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The effect of spleen dose-volume parameters on lymphopenia during chemoradiotherapy for pancreatic cancer

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ABSTRACT

Background: We aimed to investigate the relationship between the radiation dose received by the spleen and the hematological parameters of patients who underwent chemoradiotherapy after the diagnosis of locally advanced pancreatic cancer (LAPC). ***Materials and Methods:*** Patients with LAPC who were treated with chemoradiotherapy were retrospectively reviewed. Surgical status, chemoradiotherapy details, complete blood count values (baseline, mid-treatment, and end-of-treatment), mean spleen dose, and dose-volume parameters (V5, V10, V15, V20, V25, and V30) were recorded. The relationship between spleen dose-volume parameters and the development of grade 3 lymphopenia was evaluated by Spearman's rank correlation and receiver operating characteristic (ROC) analysis. ***Results:*** All dose parameters for the spleen were significantly correlated with the mid-treatment absolute lymphocyte count. In the ROC analysis, mean spleen dose (p=0.011; area under the curve [AUC]: 0.856; 95% confidence interval [CI]: 0.675-0.995), V15 (p=0.020; AUC: 0.938; 95% CI: 0.830-0.997), and V20 (p=0.002; AUC: 0.940; 95% CI: 0.811-0.1000) were significantly associated with mid-treatment grade ≥3 lymphopenia. ***Conclusion:*** A significant correlation was found between the dose received by the spleen during chemoradiotherapy in LAPC patients and the development of lymphopenia. Contouring the spleen as an organ at risk (OAR) and documenting doses is important to establish dose limitations.

INTRODUCTION

Pancreatic cancer ranks 4th in terms of cancer mortality. The outcome is poor even with multimodal therapy using surgery, chemotherapy, and radiotherapy [1]. Chemoradiotherapy is indicated as an adjuvant treatment for patients undergoing surgery or definitive treatment for patients with unresectable cases [1, 2].

During radiotherapy planning, the stomach, kidneys, spinal cord, liver, and intestines are contoured as organs at risk (OAR). The spleen is not routinely contoured [2], despite its important role in regulating the hematopoietic and immune systems [3]. The spleen also removes aged or damaged erythrocytes from circulation and acts as a source of hematopoietic cells, especially platelets. Additionally, the spleen is a major secondary organ in the immune system and is important for antibody synthesis, the phagocytosis of antibody-coated cells, and filtering. The white pulp of the spleen contains the periarteriolar lymphocyte sheath (T-cell area), adjacent follicles (B-cell area), and a marginal zone (B -cell area). T lymphocytes interact with B lymphocytes and dendritic cells in this region [4-7]. Our understanding of the spleen as an OAR has increased as immunotherapy has become increasingly common in cancer treatment [1, 2]. Moreover, the prognostic role of hematologic parameters has been demonstrated in many studies (e.g., lymphopenia is associated with decreased survival in gastrointestinal tract malignancies) [8, 9]. Lymphocyte circulation is high in the spleen, and these cells are very radiosensitive; therefore, irradiating the spleen can be consequential [10]. In general, the spleen receives a high radiation dose during radiotherapy to treat gastrointestinal tract cancers. A recent study reported that spleen V_{15} and maximum dose were associated with lymphopenia [11].

The role of the spleen in the hematological toxicities developed during chemoradiotherapy and the effect of spleen irradiation on spleen function were investigated in this study. The unclear relationship between spleen doses and hematological parameters will be discussed. In this study, the relationship between spleen dose-volume parameters and hematologic value changes was evaluated in the patient population who underwent chemoradiotherapy in the treatment of LAPC.

MATERIALS AND METHODS

Patients

Patients with pathologically confirmed LAPC treated with curative-intent chemoradiotherapy at Ankara City Hospital between January 1, 2019, and January 12, 2021, were retrospectively reviewed. Those aged ≥18 years with no other malignancies were eligible for inclusion. We excluded patients who had received granulocyte-macrophage colony-stimulating factor (GM-CSF) and those with any type of immunodeficiency.

