research article

The role of haematological parameters in predicting the response to radical chemoradiotherapy in patients with anal squamous cell cancer

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Background. Historically, the treatment of choice for anal cancer had been abdominoperineal resection (APR). Radical radiotherapy with concurrent 5-fluorouracil plus mitomycin C chemotherapy was later established as standard therapy, although with a failure rate of 20–30%. The aim of this study was to evaluate the outcomes after radical chemoradiotherapy (CRT), prognostic and predictive factors and patterns of failure.

Patients and methods. This study included 47 patients treated with radical CRT for patohistologicaly confirmed anal squamous cell carcinoma. Analysed haematological parameters included: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and haemoglobin level. The final logistic regression model included treatment break period. Tumour response was assessed at 24 weeks from CRT completion. Follow-up was performed every 3 months during the first two years, and every 6 months thereafter.

Results. A complete clinical response (CR) was detected in 30 patients (63.8%). Patients who did not achieve a 6-months CR and those who had a CR after 6 months but then relapsed were referred to surgical treatment. With combined CRT and surgical salvage treatment the CR rate was 80.9%. Patients with CR after 6 months had significantly longer disease-free survival (DFS), progression-free survival (PFS), and overall survival (OS). A significant effect on the 6-month response was confirmed for PLR ($p = 0.03$).

Conclusions. Important prognostic factors associated with CR were baseline haemoglobin level and period of treatment interruptions. Potential haematological prognostic factors could be PLR and NLR, which can be routinely determined by low-cost and minimally invasive methods.

Key words: anal cancer; chemoradiotherapy; haematological parameters

Introduction

The anal cancer is a rare malignancy in the general population globally. According to the latest official reports from 2018, it represented only 0.23% of all malignancies in Serbia.[1] However, over the last two decades there has been a steady increase in anal cancer incidence. This might be related to an increased spread of human papilloma virus (HPV) and human immunodeficiency virus (HIV) through sexual transmission, which are established risk factors of this.[2,3]

Historically, the treatment of choice for the anal cancer had been abdominoperineal resection (APR). The upfront use of surgical treatment was associated with a high percentage of local recurrence (around 40%) and a five-year survival of about 40–70%.[4] This approach also leads to serious morbidity due to permanent colostomy.[5] Studies on the application of concurrent preoperative chemoradiotherapy (CRT) treatment during the 1970s gave space for further research focused on preserving the function of the anal sphincter, with better locoregional control and longer survival.[6] As a result of multiple randomized trials, radical radiotherapy (RT) with concurrent 5-fluorouracil (5-FU) plus mitomycin C (MMC) chemotherapy was established as a standard therapy for patients diagnosed with anal cancer.[7-9] Treatment with CRT leads to preservation of the anal sphincter and a 5-year survival rate up to 80%.[10] However, failure of CRT occurs in 20–30% of patients, resulting in persistent or recurrent anal cancer.[11] Radical surgery is reserved for salvage treatment in case of partial response or recurrence.[12] Although molecular targeted therapies have redefined treatment strategies in colorectal cancer, they have shown little potential in anal cancer.[13-16]

The optimal total dose, schedule of RT and radiation delivery techniques for the anal cancer continue to be evaluated. Current state-of-the-art does not provide uniform recommendations regarding the mentioned RT parameters.[17] According to the ACCORD-03 trial no benefit was achieved using doses of > 59 Gy.[18] Due to the need to apply high RT doses to a large volume area, with combined toxicity of concomitant chemotherapy, adverse events requiring a treatment break of CRT are reported in up to 80% of patients.[19] On the other hand, some studies have shown that limited breaks in treatment are associated with increased local disease control.[20] The split-course approach with a planned treatment break can be an option to reduce treatment-related toxicity and avoid required interruptions.[21]

The aim of this study was to evaluate the outcomes after radical CRT for patients with anal squamous cell cancer, and to investigate prognostic and predictive factors using the logistic regression model, as well as patterns of failure.

