplementary Tables S1–S3 show UVA and MVA for LPFS, DMFS, and PFS. On UVA of LPFS, only larger CTV volume was associated with inferior outcome (HR = 1.01; 95% CI, 1.00–1.02; p = 0.019). As such, MVA was not performed for LPFS. On UVA of DMFS, elevated pre-SBRT CA19-9 (HR = 1.01; 95% CI, 1.000–1.001; p < 0.001) was associated with worse outcome. As such, MVA was not performed for DMFS. On MVA of PFS, only elevated pre-SBRT CA19-9 was associated with inferior outcome (HR = 1.01; 95% CI, 1.00–1.01; p < 0.001).

4. CTV is associated post-SBRT lymphocyte counts

Given that the increase in NLR was primarily due to depletion of lymphocytes (Table 2), we wanted to determine if there were any

Table 2. Pre- and post-SBRT lymphocyte, neutrophil, and NLR values

Variable	Pre-SBRT	Post-SBRT	% Change	p-value
ALC ($\times 10^3$/μL)	1.50 (0.33–3.73)	0.84 (0.32–1.93)	–36.0	<0.001
ANC ($\times 10^3$/μL)	3.19 (1.36–17.9)	2.87 (1.24–9.64)	–5.6	0.190
NLR	2.38 (0.52–44.1)	3.41 (1.32–16.9)	+35.7	0.003

Values are presented as median (range).
SBRT, stereotactic body radiation therapy; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; NLR, neutrophil to lymphocyte ratio.

Table 3. Univariate and multivariable analyses of overall survival

	Univariate			Multivariable		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (yr)	1.02	0.99–1.06	0.182			
Sex (male vs. female)	0.79	0.40–1.53	0.467			
ECOG performance status (1 vs. 0)	1.56	0.76–3.20	0.225			
Disease extent (BRPC vs. LAPC)	1.20	0.37–3.96	0.761			
Tumor location (head vs. other)	1.30	0.67–2.54	0.442			
Induction CT duration (mo)	0.79	0.60–1.04	0.094	0.75	0.57–0.99	0.048
Induction CT (FFX vs. GnP)	0.67	0.26–1.76	0.417			
Grade (I/II vs. III)	0.92	0.44–1.93	0.831			
Resected (yes vs. no)	0.66	0.34–1.29	0.229			
CTV (cm³)	1.01	0.99–1.01	0.051			
Baseline CA19-9 (U/mL)	1.00	0.99–1.00	0.309			
Pre-SBRT CA19-9 (U/mL)	1.001	1.000–1.001	0.031			
Baseline total bilirubin (mg/dL)	1.08	0.96–1.17	0.173			
Pre-SBRT						
ALC (× 10³/μL)	0.66	0.34–1.13	0.140			
NLR	1.04	0.94–1.10	0.354			
Post-SBRT						
ALC (× 10³/μL)	0.33	0.11–0.91	0.031			
NLR	1.13	1.04–1.22	0.009	1.14	1.04–1.23	0.002

ECOG, Eastern Cooperative Oncology Group; BRPC, borderline resectable pancreatic cancer; LAPC, locally advanced pancreatic cancer; CT, chemotherapy; FFX, FOLFIRINOX; GnP, gemcitabine/nab-paclitaxel; CTV, Clinical target volume; CA19-9, cancer antigen 19-9; SBRT, stereotactic body radiation therapy; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; NLR, neutrophil to lymphocyte ratio; HR, hazard ratio; CI, confidence interval.
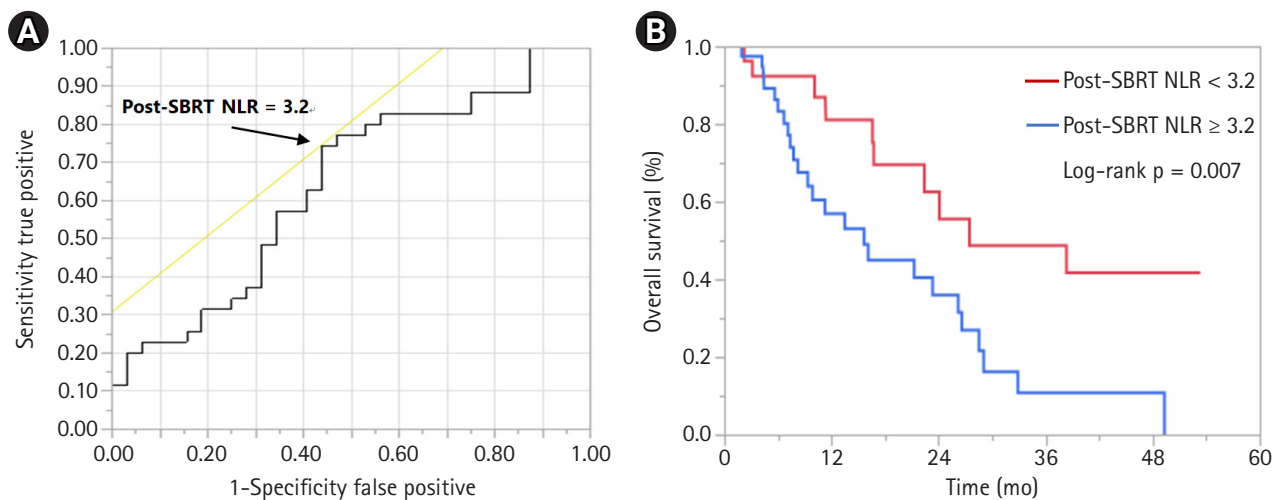

