

The Effect of Spleen Doses on the Hematological Parameters in Adjuvant Gastric Cancer Radiotherapy

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ABSTRACT

Aim: This study aimed to examine whether there is a relationship between spleen radiation doses and hematological parameters in gastric cancer patients.

Materials and Methods: The patients who received chemoradiotherapy for nonmetastatic locally advanced gastric cancer were analyzed retrospectively. The dose parameters evaluated were spleen V₅, V₁₀, V₁₅, V₂₀, V₂₅, V₃₀ and mean spleen dose (MSD). Blood tests were evaluated at the beginning of the treatment (basal), in the 12-14 fractions of the treatment (mid-treatment), in the first week after the end of the treatment (at the end of treatment) and 3rd month control after radiotherapy. The CTCAE (Common Toxicity Criteria for Adverse Events) ver 5.0 was used. The SPSS 26 (IBM Corp, Armonk, NY) was used for statistical analysis.

Results: The data of 28 patients with gastric adenocarcinoma who received curative-adjuvant radiotherapy (RT) were evaluated retrospectively. The median age of the patients was 63 years (range 33-81). The median total dose was 45 (range 41.40-55) Gy. There were not statically significant relationships between any platelet or absolute lymphocyte count (mid treatment, end of the treatment and 3. months control) and splenic doses. The statically significant relationships were found between mid-treatment absolute neutrophil count and mean splenic dose ($p=0.044$); spleen V₁₀ doses ($p=0.030$); spleen V₁₅ doses ($p=0.031$); spleen V₂₀ doses ($p=0.044$). There were statically significant difference between at the third months control after treatment absolute neutrophil count and mean splenic dose ($p=0.037$), spleen V₃₀ ($p=0.039$).

Conclusion: A significant relationship was found between mid treatment, at the third month control neutropenia and spleen mean splenic dose, spleen V₁₀ doses, spleen V₁₅ doses, spleen V₂₀ doses in gastric cancers.

Key words: gastric cancer, spleen doses, neutropenia, radiotherapy, acute toxicity

Abbreviations:

Spleen V₅: percentage of volume receiving 5 Gy for spleen

Spleen V₁₀: percentage of volume receiving 10 Gy for spleen

Spleen V₁₅: percentage of volume receiving 15 Gy for spleen

Spleen V₂₀: percentage of volume receiving 20 Gy for spleen

Spleen V₂₅: percentage of volume receiving 25 Gy for spleen

Spleen V₃₀: percentage of volume receiving 30 Gy for spleen

CTCAE: Common Toxicity Criteria for Adverse Events

SPSS: Statistical Package for the Social Sciences

G-CSF: Granulocyte colony-stimulating factor

INTRODUCTION

Adjuvant chemoradiotherapy has been recommended as a standard treatment approach for locally advanced gastric cancer since the early 2000s (1,2). The results of recent randomized studies that directly compared postoperative adjuvant chemotherapy to postoperative chemoradiotherapy have led

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practitioners to question the benefits of postoperative chemoradiotherapy, which has become one of the most controversial topics in gastro-intestinal oncology (3-7). Considering the shortcomings of these studies, chemoradiotherapy is still recommended in many centers, including ours, for patients who have node-positive gastric cancer and did not receive neoadjuvant chemotherapy.

As the target area volume in gastric cancer irradiations is large (8), the spleen is exposed to significant radiation. The spleen, which serves important functions in the hematopoietic and immune system, is not generally defined as an organ at risk in radiotherapy applications, and there is no routinely recommended dose restriction for the spleen.

The radiation dose that the spleen receives gains importance for two reasons. Firstly, studies on the relationship of hematological parameters in predicting cancer treatment outcomes and the second is that the immune system's relevance in the etiopathogenesis and treatment of cancer is growing rapidly (9-12).

Given these developments, in this study, we aimed to examine the changes in blood values during the treatment of gastric cancer patients who underwent adjuvant chemoradiotherapy and the radiation dose received by their spleens.

MATERIALS AND METHODS

The data of a gastric adenocarcinoma patient who underwent adjuvant RT in Ankara City Hospital between 01.01.2020 - 30.06.2021 were evaluated retrospectively. To obtain data; patient file information, dose volume histograms and electronic system data were used. Demographic status of the patients, spleen dose details, CT protocols, hematological assessments of the patients before, during and after RT were noted.