Patients and methods

Patients

We retrospectively reviewed medical records of 53 patients who were treated with radical CRT for anal cancer between January 2009 and December 2019 at the Institute for Oncology and Radiology of Serbia. Patients who underwent palliative therapy (n = 6) were excluded, so the final analysis was conducted on 47 patients. All patients had a patohistological diagnosis of anal squamous cell cancer confirmed by endoscopic biopsy. Prior to treatment, patients underwent physical examination, conventional radiography or computed tomography (CT) of the chest and CT or magnetic resonance imaging (MRI) of the abdomen and pelvis. Ethylenediaminetetraacetic acid (EDTA) peripheral blood was drawn by venipuncture and haematological parameters were derived from the absolute differential counts of a complete blood count (CBC). The neutrophil-to-lymphocyte ratio (NLR) was calculated as a ratio of circulating neutrophil and lymphocyte counts, and the platelet-to-lymphocyte ratio (PLR) was defined as the absolute count of platelets divided by the absolute lymphocyte count. Patients' pre-treatment haemoglobin levels were obtained from medical history. The staging of the tumour was re-evaluated according to the eighth edition of the Union for International Cancer Control (UICC) TNM staging system for cancer of the anal canal.[22] The general condition of the patients was classified using the ECOG Scale of Performance Status.[23]

Chemoradiotherapy

RT began on the first day of chemotherapy and was administered 5 times a week with a daily fraction of 1.8 Gy. External beam RT was performed with either an anteroposterior-posteroanterior (2D technique) or three-dimensional conformal RT (3D-CRT).[24] The target volumes and dose prescription were defined according to the International Commission on Radiation Units and Measurements (ICRU) Reports 50 and 62.[25,26] The gross tumour volume (GTV) encompassed the visible primary tumour on physical examination

and imaging. Gross disease clinical target volume (CTV3) includes GTV with a 2 cm margin expansion but excluding uninvolved bone, muscle, or air. Nodal GTV was defined as all nodes that are ≥ 1.5 cm, or biopsy proven nodes. The clinical target volume (CTV1) included the gross disease CTV, areas at risk for microscopic spread, and regional lymph nodes (presacral, internal and external iliac, and inguinal nodes). The prescribed dose for this volume was 36 Gy in 20 fractions. After a two-week break in treatment according to the split course approach, RT was continued with a boost dose of 14.4 Gy in 8 fractions to CTV2, for a total prescribed dose of 50.4 Gy. The CTV2 included the gross disease CTV in addition with areas at risk for microscopic spread, and regional lymph nodes inferior to the sacroiliac joint. In cases with inguinal lymph node metastases, inguinal nodes were also included in this volume. The planning target volume (PTV) was extended from CTV with margins of 1 cm in all directions. After administration of 50.4 Gy, an additional boost of 9 Gy in 5 fractions was applied to the gross disease CTV (CTV3 = PTV3). Radiation was delivered with a 10 MV linear accelerator.

Chemotherapy consisted of two cycles of 5-FU and MMC. MMC (12 mg/m^2) was administered on the first day of both parts of RT. 5-FU infusion (1000 mg/m^2) was given on days 1 to 4 at the first and the second part of RT.

The treatment compliance and acute toxicity were evaluated weekly according to the common terminology criteria for adverse events (CTCAE) v.5.0.[27]

Assessment of tumour response

Tumour response was assessed at 24 weeks from CRT completion. The response to treatment was evaluated by a digital rectal examination, rectosigmoidoscopy, and radiologic evaluation (pelvic CT or MRI). Results of the clinical assessment were reported as complete clinical response (CR) or incomplete response (partial regression [PR], stable disease [SD] or progression [PD]).[28] Patients with incomplete clinical response were referred to surgical treatment, as well as those with initial complete response who relapsed. In case of distant disease progression, patients were selected for chemotherapy.

Patient follow-up

Follow-up of patients was performed every 3 months during the first two years after completion of treatment, and every 6 months thereafter. Clinical examination and rectosigmoidoscopy were done at each follow-up. CT/MRI of the pelvis was performed every 3 months in the first year of follow-up and every 6 months thereafter.

