research article

Sequencing of chemotherapy in total neoadjuvant treatment for rectal cancer does not predict radiation-induced lymphopenia

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Background. Radiation-induced lymphopenia (RIL) is associated with an increased risk of death in solid tumors, including rectal cancer. The aim of this study was to determine whether the sequencing of chemotherapy in total neoadjuvant treatment (TNT) for rectal cancer predicts the development of RIL.

Patients and methods. We analyzed acute hematologic toxicity data from 53 patients who underwent TNT for locally or locoregionally advanced rectal cancer between July 2022 and April 2023. Twenty-eight patients received induction chemotherapy with capecitabine and oxaliplatin [CAPOX], and 25 received consolidation chemotherapy (6 cycles of CAPOX in both groups). The chemoradiation protocol consisted of Volumetric Modulated Arc Therapy with Simultaneous Integrated Boost Radiotherapy (VMAT-SIB RT) up to 48.4 Gy in 22 fractions, concomitantly with capecitabine twice a day (*lat. bis in die*, BID). The Mann-Whitney U test was performed to compare RIL between the two patient groups. Pelvic bone marrow was contoured as a non-limiting organ-at-risk to assess the received dose, and binary logistic regression was used to determine whether RIL depends on V_{5Gy}~V_{42Gy} or the planning target volume (PTV) size.

Results. Thirty-four patients (64.2%) developed RIL of any grade, which was not significantly associated with either the induction or consolidation chemotherapy TNT regimen (Wald = 3.159, p = 0.076). No significant differences were found in neutrophil counts or the neutrophil-to-lymphocyte ratio. In the logistic regression model predicting the likelihood of RIL, two variables were statistically significant: V_{10Gy} (Wald = 4.366, p = 0.037) and V_{30Gy} (Wald = 6.084, p = 0.014). These results indicate that V_{10Gy} < 71% and V_{30Gy} < 26.6% may reduce the likelihood of developing RIL.

Conclusions. In our study, the sequencing of chemotherapy in TNT for rectal cancer did not predict the development of RIL. However, the incidence of RIL may be reduced by applying RT dosimetric constraints to the pelvic bone marrow.

Key words: radiation-induced lymphopenia; rectal cancer; total neoadjuvant treatment

Introduction

Radiation-induced lymphopenia (RIL) has been associated with a poorer prognosis in patients with solid tumors.[1-3] It develops due to the direct cytotoxic effects of radiation on lymphocytes as a result of circulating blood pool exposure, as well as the impact on lymphoid tissues and bone marrow, which depends on the size and location of the treatment field.[4] The severity of RIL is additionally influenced by concurrent cytotoxic or immunosuppressive systemic therapy. Patients with persistently low absolute lymphocyte count, irrespectively of grade are at increased risk for tumor

progression and opportunistic infections due to compromised adaptive immunity.[5,6]

Total neoadjuvant treatment (TNT), which includes chemoradiation combined with either induction or consolidation chemotherapy, has become the standard of care for locally and regionally advanced rectal cancer with high risk of recurrence.[7] This approach not only improves disease control and survival outcomes but also serves as a strategy to avoid surgery and promote organ preservation.[8,9] However, little is known about the impact of chemotherapy sequencing on RIL. Since consolidation chemotherapy appears to be the preferred option for organ preservation, it is essential to examine how these two different sequencing strategies influence RIL and their potential implications for treatment outcomes.

Patients and methods

Patient selection and treatment protocol

We analyzed acute hematologic toxicity data from 53 patients who underwent TNT for locally or locoregionally advanced rectal cancer between July 2022 and April 2023 (IKONA trial, NCT05054959). Among them, 28 patients received induction chemotherapy, while 25 received consolidation chemotherapy. Induction regimen consisted of 4 cycles of capecitabine (1000 mg/m² twice a day [BID], orally) administered from the first to the 14th day of each cycle, along with a single intravenous dose of oxaliplatin (130 mg/m²) on the first day of each cycle (CAPOX), followed by chemoradiation and afterwards an additional 2 cycles of CAPOX. The consolidation regimen consisted of six cycles of CAPOX, followed by chemoradiation.