Treatment planning and contouring

Before treatment, a simulation computed tomography (Discovery™ RT, GE Healthcare, Waukesha, WI, USA) was performed from the carina to the L5 vertebra with supine position. Intravenous contrast was used at the treating physician's discretion. The Radiation Therapy Oncology Group (RTOG) guide was used for contouring (clinical target volumes and OARs, including the spleen. The PTV margin was 5 mm. The mean total prescribed dose was 45 Gy (range: 45–50.4 Gy). The ARIA® treatment planning system (Varian Medical Systems, Palo Alto, CA, USA) was used to optimize the treatment plan.

Comparison parameters

Spleen dose-volume parameters, and mean dose values were recorded. Complete blood count (CBC) testing was performed at the beginning of treatment (within one week of the first treatment), mid-treatment (between the twelfth and fourteenth fractions), and when treatment was complete (within 1 week of the last treatment). Common Terminology Criteria for Adverse Events (CTCAE) ver. 5 was used for acute side effect assessment.

Statistical analysis

SPSS software version 26 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Kolmogorov–Smirnov, and Shapiro–Wilk tests were used to analyze the normal distribution. The categorical data were compared using chi-squared and Fisher's exact tests. Spearman's rank correlation was used for univariate correlation analysis. Receiver operating characteristic (ROC) analysis was used to analyze the predictive value of spleen radiation doses for grade 3 lymphopenia, and was considered significant.

RESULTS

Plan dose parameters and CBC values of 19 patients were evaluated retrospectively. The median age at diagnosis was 62 years (range: 38–83 years old). The median total dose was 50.4 Gy (45–56 Gy). The patient and clinical characteristics are summarized in table 1. Table 2 shows the spleen dose parameters, mean dose, and CBC values.

Table 1. Patient characteristics.

Age	Median	62 (range 38-83)
Total Dose	Median	50.4 (range 45-56) Gy
Chemotherapy (CT)	None	1 (5.3%)
	Neoadjuvant	6 (31.6%)
	Adjuvant	12 (63.2%)
Surgery	None	7 (36.8%)
	Yes	12 (63.2%)
CT	Yes	19 (100%)
Concurrent CT	5- FU	5 (26.3%)
	Gemcitabine	5 (26.3%)
	Capecitabine	9 (47.4%)
RT Frc Dose	1.8 Gy	11 (57.9%)
	2 Gy	8 (42.1%)
RT Total Dose	45 Gy	3(15.8%)
	50 Gy	5 (26.3%)
	50.4 Gy	7 (36.8%)
	54 Gy	2(10.5%)
	54.2 Gy	1 (5.3%)
	56 Gy	1 (5.3%)

Gy: Gray, CT: Chemotherapy, RT: Radiotherapy, 5- FU: Fluorouracil, Frc: Fraction.

Table 2. Complete blood count and radiation dose-volume parameters.

Parameters	Median (Range)	Mean ±SE
Baseline ALC	1.38 (0.58-4.07)	1.62 ± 0.21
Mid-treatment ALC	0.43 (0.12-1.30)	0.54 ± 0.70
End of treatment ALC	0.35 (0.17-1.07)	0.44 ± 0.06
Baseline ANC	3.05(1.17-9.08)	3.66 ± 0.52
Mid-treatment ANC	3.58 (0.92-7.80)	3.49 ± 0.40
End of treatment ANC	3.95(1.31-9.19)	3.94 ± 0.52
Basale platelet count	2480000 (88000-689000)	269263±31477
Mid-treatment platelet count	152000 (88000-370000)	155194±19649
End of treatment platelet count	1725000 (87000-385000)	187875±20554
Mean dose	1357 (0-2436) cGy	1310±128.27 cGy
V_5	87 (0-99.3) cGy	76.5±6.05 cGy
V_{10}	70 (0-93.8) cGy	63±5.82 cGy
V_{15}	45 (0-87.9) cGy	43.5±5.36 cGy
V_{20}	11.6 (0-75.7) cGy	19.6±4.85 cGy
V_{25}	1.93 (0-43.9) cGy	7.3±3.04 cGy
V_{30}	0.18 (0-21.6) cGy	2.7±1.43 cGy

SE: Standard Errors, ANC: Absolute neutrophil count, Vx: Volume receiving X Gy

Absolute lymphocyte count and spleen dose-volume analysis

The mid-treatment absolute lymphocyte count was significantly correlated with the mean spleen dose (MSD), and other dose parameters, as shown in table 3. No significant correlation was found between the end-treatment absolute lymphocyte count and the spleen dose-volume parameters.

