International Journal of Colorectal Disease (2019) 34:519–525
https://doi.org/10.1007/s00384-018-03225-7

ORIGINAL ARTICLE

Prognostic significance of inflammatory-related parameters in patients with anal canal cancer

Francesca De Felice[1] · Filippo Lorenzo Rubini[1] · Luca Romano[1] · Nadia Bulzonetti[1] · Rossella Caiazzo[1] · Daniela Musio[1] · Vincenzo Tombolini[1]

Accepted: 21 December 2018 / Published online: 7 January 2019

Abstract

Purpose To investigate the correlation between inflammatory-related parameters and overall survival (OS) and disease-free survival (DFS) in anal canal cancer population.

Methods and materials Patients diagnosed with anal canal carcinoma and treated with curative intent chemoradiotherapy (CRT) were included. Data about pre-treatment complete blood count were collected. Neutrophil to lymphocyte ratio (NLR), fibrinogen (F), and a combination of these (F-NLR score) were correlated with OS.

Results A total of 58 patients were enrolled. In multivariate analysis, the strongest OS prognostic factor was NLR, with a hazard ratio (HR) for low NLR compared to high NLR of 1.30 (95% confidence interval 1.01–14.12). Kaplan-Meier survival analysis showed that patients with high NLR, F, and F-NLR had significantly shorter OS and DFS.

Conclusion To our knowledge, this is the first study providing evidence that elevated pre-treatment NLR, F, and F-NLR score significantly correlate with worse survival outcomes in patients with anal canal carcinoma. In view of our findings, future clinical trials in anal canal cancer patients are warranted to verify our results.

Keywords Anal carcinoma · Radiotherapy · Neutrophil · Lymphocyte · Fibrinogen

Introduction

Concomitant chemoradiotherapy (CRT) is nowadays the standard primary treatment for patients suffering from anal canal cancer [1]. However, the number of prognostic biomarkers for these patients lags far behind when compared with the other digestive system tumors [2]. At present, there are no recommended biomarkers for daily clinical routine in patients undergoing curative treatment regardless of their stage at diagnosis [1]. Anal cancer is a rare malignancy, representing only 0.5% of all estimated new cancer cases in 2018 [3]. Five-year overall survival (OS) rates vary considerably among patients with early-stage disease (approximately 80% in stage I) compared to the advanced stages (50% in stage III down to 15% in stage IV) [4].

In the last few years, different inflammation-related blood-derived biomarkers have been investigated for their prognostic impact in various primary solid tumors, such as lung cancer, renal cell carcinoma, and epithelial ovarian cancer [5–9]. These biomarker studies mainly focused on the prognostic role of neutrophil to lymphocyte ratio (NLR) and plasma fibrinogen (F) levels. Results have suggested that high pre-treatment NLR, as well as elevated pre-treatment F levels, can be associated with tumor progression and poor outcomes.

Nevertheless, data on inflammatory-related parameters as prognosticator for anal canal carcinoma are currently missing. Therefore, we conducted the present study to elucidate the prognostic impact of NLR and F levels in anal canal cancer patients undergoing definitive CRT. We analyzed pre-treatment levels of NLR and F and correlated them with clinical outcome data. In addition, we determined whether the F-NLR score, which combines NLR and F values, also showed considerable prognostic power in these patients. We hypothesized that elevated pre-treatment inflammatory-related parameters represent a biomarker of worse survival in patients with anal canal carcinoma.

✉ Francesca De Felice
fradefelice@hotmail.it

[1] Department of Radiotherapy, Policlinico Umberto I, "Sapienza" University of Rome, Viale Regina Elena 326, 00161 Rome, Italy

Methods and materials

Study design All patients originated from the Department of Radiotherapy, Policlinico Umberto I, "Sapienza" University of Rome. The protocol was reviewed and approved by the institutional review board and the scientific review committee. Written informed consent was obtained from all patients before the initiation of therapy. Patients underwent a complete physical examination, including digital ano-rectal exploration and inguinal node palpation, anoscopic examination, and total body contrast-enhanced computed tomography. Magnetic resonance imaging of the pelvis was performed in case of an uncertain diagnosis until January 2015 and routinely thereafter. Gynecologic exam was performed in female patients. The main eligibility criteria were histological confirmation of primary squamous cell carcinoma of the anal canal, staged tumor (T) 1–4, with or without lymph nodes (N) positive at diagnosis, without evidence of distant metastases. All patients were re-staged according to the American joint committee on cancer (AJCC) staging system, eighth edition [10]. Mitomycin/5-fluorouracil were administered concurrently with RT. Details of CRT were described in our previous study [11]. Patient exclusion criteria consisted of coexistent hematologic disorders or known active infection, synchronous tumors, cardiovascular disease, history of neurological or psychiatric disorders, or previous pelvic radiotherapy. Following definitive CRT, patients were followed-up with serial complete physical examination at 6-week intervals. If clinical evidence of disease was absent, evaluation was then performed at three monthly intervals for the additional 2 years and every 6 months for subsequent years. In case of persistent disease, consideration of abdominoperineal resection was recommended [12].