Patient selection

Patients with gastric adenocarcinoma who had not previously used granulocyte-colony stimulating factor (G-CSF) treatment, did not have immune deficiencies or hematologic malignancies, and did not have a history of previously received radiotherapy (RT) were included.

Blood test details

Blood test results were evaluated at the beginning of the treatment (basal), after 12–14 fractions of the treatment (mid-treatment), in the first week after the end of treatment (at the end of treatment), and 3

months after radiotherapy (control). Our patients have a weekly blood check. Complete blood count (CBC) is requested as a blood test. In the CBC, neutrophil, lymphocyte and platelet counts were noted. The Common Toxicity Criteria for Adverse Events (CTCAE) ver. 5.0 was used for side effect assessment (13). For each patient; hemogram results were noted at 24 hours after the first fraction, at 48 hours after the first fraction and 7 days after the end of RT. For lymphopenia; If Absolute Lymphocyte Count (ALC) 500 cells/mL is defined as grade 3 lymphopenia; and ALC < 200 cells/mL was defined as grade 4 lymphopenia. For neutropenia; If (ANC) absolute neutrophil count between 500 - 1000/microL is defined as grade 3 neutropenia if <500/microL is defined as grade 4 neutropenia. For platelet; if the platelet value is between 25,000 and 50,000/microL, it is defined as grade 3 thrombocytopenia; if <25,000/microL is defined as grade 4 thrombocytopenia.

Treatment Planning and Contouring

The computerized tomography (CT) simulation used for planning was made in the supine position with simulation CT (Discovery™ RT GE Medical System) using an alpha cradle. Non-contrast images were obtained for the simulation with a cross-sectional interval of 2.5 mm, the upper border at the carina, and the lower border at the L5 vertebra. The contouring is based on the 2012 publication of Wo et al (14). The planning target volume (PTV) was created with an additional 5 mm margin around the clinic target volume (CTV). The mean total prescribed dose was 45 Gy (range: 41.4–54). Spleen contouring was performed on the treatment plan tomography and the doses received were calculated with the Varian ARIA planning system.

Spleen doses

The spleen contour of each patient was checked by the responsible and assistant investigators before the study. Subsequently, the doses received by the spleen were noted. The dose parameters evaluated were spleen V₅, V₁₀, V₁₅, V₂₀, V₂₅, V₃₀, and mean spleen dose (MSD).

Statistics

We used SPSS 26 (IBM Corp, Armonk, NY) for the analysis. Patients' categorical demographic characteristics were calculated with chi-squared and Fisher's exact tests. The data's fit to the normal distribution was evaluated visually and analytically, and nonparametric

tests were used because they did not fit the normal distribution. Correlation analysis of two numerical variables, Spearman correlation test was used. The statistical significance limit was accepted as less than 0.05.

RESULTS

The data from 28 patients with gastric adenocarcinoma who received adjuvant RT in Ankara City hospital between 12.05.2020 and 22.06.2021 were evaluated retrospectively. Seventeen (60.7%) of the patients were male and 11 (39.3) were female. The median patient age at the time of diagnosis was 63 years (range: 33–81). The median total dose was 45 (range: 41.40–55) Gy. Of the total, 22 (78.6%) patients received concurrent CT. The 3D technique was used for two patients (7.1%); the VMAT technique was used for 26 (92.7%) patients. The most frequently used energy is 6 MV (64.3%). For one obese patient (3.6%), 15 MVs were used. In the stage evaluation, the most common stage of the patients was 3A (n:11; 39.3%). The dose parameters were noted as mean splenic dose values (V₅, V₁₀, V₁₅, V₂₀, V₂₅, V₃₀). The patient and treatment details are given in *table 1*. The change in blood parameters of the patients with the RT process is summarized in the *table 2* and *fig. 1*.