Overall survival (OS) was defined as time from the date of beginning of CRT to the date of the last clinical control or the date of death. Anal cancer specific overall survival (ACSOS) excluded patients in whom death occurred for other reasons, and was calculated like OS. Progression-free survival (PFS) was calculated for patients whose response was assessed as PR, CR, or SD on initial evaluation 6 months after treatment completion, and was defined as time from the date of follow-up 6 months after treatment until the onset of progression, death or last follow-up for patients who did not progress. Disease-free survival (DFS) was based on the time from achieving a CR to the onset of progression, death, or the date of the last follow-up for patients who did not progress. Colostomy-free survival (CFS) was calculated only for patients in whom no colostomy was placed at the time of beginning of CRT, and was defined as the time from the start of treatment to the date of placement of a colostomy, death, or the date of the last follow-up for patients who did not have a colostomy. Overall treatment time (OTT) was measured as the number of days from the start of CRT to the end of treatment.

Statistical analysis

For normal distribution data testing, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used. Descriptive methods (frequencies, percent, mean, median, standard deviation [SD] and range) were used to summarize the data. The statistical significance level was set at $p < 0.05$. For comparison of disease and treatment characteristics among different risk subgroups the Wilcoxon rank sum, Pearson chi-square and Fisher exact tests were used. Methods of survival analysis were used for DFS, OS, PFS, ACSOS, CFS (median with corresponding 95% confidence interval (CI) for description, Kaplan-Meier product-limit method for illustration and log-rank test). Also, for evaluating potential predictors of the response, univariate and multivariate logistic regression was used (odds ratio (OR) with 95% CI for description, Likelihood Ratio and Wild test), and the CR after 6 months (coded as 0) *vs.* non-CR (coded as 1) was set as a dependent variable. The receiver operating characteristics (ROC) curve methods were applied to investigate the discriminative potential of NLR and

TABLE 1. Patients' disease, treatment and outcomes characteristics

Characteristics	N (%)	Characteristics	N (%)
Age (years)		**NLR**	
Mean (SD)	61.9 (10.0)	Mean (SD)	2.7 (1.7)
Median (Range)	63.0 (40.0–81.0)	Median (Range)	2.1 (0.8–7.0)
Gender		**PLR**	
Female	36 (76.6%)	Mean (SD)	159.4 (92.1)
Male	11 (23.4%)	Median (Range)	132.9 (51.7–401.2)
Performance status (PS)[1]		**RT technique**	
ECOG 0	13 (27.7%)	2D	23 (48.9%)
ECOG 1	33 (70.2%)	3D	24 (51.1%)
ECOG 2	1 (2.1%)	**The first RT part-dose (Gy)**	
T in clinical TNM		Mean (SD)	36.1 (1.6)
T2	18 (38.3%)	Median (Range)	36 (30–45)
T3	24 (51.1%)	**The second RT part-dose (Gy)**	
T4	5 (10.6%)	Mean (SD)	22.8 (2.5)
N in clinical TNM		Median (Range)	23.4 (9–26)
N0	17 (36.2%)	**Total dose (Gy)**	
N1	30 (63.8%)	Mean (SD)	58.9 (1.6)
UICC staging		Median (Range)	59.4 (52–59.4)
IIA	10 (21.3%)	**OTT (days)**	
IIB	7 (14.9%)	Mean (SD)	74.7 (14.2)
IIIA	8 (17.0%)	Median (Range)	77 (51–134)
IIIC	22 (46.8%)	**Acute toxicity-first part**	
Tumour differentiation		Without or gr. I/II	26 (55.3%)
well	24 (51.1%)	Grade III/IV	21 (44.7%)
moderate	13 (27.7%)	**Acute toxicity-second part**	
poor	4 (8.5%)	Without or gr. I/II	32 (68.1%)
without data	6 (12.8%)	Grade III/IV	15 (31.9%)
Tumour size (cm)		**Tumour response at 6 months**	
Mean (SD)	5.2 (2.0)	CR	30 (63.8%)
Median (Range)	5.4 (2.1–10.0)	PR	15 (31.9%)
Initial haemoglobin level (g/L)		SD	1 (2.1%)
Mean (SD)	116.3 (20.3)	PD	1 (2.1%)
Median (Range)	124 (66–154)	**Follow-up period (months)**	
Pretreatment colostomy		Mean (SD)	53.0 (30.9)
No	42 (89.4%)	Median (Range)	44 (11–136)
Yes	5 (10.6%)	**Total**	47 (100%)