The chemoradiation protocol included Volumetric Modulated Arc Therapy with Simultaneous Integrated Boost Radiotherapy (VMAT-SIB RT). Pelvic lymph nodes received 41.8 Gy in 22 fractions, with a Simultaneous Integrated Boost (SIB) to the gross tumor volume (GTV) up to 46.2 Gy, or 48.4 Gy in the case of T4 tumors (Figure 1). RT was administered concomitantly with capecitabine (825 mg/m² BID), which was given continuously from the first to the last day of radiotherapy, including weekends.

Data collection

Absolute lymphocyte and neutrophil counts were obtained from the hospital information system as part of routine peripheral blood work, collected at both the beginning and end of treatment. The neutrophil-to-lymphocyte ratio (NLR) was calculated from these values. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 (grade 1 < lower level of normal - 0.8 x 10^9/L; grade 2 < 0.8–0.5 x 10^9/L; grade 3 < 0.5–0.2 x 10^9/L; grade 4 < 0.2 x 10^9/L).

In the planning CT scans, pelvic bone marrow was contoured as a non-limiting organ-at-risk to assess the received radiation dose and its correlation with the planning target volume (PTV) size. Dosimetric data for bone marrow volumes receiving 5 to 42 Gy (V_{5Gy}–V_{42Gy}) were extracted from dose-volume histograms using the Varian Eclipse treatment planning system, version 15.1.

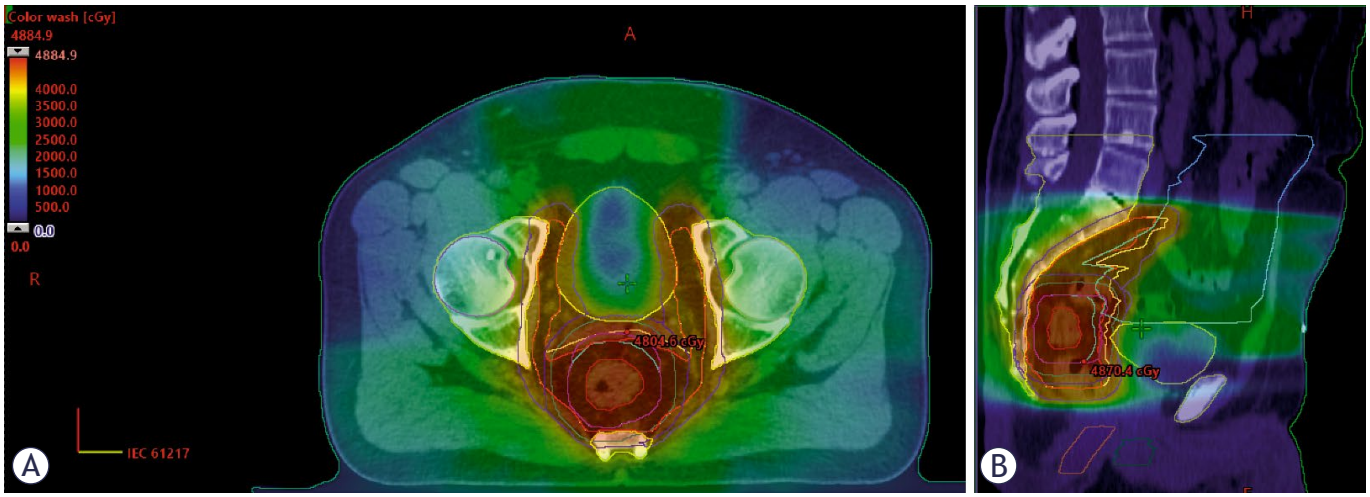

FIGURE 1. Dose distribution of volumetric modulated arc therapy with simultaneous integrated boost in a representative patient with locally advanced rectal cancer undergoing total neoadjuvant treatment. Bone marrow contour is in yellow. **(A)** Axial view. **(B)** Sagittal view.