Analysis of absolute lymphocyte count

There was no statistically significant difference between the mid-treatment absolute lymphocyte count and the mean splenic dose ($p=0.361$); by splenic dose, the results were V₅ ($p=0.672$), V₁₀ ($p=0.883$), V₁₅ ($p=0.727$), V₂₀ ($p=0.956$), V₂₅ ($p=0.214$), and V₃₀ ($p=0.131$). There was no statistically significant difference between the end-of-treatment absolute lymphocyte count and the mean splenic dose ($p=0.778$); by dose, V₅ ($p=0.329$), V₁₀ ($p=0.336$), V₁₅ ($p=0.863$), V₂₀ ($p=0.704$), V₂₅ ($p=0.403$), and V₃₀ ($p=0.377$). Similarly, there was no

Table 1 - Patients and Treatment Details

Gender	Female	11 (39.3%)
	Male	17 (60.7%)
Stage	1B	1 (3.6%)
	2B	4 (14.3%)
	3A	11 (39.3%)
	3B	8 (28.6)
	3C	2 (7.1%)
	4	2 (7.1%)
RT Technique	3D	2 (7.1%)
	VMAT	26 (92.9%)
RT Energy	6 MV	18 (64.3 %)
	10 MV	9 (32.1%)
	15 MV	1 (3.65)
Chemotherapy	None	7 (25%)
	Neoadjuvant	8 (28.6%)
	Adjuvant	13 (46.4)
Concurrent CT	No	6 (21.4%)
	Yes	22 (78.6%)
Concurrent CT agent	Cisplatin + etoposid	1 (3.6%)
	Capecitabine	7 (25%)
	5 FU	14 (50%)
Mean splenic dose	Median (range)	2686 (340-3488)
	Mean (SE)	2530.88 (183.14)
Spleen V ₅	Median (range)	100 (10-100)
	Mean (SE)	91.12 (4.80)
Spleen V ₁₀	Median (range)	99.40 (0-100)
	Mean (SE)	87.08 (5.45)
Spleen V ₁₅	Median (range)	92 (0-100)
	Mean (SE)	78.31 (6.01)
Spleen V ₂₀	Median (range)	77(0-100)
	Mean (SE)	67.35(6.57)
Spleen V ₂₅	Median (range)	54(0-97)
	Mean (SE)	52.20(6.44)
Spleen V ₃₀	Median (range)	41(0-70)
	Mean (SE)	35.44(4.95)

Abbr. SE: Standard Errors; MV: Megavoltage; V₅: Volume Receiving 5 Gy; V₁₀: Volume Receiving 10 Gy; V₁₅: Volume Receiving 15 Gy; V₂₀: Volume Receiving 20 Gy; V₂₅: Volume Receiving 25 Gy; V₃₀: Volume Receiving 30 Gy.

statistically significant difference between the three-month control after treatment absolute lymphocyte count and the mean splenic dose ($p=0.110$); by dose, V₅ ($p=0.507$), V₁₀ ($p=0.881$), V₁₅ ($p=0.342$), V₂₀ ($p=0.274$), V₂₅ ($p=0.440$), and V₃₀ ($p=0.710$).

Table 2 - Patients' blood values

		Basal	Mid-treatment	The end of treatment	Third months control
Absolute lymphocyte count	Median (range)	1,36 (0.56-3.92)	0.41 (0.14-1.97)	0.28 (0.08-2.89)	0.85 (0.10-1.78)
	Mean (SE)	1,62 (0.16)	0.49 (0.07)	0.54 (0.12)	0.89 (0.10)
Absolute neutrophil count	Median (range)	3.65 (0.95-22.5)	3.34 (1.03-6.75)	2.56 (1.77-5.81)	2.29 (0.04-6.53)
	Mean (SE)	4.51 (0.77)	3.52 (0.28)	2.94 (0.19)	2.67 (0.33)
Platelet count	Median (range)	223.000 (87.000-720.000)	181.000 (50.000-550.000)	156.500 (58.000-483.000)	199.000 (64.000-344.000)
	Mean (SE)	264.530 (25.95)	202.920 (20.32)	178.785(18.98)	199.250 (15.65)