CR = complete clinical response; NLR = neutrophil-to-lymphocyte ratio; OTT = overall treatment time; PD = disease progression; PR = partial regression; PLR = platelet-to-lymphocyte ratio; RT = radiotherapy; SD = stable disease; SD = standard deviation; UICC = Union for International Cancer Control; [1] ECOG PS = The Eastern Cooperative Oncology Group performance status

PLR for the presence/absence of CR (Area Under the ROC curve [AUC ROC] according DeLong's method; Likelihood ratio test for AUC ROC; the best cut-off value was set as value with maximum sensitivity and specificity). The statistical analysis was performed using the program R (version 3.3.2 (2016-10-31) –"Sincere Pumpkin Patch"; Copyright (C) 2016 The R Foundation for Statistical

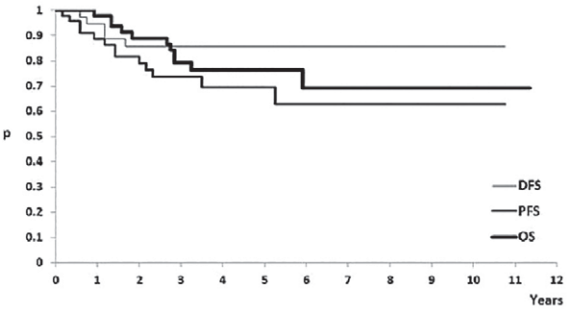

FIGURE 1. Disease-free survival (DFS), progression-free survival (PFS), and overall survival (OS) for the whole patient group.

Computing; Platform: x86_64-w64-mingw32/× 64 (64-bit); downloaded: January 21, 2017).

Ethics approval

All analyses presented in this study are part of routine clinical practice approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia and were performed in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Results

Patients' disease, treatment and outcomes characteristics are presented in Table 1. Radical CRT according to protocol was completed in 39 patients. All 47 patients completed the planned RT treatment. RT alone was performed in 3 patients. Two of them didn't receive chemotherapy due to significant medical comorbidities, and 1 patient refused the proposed chemotherapy treatment. Five patients didn't receive a second cycle of chemotherapy due to toxicities Grade 3 or 4 after the first course. The three-dimensional conformal radiotherapy (3D-CRT) was delivered in twenty-four patients, while the remaining 23 patients received the 2D technique. In the first part, majority of patients (95.7%) received the dose of 36 Gy. After a two-week break in treatment according to the split course approach, median planned dose of radiation was 23.4 Gy. The median total dose of radiation was 59.4 Gy. (Table 1).

Most patients (80.85%) had treatment pause due to toxicities. Because of treatment interruptions the median OTT was 77 days. The most common non-haematological acute toxicity was radiation dermatitis. Any grade of haematological acute complications was registered in 33 patients (70.21%). The

TABLE 2. Comparison of characteristics of complete responders (CR) and non-complete responders (non-CR) to chemoradiotherapy