Results of the ROC curve analyses of the relationship between mid-treatment lymphopenia grade ≥3 and mean spleen dose, V_{15}, V_{20}, and V_{25} are shown in Table 4 and Figure 1. V_5, V_{10}, and V_{30} were not significantly associated with mid-treatment lymphopenia grade ≥3.

Table 3. Mid-treatment absolute lymphocyte count and spleen dose-volume parameters.

Parameters	Spearman Rho Coefficient	P-value
Mean dose	-0.630	0.005
Spleen V_5	-0.466	0.050
Spleen V_{10}	-0.482	0.043
Spleen V_{15}	-0.758	<0.001
Spleen V_{20}	-0.740	<0.001
Spleen V_{25}	-0.613	0.007
Spleen V_{30}	-0.542	0.020

Vx: Volume receiving X Gy

Table 4. Relationship between mid-treatment lymphopenia grade ≥ 3 and mean spleen dose, V15, V20, and V25 analyzed using ROC analysis.

	P-value	AUC	%95 CI	Cut Off	Sensitivity	Specificity
MSD	0.011	0.856	0.675-0.995	13.78	% 60	% 25
V_{15}	0.020	0.938	0.830-0.997	43.50	%90	%25
V_{20}	0.002	0.940	0.811-0.100	10.30	%90	%12.5
V_{25}	0.016	0.838	0.633-0.998	2.46	%80	%15

MSD: Mean spleen doses, AUC: Area under the ROC curve, CI: Confidence interval, Vx: Volume receiving X Gy