Fig. 1. Receiver operating characteristic (ROC) curve showing (A) optimal post-SBRT NLR value predicting overall survival and (B) Kaplan-Meier curve showing overall survival stratified by post-SBRT NLR of 3.2. Intersection of yellow line and ROC curve in Fig. 1A corresponds to the optimal NLR cut-off value of 3.2. SBRT, stereotactic body radiation therapy; NLR, neutrophil to lymphocyte ratio.

variables, including modifiable treatment characteristics, associated with post-SBRT ALC. There was a negative correlation between $\log_{10}$CTV and post-SBRT ALC (r = -0.284, p = 0.020) (Fig. 2A). To determine if this association existed prior to radiation or was radiation induced, $\log_{10}$CTV was plotted against pre-SBRT ALC, with no correlation found (r = -0.031, p = 0.800) (Fig. 2B). Similarly, there was no correlation between $\log_{10}$CTV and post-SBRT ANC (r = 0.052, p = 0.675) (Fig. 2C). Table 4 shows univariate and multivariable linear regression of variables associated with post-SBRT ALC. On MVA, when accounting for age, sex, ECOG, disease extent, in-
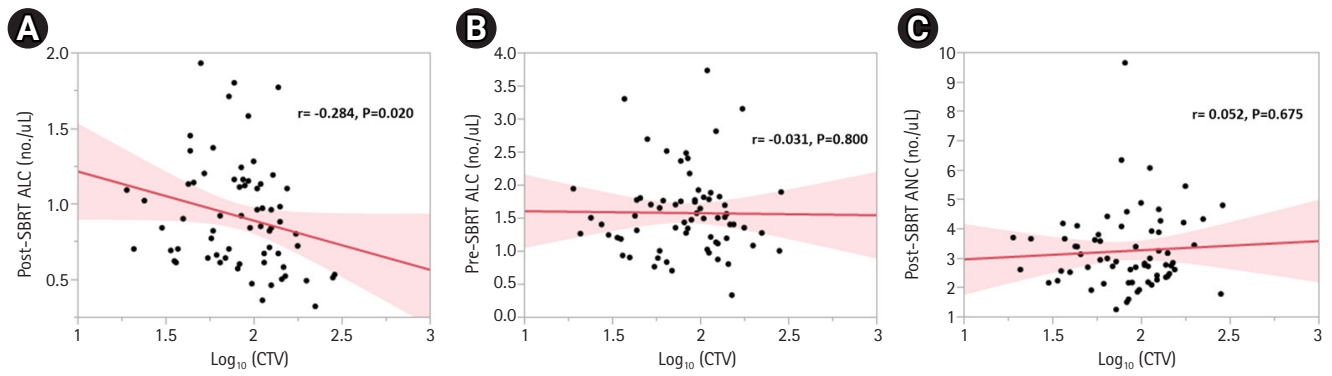
Fig. 2. Correlations among (A) post-SBRT ALC, (B) pre-SBRT ALC, and (C) post-SBRT ANC with log$_{10}$ (CTV). Spearman correlation coefficients (r) and p-values are displayed. SBRT, stereotactic body radiation therapy; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CTV, clinical target volume.