Characteristic	The response to treatment after 6 months		
	CR	non-CR	Wilcoxon rank sum test
Age (years)			
Mean (SD)	60 (10.7)	65.1 (7.8)	ns
Median (Range)	59.5 (40.0–80.0)	65.0 (52.0–81.0)	
Gender			
Male	6 (20%)	5 (29.4%)	ns*
Female	24 (80%)	12 (70.6%)	
T in clinical TNM			
T2	13 (43.3%)	5 (29.4%)	
T3	14 (46.7%)	10 (58.2%)	ns#
T4	3 (10.0%)	2 (11.8%)	
N in clinical TNM			
N0	15 (50.0%)	2 (11.8%)	$p^* < 0.05$
N1	15 (50.0%)	15 (88.2%)	
Tumour size (cm)			
Mean (SD)	4.7 (1.8)	6.0 (2.1)	$p < 0.05$
Median (Range)	4.9 (2.1–8.0)	5.8 (2.3–10.0)	
Initial haemoglobin level (g/L)			
Mean (SD)	124.2 (16.9)	103.0 (18.8)	$p < 0.01$
Median (Range)	127.0 (66.0–154.0)	101.0 (68.0–132.0)	
Pretreatment colostomy			
No	28 (93.3%)	14 (82.3%)	ns#
Yes	2 (6.7%)	3 (17.6%)	
Neutrophil-to-lymphocyte ratio			
N (%)	16/30 (50%)	16/17 (50%)	
Mean (SD)	2.4 (1.8)	3.1 (1.6)	ns
Median (Range)	1.9 (0.8–7.0)	3.2 (0.9–5.6)	
Platelet-to-lymphocyte ratio			
N (%)	16/30 (50%)	16/17 (50%)	
Mean (SD)	118.3 (54.9)	200.5 (104.4)	$p < 0.05$
Median (Range)	108.3 (51.7–256.6)	158.9 (79.5–401.2)	
DFS (months)			
Median (95% CI)	NR	NR	$p^\$ < 0.05$
PFS (months)			
Median (95% CI)	NR	26 (> 17)	$p^\$ < 0.01$
OS (months)			
Median (95% CI)	NR	71 (> 33)	$p^\$ < 0.01$
ACSOS (months)			
Median (95% CI)	NR	NR	$p^\$ < 0.05$
CFS (months)			
Median (95% CI)	NR	11 (> 10)	$p^\$ < 0.01$
Total	**30 (100%)**	**17 (100%)**	-

ACSOS = anal cancer specific overall survival; CFS = colostomy-free survival; DFS = disease-free survival; CI = confidence interval; DFS = disease-free survival; NR = not reached; ns = not statistically significant; OS = overall survival; PFS = progression-free survival; SD = standard deviation; * = Pearson χ2 test; # = Fisher exact test; $ = log-rank test

TABLE 3. Logistic regression analysis of the response to treatment after 6 months

Characteristic	Logistic regression			
	Univariate		Multivariate	
	OR (95%CI)	Wild test	OR (95%CI)	Likelihood Ratio test
Age				
> 63 y vs. ≤ 63 y	1.7 (0.4–6.6)	p = 0.213	-	p = 0.884
Gender				
Male vs. Female	2.1 (0.6–7.2)	p = 0.468	-	p = 0.082
T in clinical TNM				
T3 vs. T2	1.9 (0.5–6.9)	p = 0.634	-	p = 0.940
T4 vs. T2	1.7 (0.2–13.7)			
N in clinical TNM				
N1 vs. N0	7.5 (1.4–38.7)	p = 0.006	-	p = 0.133
Tumour size (cm)				
> 4 cm vs. ≤ 4 cm	6.6 (1.3–33.8)	p = 0.011	-	p = 0.602
Initial haemoglobin level (g/L)				
< 120 g/L vs. ≥ 120 g/L	8.9 (2.2–35.6)	p = 0.001	13.4 (2.4–74.3)	p* = 0.003
RT technique				
2D vs. 3D	1.3 (0.4–4.2)	p = 0.679	-	p = 0.784
Treatment break				
> 10 days vs. ≤ 10 days	6.0 (1.6–22.3)	p = 0.005	9.6 (1.7–52.5)	p* = 0.009
Completed chemotherapy				
No vs. Yes	2.0 (0.4–9.3)	p = 0.379	-	p = 0.555

CI = confidence interval; OR = odds ratio; RT = radio therapy; * = wild test

most frequent serious haematological toxicity (gr. III/IV) was leukopenia.