Abbr. SE: Standard Errors

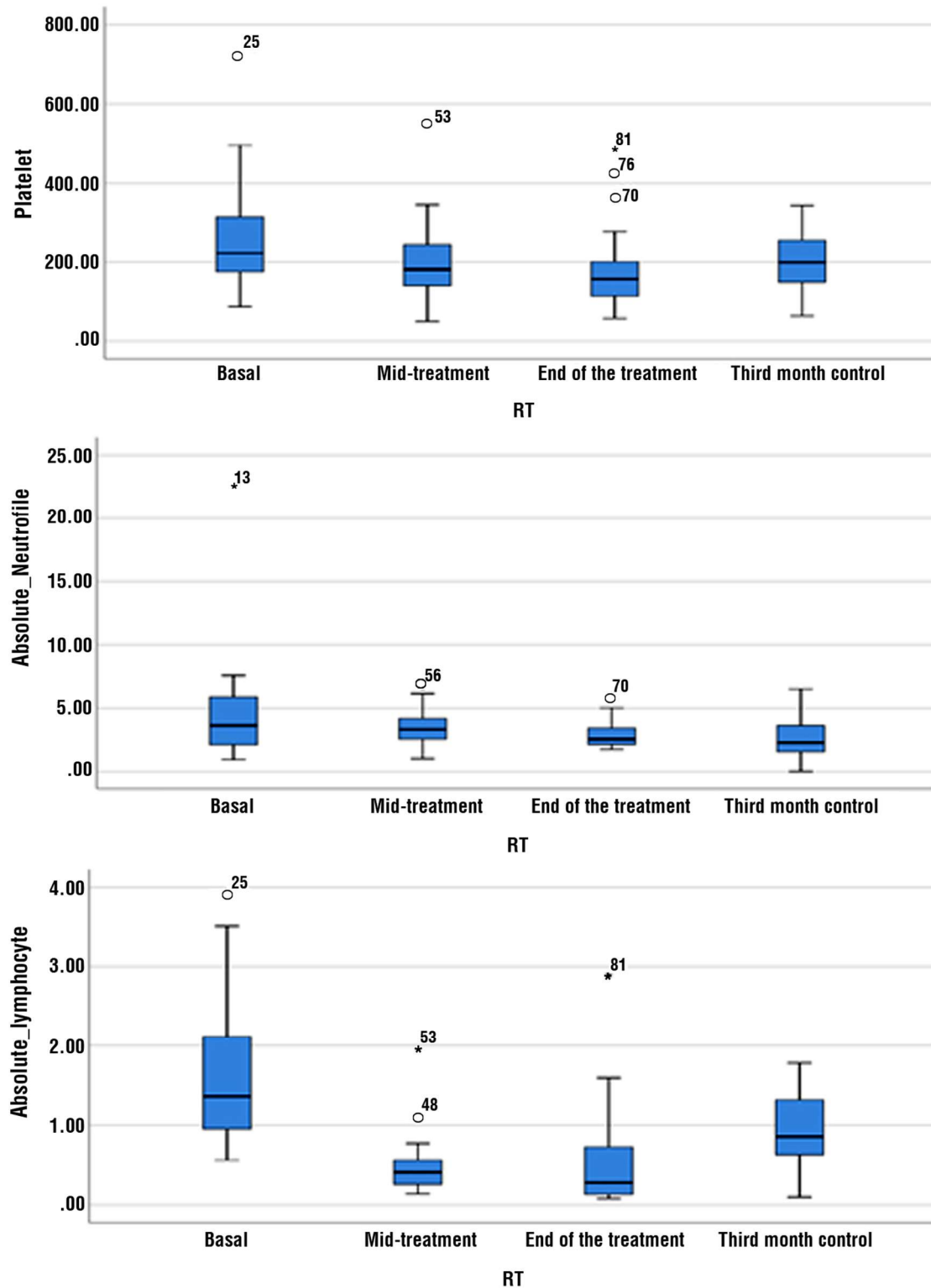


Figure 1 - The change of the blood parameters of the patients with the RT process

Analysis of absolute neutrophil count

There was no statistically significant difference between the mid-treatment absolute neutrophil count

and the splenic doses V_5 ($p=0.174$), V_{25} ($p=0.107$), or V_{30} ($p=0.124$). Statistically significant relationships were found between the mid-treatment absolute neutrophil count and the mean splenic dose (Spearman's