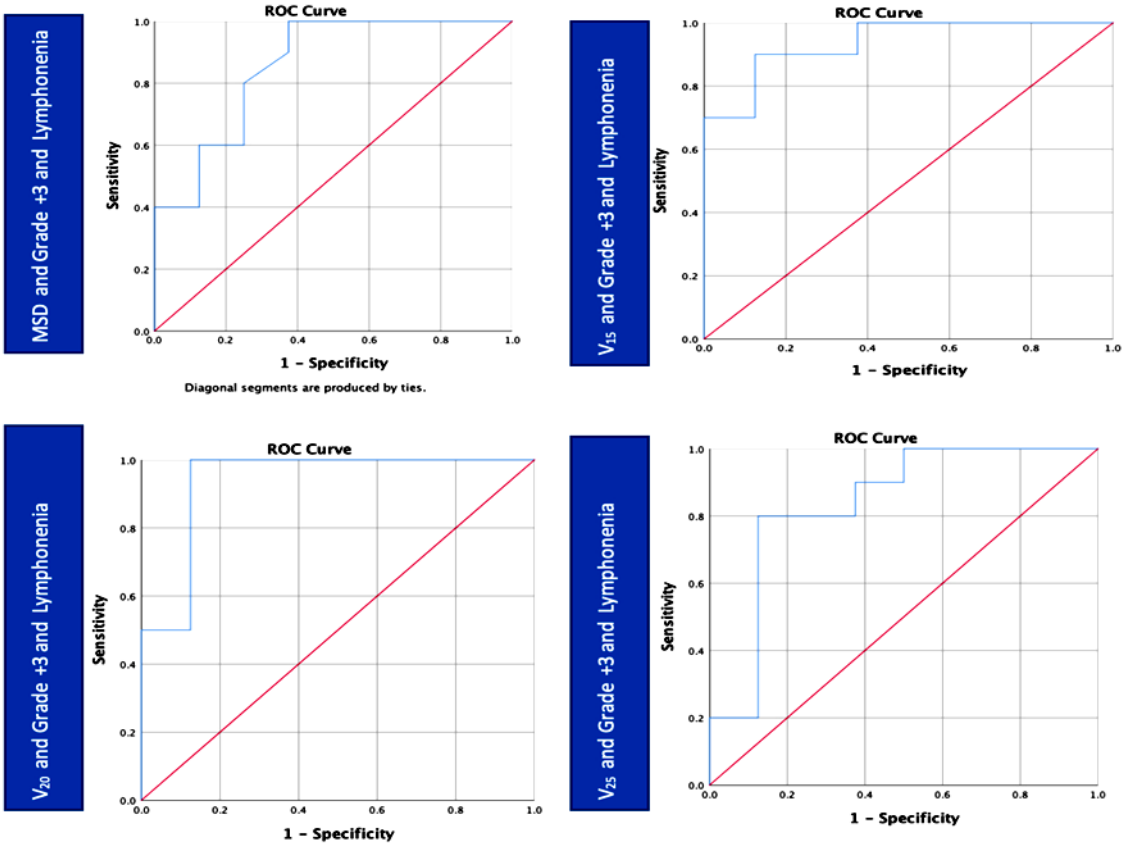

Figure 1. Receiver operating characteristic (ROC) curve analysis of the MSD, V15, V20, and V25 values respectively.

The results of the ROC curve analyses of the relationship between mid-treatment grade ≥ 3 lymphopenia and mean spleen dose, V_{15}, V_{20}, and V_{25} are shown in table 4 and figure 1. Spleen doses V_5, V_{10}, and V_{30} were not significantly associated with mid-treatment grade ≥3 lymphopenia.

Absolute neutrophil count and spleen dose-volume analysis

The mid-and end-of-treatment absolute neutrophil counts were not significantly correlated with the mean spleen dose or any dose-volume parameter.

Platelet count and spleen dose-volume analysis

No significant correlation was found between the spleen dose-volume parameters and mid-treatment platelet count. The end-of-treatment platelet count was significantly correlated with V_{25} (Spearman's rho: -0.503; p=0.047), but not with the mean spleen dose, V_5, V_{10}, V_{15}, V_{20}, or V_{30}.

DISCUSSION

The crelationship etween the dose received by the spleen during radiotherapy and the hematological parameters was evaluated in patients with locally advanced pancreatic cancer. There is a negative correlation between the spleen dose parameters and mid-treatment absolute lymphocyte count. Low specificity values were noted in the ROC analysis. As in the correlation analysis, the ROC analysis indicated that V_{15} and V_{20} were the most strongly related to lymphopenia (AUC = 0.938 and 0.940, respectively).

Immunotherapy is used to treat many cancers [18]. While current studies show the critical role of the immune system in cancer treatment, the effect of

lymphopenia on cancer treatment outcomes is also under investigation. [13-17]. In addition, many factors are associated with lymphopenia development in patients who are treated with chemoradiotherapy, including patient age, the type of chemotherapy, the size of the irradiated target volume, and bone marrow or splenic irradiation. In patients undergoing abdominal irradiation, the spleen receives substantial radiation; however, it is not routinely contoured and a standard dose limitation has not been defined. Dose limits differ between studies and have been based on the development of lymphopenia. New studies evaluating this issue are summarized in table 5. It is not possible to reach a general conclusion due to the heterogeneity of parameters and the patient groups who were evaluated. Moreover, some studies do not mention GM-CSF use, which can affect the degree of lymphopenia. The timing of lymphopenia development and its clinical significance is not known [8-12].