Survival curves for the whole patient group are presented in Figure 1. The follow up period had a median of 44 months. The median times for DFS, PFS and OS were not reached.

Evaluation of response after 6 months

The response to treatment was evaluated after 6 months of completion of therapy. The CR was detected in 30 patients (63.8%), 24 females (80%) and 6 males.

Comparison of characteristics of complete responders and non-complete responders to chemoradiotherapy are presented in Table 2. Patients with N0 responded to treatment significantly better than patients with N1. Primary tumour size also influenced the response.

Although median times to events for DFS/PFS/OS were not reached, patients with CR after 6 months had significantly increased DFS, PFS, and OS after treatment completion compared to patients with non-CR response (Figure 2, Table 2).

Predicting the response to treatment after 6 months

A logistic regression model included nine variables (gender, age, clinical T stage, clinical N stage, tumour size, haemoglobin level, RT technique, treatment break period, and chemotherapy completion) (Table 3). It was found that patients with shorter period of treatment interruptions, a tumour size ≤ 4cm, the initial haemoglobin level more than 120 g/L, and lymph node negative patients, responded significantly better to treatment. The final model included pretreatment haemoglobin level and treatment break period.

Evaluating the potential of NLR and PLR in predicting the response to treatment after 6 months

Next, we examined if there were differences in the response to treatment after 6 months according to the cut-off values obtained by ROC analysis for NLR and PLR. (Figure 3, Table 4) According to the cut-off value obtained by ROC analysis (145.2), a

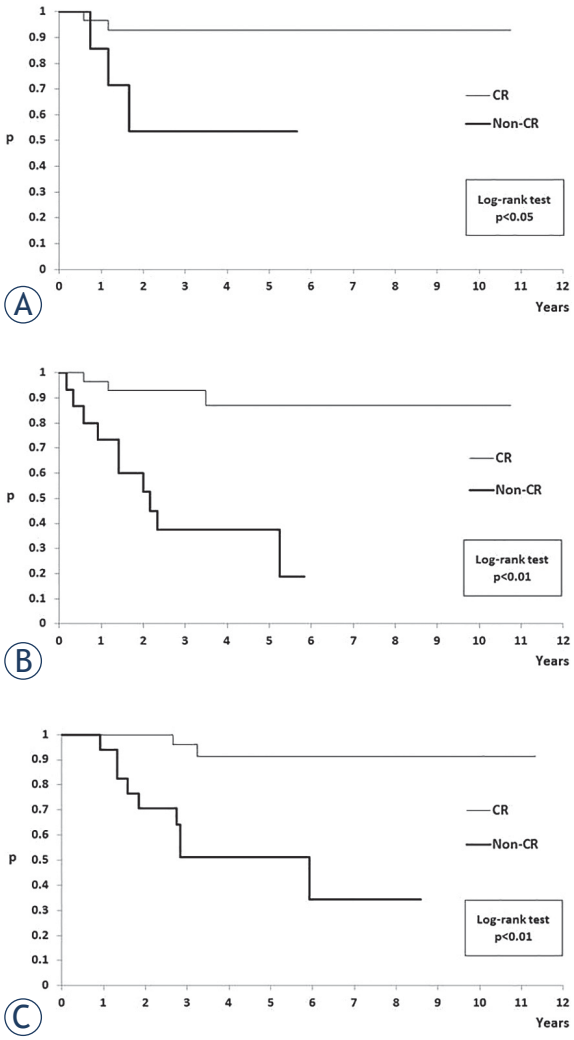

FIGURE 2. (A) Kaplan-Meier plots for Disease-free survival (DFS), **(B)** progression-free survival (PFS), and **(C)** overall survival (OS) in relation to response to treatment after 6 months.