Table 4. Univariate and multivariable linear regression of post-SBRT ALC

	Univariate			Multivariable		
	Regression coefficient	95% CI	p-value	Regression coefficient	95% CI	p-value
Age (yr)	-0.005	-0.014 to 0.004	0.297			
Sex (male vs. female)	-0.035	-0.124 to 0.054	0.439			
ECOG performance status (1 vs. 0)	-0.005	0.098 to 0.088	0.914			
Disease extent (BRPC vs. LAPC)	0.080	-0.055 to 0.214	0.242			
Tumor location (head vs. other)	0.056	-0.031 to 0.143	0.203			
Induction CT duration (mo)	-0.016	-0.083 to 0.051	0.643			
Induction CT (FFX vs. GnP)	0.056	-0.102 to 0.213	0.483			
Grade (I/II vs. III)	0.003	-0.010 to 0.103	0.959			
Log$_{10}$CTV	-0.326	-0.661 to 0.010	0.057	-0.314	-0.626 to -0.003	0.048
Pre-SBRT ALC (× 10^3/μL)	0.220	0.090 to 0.349	0.001	0.217	0.057 to 0.344	0.001

SBRT, stereotactic body radiation therapy; ALC, absolute lymphocyte count; ECOG, Eastern Cooperative Oncology Group; BRPC, borderline resectable pancreatic cancer; LAPC, locally advanced pancreatic cancer; CT, chemotherapy; FFX, FOLFIRINOX; GnP, gemcitabine/nab-paclitaxel; CTV, clinical target volume; CI, confidence interval.

duction chemotherapy duration and regimen, and disease grade, log$_{10}$CTV (regression coefficient = -0.314; 95% CI, -0.626 to -0.003; p = 0.048) had a significant negative correlation with post-SBRT ALC, while pre-SBRT ALC (regression coefficient = 0.217; 95% CI, 0.057 to 0.344; p = 0.001) had significant positive correlation with post-SBRT ALC.

Discussion and Conclusion

In this report, we show that elevated NLR after SBRT is a result of depletion of lymphocytes and associated with inferior survival outcomes in localized pancreatic cancer treated with anti-PD-1 antibody. Furthermore, larger CTVs was associated with decreased post-SBRT lymphocyte counts. These findings may have implications on radiation field design for localized pancreatic cancer, particularly in the setting of combination therapy with immunotherapeutic agents.

The role of ICIs in pancreatic cancer is still under investigation. Only a handful of studies exist, which suggest that ICI monotherapy may have limited benefit [8,9,21,22]. This is likely due to immunosuppressive and hypoxic environment of the pancreatic TME [10]. Additionally, pancreatic adenocarcinoma is associated with low mutational burden, affecting neoantigen production and immune recognition [23,24]. To increase tumor immunogenicity, trials are investigating combination therapy of ICI with vaccines, chemokine inhibitors, oncolytic viruses, and SBRT [11-16,25]. Prognostic markers that take into account immunogenicity may aid in selection of these personalized therapies.

It is widely known that inflammation and cancer are inextricably linked [26]. The NLR is a marker of inflammation and immunogenicity, with elevated levels associated with poor outcomes in various malignancies [18]. The NLR is practical measure since it can be calculated from routine blood counts. Although the exact mechanism is unknown, it is thought that the NLR takes into account

both the pro-tumorigenic and anti-tumorigenic activity of neutrophils and lymphocytes, respectively. Neutrophils have been shown to secrete signaling molecules which promote tumor angiogenesis and evasion while suppressing cytotoxic T lymphocytes [27,28]. Lymphocytes, however, are associated with direct killing of cancerous cells [29]. The NLR has been validated as a prognostic and predictive factor in a wide range of cancers including pancreatic adenocarcinoma [18,30]. Recently, the prognostic value of NLR has been confirmed in various cancers treated with immunotherapy, including ICIs [19]. A report by Li et al. [31] showed that baseline and on-treatment NLR ≥5 predicted for worse OS in advanced cancers treated with ICIs. However, there have been no reports investigating the role of NLR in localized pancreatic cancer treated with ICIs and SBRT.