$\rho=0.433$; $p=0.044$), as well as doses V_{10} (Spearman's $\rho=0.462$; $p=0.030$), V_{15} (Spearman's $\rho=0.461$; $p=0.031$), and V_{20} (Spearman's $\rho=0.434$; $p=0.044$) (table 3) was no statistically significant difference between the end-of-treatment absolute neutrophil count and the mean splenic dose ($p=0.901$); by dose, V_5 ($p=0.441$), V_{10} ($p=0.593$), V_{15} ($p=0.891$), V_{20} ($p=0.780$), V_{25} ($p=0.959$), and V_{30} ($p=0.730$). Similarly, there was no statistically significant difference between the three-month control after treatment absolute neutrophil count and the splenic doses V_5 ($p=0.372$), V_{10} ($p=0.127$), V_{15} ($p=0.103$), V_{20} ($p=0.29$), V_{25} ($p=0.056$), and V_{30} ($p=0.730$). There were statistically significant relationships between the three-month control after treatment absolute neutrophil count and the mean splenic dose (Spearman's $\rho=-0.494$; $p=0.037$) and V_{30} (Spearman's $\rho=-0.490$; $p=0.039$) (table 4).

Analysis of platelet count

There was no statistically significant difference between the mid-treatment platelet count and the mean splenic dose ($p=0.828$); by splenic dose, V_5 ($p=0.717$), V_{10} ($p=0.442$), V_{15} ($p=0.351$), V_{20} ($p=0.392$), V_{25} ($p=0.996$), and V_{30} ($p=0.988$). There was also no statistically significant difference between the end-of-treatment platelet count and the mean splenic dose ($p=0.748$); by dose, V_5 ($p=0.972$), V_{10} ($p=0.524$), V_{15} ($p=0.804$), V_{20} ($p=0.917$), V_{25} ($p=0.701$), and V_{30} ($p=0.598$). There was no statistically significant difference between the three-month control platelet count and the mean splenic dose ($p=0.494$); by dose, V_5 ($p=0.484$), V_{10} ($p=0.739$), V_{15} ($p=0.750$), V_{20} ($p=0.997$), V_{25} ($p=0.559$), and V_{30} ($p=0.570$).

DISCUSSION

Only the mean spleen dose was associated with the mid-treatment and three-month control neutrophil value decreases. Changes in the mid-treatment neutrophil count were also associated with spleen doses V_{10} , V_{15} , and V_{20} , while the three-month post-treatment neutrophil count was associated with V_{30} .

The spleen was not initially considered at risk due to radiotherapy. However, it is important in the regulation of the hematopoietic and immune systems (15). It affects the first by serving as erythrocyte quality control and removing aged erythrocytes. Second, the spleen acts as a pool for hematopoietic cells, especially platelets. Physiological platelet sequestration occurs in the spleen, and approximately one-third of the platelet mass is collected there. Based on this data, the relation-

Table 3 - Analysis of mid-treatment absolute neutrophil count and splenic dose variables

Parameters	Spearman Rho Coefficient	P-value
Mean splenic dose	-0.433	0.037
Spleen V_{10} doses	-0.462	0.030
Spleen V_{15} doses	-0.461	0.031
Spleen V_{20} doses	-0.434	0.044

Abbr. V_{10} : Volume Receiving 10 Gy; V_{15} : Volume Receiving 15 Gy; V_{20} : Volume Receiving 20 Gy

ship between platelet count and spleen dose was evaluated, but no significant relationship was found.

The spleen also contributes to erythrocyte regulation. The main erythrocyte regulation task in adults is cleaning up unsuitable erythrocytes and erythrocytes that have completed their lifespans; in other words, performing quality control. This is why an increase in erythrocytes containing siderotic granules is observed in splenectomy cases (16,17). Whether this function of the spleen was affected by radiotherapy could not be evaluated due to the retrospective nature of this study.

The spleen is involved in immune system regulation through its role in lymphocyte regulation. T lymphocytes interact with dendritic cells and B lymphocytes in the spleen. The replication and differentiation of activated B lymphocytes also occur in the white pulp of the spleen. Because of these activities, the spleen contributes to adaptive immunity (18). In particular, the fact that immune therapies are increasingly applied concurrently or in sequential treatment schemes with RT makes evaluating the doses received by the spleen important. However, evaluating this interaction via lymphocyte count alone may be insufficient. In our study, no significant relationship was found between the change in lymphocyte count and spleen dose parameters.