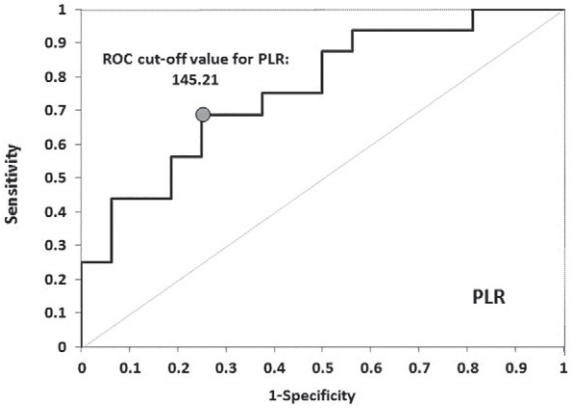

FIGURE 3. Receiver Operating Characteristics (ROC) curve for the platelet-to-lymphocyte ratio (PLR) in relation to response to treatment after 6 months.

statistically significant difference in the response was confirmed for PLR (p = 0.03). For NLR, a statistically significant effect on the response was not confirmed (p = 0.23). The patients were further divided into two group based on literature cut-off value for NLR in rectal cancer (0–3 *vs*. ≥ 3).[29] A positive trend (p = 0.06) was found when 0–3 *vs*. ≥ 3 groups were tested, in regard to the nonCR *vs*. CR response. (Table 5)

Evaluation of long term outcomes

The one patient who had disease progression was selected for chemotherapy. All other patients (n = 16) who did not achieve a CR after 6 months were referred to surgical treatment, as well as two patients who had a CR after 6 months but then relapsed. From the 11 patients that underwent surgery (APR), 9 were incomplete responders to CRT and 2 initial complete responders that relapsed. Within this surgically treated group, 9 patients (82%) achieved a complete response after surgical treatment. One patient died in early postsurgical period due to acute renal failure. Four patients relapsed after surgery, 3 of them were initial complete responders to surgery, but presented with distant metastases within two years of follow-up. All patients who relapsed after surgery were treated with postoperative chemotherapy and continued follow-up. With combined CRT and surgical salvage treatment CR rate was 80.9%. Two patients had palliative postCRT colostomy, due to medical comorbidities which didn't allow radical surgical treatment. The remaining five patients were closely followed-up without any additional treatment because they had a poor general condition or refused surgery.

Six patients developed distant metastasis during the follow-up period and five of them were referred to chemotherapy. Two of them had dissemination to the lungs, 2 to the liver and the other sites were retroperitoneum, bones, and peritoneal metastasis. Three patients had multiple metastases.

Discussion

To the best of our knowledge, no study has evaluated the long-term outcomes after CRT, as well as predictors of the response after 6 months of CRT completion in patients with anal cancer in the Balkan region. In this study, we focused on the as-

TABLE 4. Results of the ROC analysis for neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) and relevant events

Characteristics	NLR	PLR
AUC ROC[a] (95% CI)	65.2% (45.3–85.2%)	76.2% (59.5–92.9%)
Likelihood ratio test[b]	*ns*	$p < 0.05$
ROC-cut-off value[c]	-	145.2
Sensitivity (95% CI)	-	68.7% (43.7–87.5%)
Specificity (95% CI)	-	75.0% (56.1–93.7%)

AUC ROC[a] = Area Under the Receiver Operating Characteristics Curve (DeLong's method); [b] = Likelihood ratio test for AUC ROC; [c] = Value with maximum sensitivity and specificity; CI = confidence interval; ns = not statistically significant

TABLE 5. The value of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in prediction of CR *vs*. non-CR

Characteristic	The response to treatment after 6 months		
	CR	non-CR	Fisher Exact Test
NLR (literature cut-off value)			
< 3.0	13 (81.2%)	7 (43.8%)	p = 0.06
≥ 3.0	3 (18.8%)	9 (56.2%)	
PLR (literature cut-off value)			
< 160.0	13 (81.2%)	9 (56.2%)	p = 0.25
≥ 160.0	3 (18.8%)	7 (43.8%)	
PLR (ROC cut-off value)			
< 145.2	12 (75%)	5 (31.3%)	p = 0.03
≥ 145.2	4 (25%)	11(68.7%)	
Total	**16 (100%)**	**16 (100%)**	-

CR = complete clinical response, non-CR = non-complete clinical response; ROC = Receiver Operating Characteristics

sessment of new prognostic and predictive factors for CRT, evaluating demographic, clinico-pathological and haematological parameters.