TABLE 1. Patient, lymphocyte count and dosimetric characteristics

	Induction chemotherapy	Consolidation chemotherapy
Number of patients	28	25
Sex	25 M, 3 F	20 M, 5 F
Mean age at the start of treatment (years)	59.1 (37-76)	54.3 (33-72)
Mean absolute lymphocyte count at the start of treatment	2.13 x 10^9/L (0.78 IQR)	1.95 x 10^9/L (1.03 IQR)
Mean absolute lymphocyte count at the end of treatment	1.12 x 10^9/L (0.52 IQR)	0.92 x 10^9/L (0.37 IQR)
Mean change in lymphocyte count	-1.05 x 10^9/L (0.77 IQR)	-1.10 x 10^9/L (1.02 IQR)
Number of patients with RIL	15 (53.6%)	19 (76.0%)
Mean PTV size	1273 mL (299 IQR)	1259 mL (378 IQR)
Mean pelvic bone marrow volume	1767 mL (304 IQR)	1693 mL (362 IQR)
Mean dose to pelvic bone marrow	20.38 Gy (3.04 IQR)	20.43 Gy (2.16 IQR)
V_{5Gy}	80.23% (8.48 IQR)	80.26% (8.75 IQR)
V_{10Gy}	71.34% (10.0 IQR)	69.43% (11.1 IQR)
V_{20Gy}	50.95% (9.1 IQR)	51.06% (8.6 IQR)
V_{30Gy}	26.65% (8.2 IQR)	26.56% (6.9 IQR)
V_{40Gy}	10.25% (3.6 IQR)	10.10% (2.9 IQR)
V_{42Gy}	5.47 % (2.1 IQR)	4.90% (2.5 IQR)

F = female, Gy = Gray, IQR = interquartile range, L = liter, M = male, PTV = planning target volume, RIL = radiation-induced lymphopenia, TNT = total neoadjuvant treatment, V_x = volume of pelvic bone marrow that receives X Gy

TABLE 2. Neutrophil-to-lymphocyte ratio (NLR) pre- and post-treatment

	Induction chemotherapy	Consolidation chemotherapy
Mean absolute neutrophil count at the start of treatment	5.15 x 10^9/L (1.94 IQR)	5.79 x 10^9/L (2.12 IQR)
Mean absolute neutrophil count at the end of treatment	2.79 x 10^9/L (0.88 IQR)	3.03 x 10^9/L (1.53 IQR)
Mean change in neutrophil count	-2.35 x 10^9/L (1.63 IQR)	-2.76 x 10^9/L (2.88 IQR)
Mean pre-treatment NLR	2.76 (1.18 IQR)	3.27 (2.33 IQR)
Mean post-treatment NLR	2.83 (1.64 IQR)	3.73 (1.49 IQR)
Mean change NLR	+0.07 (1.49 IQR)	+0.46 (2.44 IQR)

IQR = interquartile range, NLR = neutrophil-to-lymphocyte ratio

Statistical analysis

Statistical analysis was conducted using SPSS software (Statistical Package for the Social Sciences, version 29.0, IBM Corp., Armonk, NY, USA). The Mann-Whitney U test was used to compare RIL between the two patient groups, with a p-value of less than 0.05 considered statistically significant. Additionally, binary logistic regression was performed to identify factors influencing the likelihood of RIL development.

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (Ref. No. 0120-214/2021).

Results

Lymphopenia

Patient and dosimetric characteristics are shown in Table 1. Thirty-four patients (64.2%) developed RIL of any grade, which was not significantly as-

sociated with either the induction or consolidation chemotherapy TNT regimen (Wald = 3.159, p = 0.076). Grade 1, 2, and 3 lymphopenia were developed by 20, 12, and 2 patients, respectively, while no Grade 4 lymphopenia was recorded.

In the logistic regression model predicting the likelihood of RIL, two variables were statistically significant: V_{10Gy} (Wald = 4.366, p = 0.037) and V_{30Gy} (Wald = 6.084, p = 0.014). These results indicate that $V_{10Gy} < 71\%$ and $V_{30Gy} < 26.6\%$ may reduce the likelihood of developing RIL. The size of PTV did not predict RIL.