We show that elevated post-radiation NLR is predictive of worse OS in BRPC/LAPC treated with anti-PD-1 antibody and SBRT. The mechanism by which elevated post-radiation NLR is associated with worse OS in these patients is unknown. A focus on lymphocyte counts could provide some insight. Our data suggest that the increase in NLR after SBRT is largely due to depletion of lymphocytes, which is in agreement with a recent study by Wolfe et al. [32]. Radiation induced lymphopenia is associated with worse survival outcomes in both resected and locally advanced pancreatic cancer [33,34]. Furthermore, anti-PD-1 antibody induce immunogenic cell death through direct activation of cytotoxic T lymphocytes. Therefore, a possible explanation is that elevated post-radiation NLR is associated with inferior survival as a result of radiation-induced depletion of lymphocytes, with decreased efficacy of ICI therapy as a result. However, lymphocytes alone cannot fully explain this interaction, as ALC was not associated with outcomes in our study, suggesting that neutrophils may play a role as well. Indeed, a recent report showed that neutrophilia was associated with inferior outcomes in LAPC treated with chemoradiation [35]. Therefore, further studies are needed to further elucidate the roles of neutrophils and lymphocytes in the pancreatic cancer TME and how they interact with SBRT and ICIs.

Our data also demonstrate that larger CTVs correlated with decreased lymphocyte counts, which is in agreement with findings from others [36,37]. This suggests that field design is crucial in the radiation planning process for the treatment of pancreatic cancer. Currently, there is varying consensus on the appropriate target volumes for the treatment of intact pancreatic cancer. Some advocate for coverage of smaller volumes including gross disease and adjacent vasculature, while others suggest that treating larger volumes with elective nodal irradiation may improve outcomes [38-41]. A prior analysis from our institution (results not published), for exam-

ple, showed that nearly all local failures mapped to a "triangle volume" bordered by peri-pancreatic vasculature, which supports irradiation of a larger volume beyond gross disease that contains perineural tracts and lymphatic channels at risk of microscopic residual disease. However, the immunologic implications of targeting such a volume should be explored. One important consideration is whether radiation-induced lymphopenia is a result of irradiation to circulating blood/lymphatic channels or hematopoietic organs such as the spleen and vertebral bodies or a combination of both. If post-radiation lymphopenia is primarily due to irradiation of hematopoietic organs, then treating larger volumes such as the aforementioned "triangle volume" with optimization of splenic and vertebral body dosimetry may be appropriate. Prior reports do suggest that splenic and vertebral body dose is an important contributor to lymphopenia and that meeting specific dose constraints can mitigate this effect [42,43].

The current study has several limitations, including its retrospective nature. Additionally, patients were treated with heterogeneous induction chemotherapy regimens and different experimental immunotherapy agents in combination with anti-PD-1 antibody, which in turn, could influence peripheral blood markers, tumor inflammation, interaction with SBRT, and clinical outcomes. Similarly, the duration and timing of immunotherapy were also variable. Finally, post-SBRT peripheral blood markers were collected anywhere from 1–6 weeks after completion of treatment. It is possible that these values may have fluctuated during this time. The strengths of this study include its large sample size, homogeneity in SBRT dose/fractionation, and long follow-up time. Despite the limitations, this is the first report on this subject and provides new information regarding the role of NLR in pancreatic cancer treated with anti-PD-1 antibody and SBRT.

In summary, we demonstrate that elevated NLR after SBRT is primarily due to depletion of lymphocytes and is associated with inferior survival in localized pancreatic adenocarcinoma treated with anti-PD-1 antibody. Additionally, larger CTVs were associated with decreased post-SBRT lymphocyte counts. Further investigation into the complex relationship between SBRT, NLR, and ICI activity is warranted.

Conflict of Interest

Dr. Joseph M Herman is a former employee of PANCAN and current employee of 1440 Foundation. Dr. Jeffrey Meyer receives royalties from Uptodate and Springer and honorarium from Springer. No other conflicts of interest to disclose.

Author Contribution

Conceptualization: AVR, AKN; Investigation and methodology: AVR, AKN; Resources: AKN; Supervision: AKN, LZ, DAL, ADJ, JMH, JM; Writing of the original draft: AVR; Writing of the review and editing: AVR, AKN, CSH, SS, LZ, DAL, ADJ, JMH, JM; Formal analysis: AVR; Data curation: AVR, CSH, SS. All the authors have proofread the final version.

Supplementary Materials

Supplementary materials can be found via https://doi.org/10.3857/roj.2021.01060.

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