Table 5. Studies evaluating the relationship between spleen radiation dose and hematological parameters

Study	Number of patients	Primary Disease	Specified spleen dose parameter	Evaluated Blood Parameter	Results
Wolfe *et al*. 2021[8]	101	PC	MSD V_5 V_{10} V_{15} V_{20}	Delta NLR	Optimal radiation cutoff points to predict a DNLR 2.5 were splenic Dmean of 308 cGy and V_5 of 10.3%.
Liu *et al*. 2017 [9]	59	HCC	MSD V_5 V_{10} V_{20} V_{25} V_{30}	Min ALC	Spleen V5 and MSD values were found to be important in predicting min ALC
Yalamanchali *et al*. 2021 [10]	140	PC (n= 67), gastroesophageal (n=61), biliary tract(n=12) adenocarcinoma	MSD V_5 V_{10} V_{15} V_{20} V_{25}	ALC and ALC loss rate	It has been reported that each 1-Gy increase in MSD increases the probability of grade 3 RIL by 18.6%.

Vx: Volume receiving X Gy, PC: Pancreas cancer, MSD: Mean spleen dose, Min ALC: minimum value of absolute counts for peripheral blood lymphocytes, RIL: Radiation-induced lymphopenia.

Although the spleen is generally evaluated using the mean dose, volumetric V_5, V_{10}, V_{15}, and V_{20} have also been used. In a recent study by Alexandriu *et al.* [11], which used the nadir lymphocyte value as the baseline parameter, rather than the value at the beginning of treatment, a significant correlation was found between lymphopenia and V_{15}, similar to our study. Liu *et al.* [9] analyzed HCC patients and reported that MSD and spleen V_5-V_{25}-V_{30}, correlated with the minimum (Absolute Lymphocyte Count) ALC. In a study evaluating patients with esophageal cancer, an increase of 1 Gy in the mean spleen dose predicted a 2.9% decrease in the lowest absolute lymphocyte count [12]. In another study published in 2021, a similar result of each 1-Gy increase in MSD increasing the odds of grade 3 radiation-induced lymphopenia (RIL) by 18.6% was reported [10]. Publications on this subject in the literature are limited and difficult to compare. However, the importance of spleen doses has been emphasized in nearly all of them. The importance of spleen dose in the irradiation of pediatric cases was also noted. In a study on spleen irradiation in the pediatric patient group, antibiotic prophylaxis and vaccination were recommended for patients with MSD ≥10 Gy [19].

Although the increase in spleen dose has been argued to increase hematological side effects in general, Chin *et al*. [20] claimed the opposite. In this study, spleen dose-volume values and hematological toxicity were examined in 60 esophageal cancer patients, and an inverse relationship was found between these parameters. Contrary to what was expected in this study, a decrease in the development of leukopenia was found with the increase in the spleen's radiation dose. The authors explained this inverse relationship by suggesting that increasing spleen doses increased the release of defense cells sequestered in the spleen. Hematological toxicity then becomes more moderate during chemoradiotherapy. Prospective studies evaluating patients receiving homogeneous therapy are required to evaluate the effect of spleen doses on hematological parameters.

Our study's strengths include its homogeneous study population, which was diagnosed with a single type of cancer, and the exclusion of patients who had previously received GM-CSF. Limitations of the study are its retrospective design, single-center design, number of patients, and varying chemotherapy regimens.

CONCLUSION

Our findings show that spleen dose-volume parameters appear to affect lymphopenia in patients undergoing chemoradiotherapy for LAPC. Routine contouring of the spleen and its documentation is recommended.

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***Conflict of Interest Statement*:** The authors have no conflicts of interest to declare.

***Author Contribution*:** Conceptualization: GAI, SAA, YT; Investigation and methodology: GAI, IPA, ZG; Project administration: GAI; Resources: GAI, IPA, HFO; Supervision: GAI, YT; Writing of the original draft: GAI, IPA; Writing of the review and

editing: SAA, YT, HFO; Software: IPA; Validation: SAA; Formal analysis: IPA; Data curation: ZG, GAI; Visualization: GAI, SAA. All the authors have proofread the final version.

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***Ethical statement*:** This study was conducted according to the Helsinki Declaration. The ethical suitability of the study was approved by the Ankara City Hospital Ethics Committee No. 2 on November 10, 2021, with the number E2-21-986.

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