Neutropenia and NLR

No cases of neutropenia below 1.5 × 10⁹/L were recorded at the end of treatment. Pre- and post-treatment neutrophil-to-lymphocyte ratio (NLR) values are presented in Table 2. No significant differences were observed between the groups.

Discussion

This is the first study, to our knowledge, to explore the relationship between the sequencing of chemotherapy within TNT for rectal cancer and the development of RIL. Our data indicate no significant association between induction or consolidation chemotherapy and the risk of RIL. This finding is particularly important because several studies have shown that starting with radiotherapy, followed by consolidation chemotherapy, increases the likelihood of a complete clinical response of the tumor and, consequently, the number of patients who can undergo a 'watch-and-wait' approach as an organ preservation strategy.[10,11]

The two most notable phase 2 randomized trials, CAO/ARO/AIO-12 and OPRA, that both compared induction and consolidation chemotherapy did not report lymphopenia rates, but acute hematologic toxicity described by neutropenia, febrile neutropenia and low platelets count. No differences were observed between groups.[12-15] Lymphopenia as a side effect of radiotherapy in rectal cancer was recently assessed in a retrospective study comparing fractionation schedules.[16] Lymphocyte count declines were greater in normofractionation groups (1.8 Gy or 2 Gy per fraction) than in hypofractionation groups (3.4 Gy or 5 Gy per fraction). In their analysis, $V_{30Gy} < 30\%$ of bone marrow volume was associated with a reduced risk of RIL, which is consistent with our observation of $V_{30Gy} < 26.6\%$. Our other suggested constraint ($V_{10Gy} < 71\%$) appears to be slightly more conservative as studies exploring haematological toxicity in cervical cancer recommended cut-off values of V_{10Gy} in the 75–95% range.[17]

Our results should be interpreted solely in the context of acute toxicity outcomes, not the potential long-term impact on bone marrow function, which represents a limitation of this study. Research on patients with advanced oral cancer has shown that, unlike surgery, lymphopenia following radiotherapy can persist for at least one year after treatment completion.[18,19] It would therefore be valuable to investigate whether recovery rates after the subacute toxicity period differ between the two TNT groups. This area of research could also prove valuable with the adoption of immunotherapy in this setting.[20] For instance, in the preliminary results of the PKUCH 04 trial that incorporated PD-1 blockade in the TNT protocol, Grade 3 lymphopenia occurred in 24% of the patients.[21]

It should be noted that the term 'radiation-induced' may be potentially misleading, since lymphopenia can also be exacerbated by systemic treatment. A more appropriate general term might be 'treatment-related lymphopenia.'[22] However, our study also aimed to evaluate the influence of the radiation dose to the bone marrow, the organ at risk, which explains our deliberate choice to use the term radiation-induced lymphopenia (RIL).

We acknowledge that there is likely underreported variability in bone marrow contouring. Typically, whole pelvic bones, including the fifth lumbar vertebra (L5), are delineated as a surrogate for pelvic bone marrow.[23] However, in cervical cancer, freehand contouring of low-density bone marrow regions has been shown to better predict higher-grade hematologic toxicity. In our study, we used whole pelvic bone contouring, currently more widely adopted technique in clinical practice.[24]

Conclusions

In conclusion, our study provides novel insights into the relationship between TNT sequencing and RIL in rectal cancer, demonstrating no significant impact of induction versus consolidation chemotherapy on acute lymphopenia risk, alongside identifying bone marrow dose constraints ($V_{10Gy} < 71\%$, $V_{30Gy} < 26.6\%$) that may mitigate this toxicity. These findings align with recent evidence on fractionation and dosimetry effects. Future studies should explore long-term bone marrow recov-

ery in this setting, refine bone marrow contouring techniques, and assess the implications of RIL for the emerging immunotherapy era in organ preservation treatment strategies.

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