Studies evaluating the effects of spleen doses on spleen functions are limited in the literature. The differences in parameters that studies examine and the heterogeneity of the evaluated patient groups make evaluating these studies together difficult. Because of the different technical characteristics (energy, device used, irradiation time, etc.) of radiotherapy, the patients' chemotherapy schemes are not standardized

Table 4 - Analysis of at the third months control absolute neutrophil count and splenic dose variables

Parameters	Spearman Rho Coefficient	P-value
Mean splenic dose	0.494	0.044
Spleen V_{30} doses	0.490	0.039

Abbr. V_{30} : Volume Receiving 30 Gy.

and the parameters used for hematological evaluation are different; additionally, the patient populations in the studies are heterogeneous, so a dose limit value for the spleen cannot be determined from a common study pool.

Among the hematological parameters examined in this study, only neutrophil count and spleen dose parameters were found to be significantly correlated. Contrary to expectations, no significant correlation was found using lymphocyte or platelet counts. Some retrospective studies in the literature report that the radiation dose received by the spleen adversely affects the lymphocyte counts in particular. In Alexandriu et al.'s study evaluating the spleen doses of 46 patients with GIS tumors, a 1 Gy increase in the mean spleen dose was associated with a 1% decrease in the absolute lymphocyte count (11). In the same study, a correlation was found between the spleen V15 value and the rare lymphocyte count. However, the presence of different cancer types and, accordingly, different chemotherapy schemes in that study make it difficult to evaluate. Again, data on whether the patients received G-CSF treatment were not reported.

Yalamanchali et al. also studied the relationship between the spleen radiation dose and lymphocyte counts in a larger set of patients with GIS malignancies (n=140) (10). In that study, the fractional lymphocyte loss rate (FLL) and total percent change in absolute lymphocyte count (% Δ ALC) were used to evaluate lymphocyte count kinetics. One of the important results of this study is the identification of a significant relationship between the development of grade 4 lymphopenia and spleen V5 values. Additionally, a 1 Gy increase in the mean spleen dose increased the risk of developing grade 3 and above lymphopenia by 18.6%, and FLL was an important predictor of the development of lymphopenia.

In studies conducted with patient groups who have the same cancer diagnosis, a relationship between spleen dose and lymphopenia was reported. Liu et al. reported that mean spleen dose and V5 values were effective in predicting the absolute lymphocyte count in their study of 59 patients with HCC (19). Another study focusing on the same issue in patients with locally advanced pancreatic cancer reported that mean spleen dose was the most important predictor of the development of lymphopenia (20). Saito et al. evaluated the background data of 61 patients with esophageal cancer and reported that an increase of 1 Gy in MSD caused a 2.9% decrease in the absolute rare lymphocyte value (12).

To our knowledge, no publication in the literature

evaluates the relationship between spleen doses and lymphopenia during gastric cancer radiotherapy alone, and our results differ from existing publications in terms of the development of lymphopenia. We believe the difference in the results is due to the small number of patients and the fact that other factors (initial bone marrow reserve, patient age, comorbid diseases, previous viral infections, G-CSF use, etc.) were not adequately investigated in other studies.

The small number of patients evaluated in our study, the order of chemotherapy schemes, and the different agents used weakened the study's evaluation of the relationship between the spleen radiation dose and hematological parameters. Despite these limitations, the fact that only gastric cancer cases were evaluated and patients were included according to their use of G-CSF are strengths in this study.

CONCLUSION

In our study, unlike reports in the literature, no significant correlation was found between the radiation dose received by the spleen and the change in lymphocyte measurements. A significant correlation was found between the change in mean spleen dose and the neutrophil count. To optimally evaluate the relationship between the change in spleen radiation dose and the hematological parameters, certain criteria that may affect the lymphocyte count must be standardized, and prospective and radiobiological studies must be performed.

Conflicts of interest and source of funding

There is no conflict of interest and funding.

Ethics approval

Ethical approval of this study was obtained by the Ethics Committee of Ankara City Hospital number. 2 with the date 1071172021 and number E2-21-985.

Authorship

All authors must confirm that they have met the criteria for authorship as established by the International Committee of Medical Journal Editors.

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