There is no precise information about the optimal waiting time period for complete response after CRT. The ACT II trial showed that, in the 29% of patients who did not achieve a complete response at 11 weeks, a complete response occurred at 26 weeks.[30] In this study, the assessment of tumour response was performed 6 months after completion of CRT and CR was found in 63.8% of patients. CR was confirmed as a strong predictor of favourable long term clinical outcome. Moreover, it was also found that baseline haemoglobin level and period of treatment interruptions were independent predictors, as low initial haemoglobin level and prolonged period of treatment were related with significantly lower likelihood for CR response.

The optimal dose and schedule of RT for anal cancer also continue to be explored. The median dose in our study was 59.4 Gy, and all patients got the split-course approach. All patients included in our study were in II or III stadium of disease. Recent research suggested that for early-stage tumours < or = 10 mm, optimal radiotherapy dose should be between 40 and 50 Gy for subclinical lesions and 50–60 Gy for T1.[31] For patients with locally advanced disease (T3, T4, or lymph node-positive tumours) doses of ≥ 54 Gy administered with limited treatment breaks (less than 60 days) were associated with increased local control.[20] The results from the RTOG 92-08 trial147 suggested that doses of > 59 Gy provide no additional benefit to patients with anal cancer.[18,32]

In our study the majority of patients had treatment interruptions due to acute toxicities, which might correlate with the used RT technique. The development of an advanced technique of RT has enabled the safe application of high RT doses while reducing the dose to surrounding normal tissues like skin, small bowel, bladder, femoral heads, external genitalia, and bone marrow.[33] This leads to low rates of acute and late toxicity and excellent local control, disease-free survival, and overall survival.[34]

The relationship between inflammation and cancer has been investigated in many studies. Systemic inflammation-based scores extracted from the absolute blood cell count of peripheral blood have the potential to predict the response to various therapeutic approaches, but have still not been validated in larger patient cohorts. Advantages of blood biomarkers lay in the inexpensiveness of analyses and quick availability, as well as minimal invasiveness, and availability in initial assessment. Our recent study successfully evaluated the role of haematological parameters in predicting the survival and toxicity to specific treatment in the lung cancer setting.[35] Several studies have reported that an elevated NLR is associated with poor clinical outcome in patients with colorectal cancer.[36] To the best of our knowledge, this is the first study which aimed to analyse the prognostic role of NLR and PLR in patients with anal cancer. We analysed the discriminative potential of NLR and PLR in regard to CR *vs*. non-CR 6 months after CRT completion. It was found that patients with PLR higher than 145.2 had significantly worse CR rate after 6 months. Meta-analysis conducted by Zhnag *et al*. found that elevated NLR, PLR and platelet counts may be associated with worse survival in colorectal cancer patients.[37] However, in this study, lower NLR was

not correlated with better response, which might be due to a low number of patients, population-specific differences or differences in the analysed cancer type.

The limitations of the study include the retrospective approach, the fact that this was a single institution analysis and that the sample size was relatively low, which calls for caution in data interpretation. However, the number of analysed patients is considerable taking into consideration that anal cancer is a rare disease. Further studies should be performed on patients treated with novel RT techniques considering shorter treatment interruptions and taking into account both clinical parameters and genetic characteristics of patients, as has been suggested for other cancer types.[38,39]

Conclusions

Based on the logistic regression model important prognostic factors associated with CR in this study were baseline haemoglobin level and period of treatment interruptions. Potential haematological prognostic factors could be PLR and NLR, which can be routinely determined by low-cost and minimally invasive methods.

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