The neutrophil to lymphocyte ratio and plasma fibrinogen measurements Blood samples were taken at the time of admission to the hospital within 1 week before treatment, in order to rule out any unspecific alteration due to CRT. Each patient blood sample was collected in ethylenediaminetetraacetic acid (EDTA)-treated tube and then analyzed for routine peripheral blood cells, including lymphocytes, neutrophils, monocytes, eosinophils, basophils, and platelets counts. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The cut-off of NLR was calculated with the receiver operating characteristic (ROC) curve and was utilized to dichotomize patients into an elevated NLR and a low-NLR group. Plasma F levels were measured according to the Claus method [13]. The F cut-off value, established on the ROC curve, was used to dichotomize the patients into high-F and low-F groups.

F-NLR prognostic score The F-NLR prognostic score was calculated as follows: patients with low NLR and low F value were allocated to F-NLR score 0, patients with either heightened NLR or F were allocated to the F-NLR score 1 group, and patients with both, high NLR and F, were allocated to the F-NLR score 2 group.

Statistical analysis Statistical analysis was performed using RStudio-0.98.1091 software. Standard descriptive statistics was used to evaluate the distribution of each variable. Continuous variables were reported as median and interquartile range, and categorical variables were presented as counts and percentages. The association of all analyzed inflammatory biomarkers (NLR, F, and F-NLR score) with main categorical baseline patients' characteristics (age, gender, eastern cooperative oncology group—ECOG—performance status, body mass index, human papillomavirus—HPV—status, human immunodeficiency virus—HIV—status, T stage, N stage, inappropriate surgery at diagnosis) was evaluated using the two-sided χ^2 test. Diagnostic power of both NLR and F values to predict survival was evaluated using ROC curve analysis by calculating the areas under the ROC curve (AUCs). The optimal cut-off value was defined as the point on the ROC curve that maximizes the sensitivity and specificity. OS and disease-free survival (DFS) were calculated in months from the date of the end of CRT to the first event, including date of the last follow-up or death (OS) and/or relapse (DFS). OS and DFS were estimated by the Kaplan-Meier method, and survival curves were compared by the log-rank test. p values lower than 0.05 were considered significant.

Results

Baseline patients' characteristics This study cohort consisted of 58 anal canal cancer patients. The main characteristics are detailed in Table 1. The vast majority of patients were female ($n = 46$, 79.3%). At diagnosis, most tumors presented regional node metastasis ($n = 35$; 60.3%). All patients completed the programmed CRT. Globally, CRT was associated with a high complete response rate within tolerable toxicity. In total, 37 patients (63.8%) were early responders, 17 patients (29.3%) achieved complete clinical remission within 9 months the end of CRT, and 4 patients (6.9%) underwent salvage abdominoperineal resection.

Inflammatory-related parameters Three inflammatory parameters were considered as follows: (i) NLR, (ii) F, and (iii) F-NLR score. NLR was available in all patients. The NLR ranged from 0.71 to 13.37, with a mean value of 3.02 and a median value of 2.65. Based on the ROC curve analysis, patients were categorized into two groups by the optimal NLR cut-off value of 2.50 (sensitivity 64%, specificity 59%; AUC = 0.55). Levels of NLR greater than 2.50 were considered high ($n = 31$; 53.4%), whereas NLR values equal to or less than 2.50 were defined as low ($n = 27$; 46.6%).

Table 1 Baseline patients' characteristics

Characteristic	Value (%)
Age (years), median (range)	62 (38–91)
Gender	
Male	12 (20.7)
Female	46 (79.3)
ECOG performance status	
0	49 (84.5)
1	8 (13.8)
2	1 (1.7)
Body mass index	
≤ 25	34 (58.6)
> 25	24 (41.4)
HPV infection	
No	49 (84.5)
Yes	9 (15.5)
HIV infection	
No	52 (89.7)
Yes	6 (10.3)
Clinical T stage	
1	15 (25.9)
2	15 (25.9)
3	8 (13.8)
4	20 (34.4)
Clinical N stage	
0	23 (39.7)
1a	35 (60.3)
1b	0 (0)
1c	0 (0)
Inappropriate surgery before CRT	
No	40 (69)
Yes	18 (31)

%, percentage; *HPV*, human papillomavirus; *HIV*, human immunodeficiency virus; *T*, tumor; *N*, node; *CRT*, chemoradiotherapy

F value was available in 51 patients (88% of the study population). Mean F level prior to CRT was 3.58 (± 0.74) g/L. The cut-off value of 3 g/L (sensitivity 81%, specificity 41%; AUC = 0.60) was used to dichotomize patients into high-F and low-F groups. Twenty-nine patients (56.8%) had a F level higher than 3 g/L. Twenty-two patients (43.2%) were classified as low-F group.

Then, NLR and F values were combined and the F-NLR score was calculated. Considering that in 7 patients, the fibrinogen value was missing in the medical records, F-NLR score could not be calculated in these patients. Patients were stratified in 3 groups, according to elevated NRL and high F (F-NLR 2 $n = 15$; 29.4%), elevated NRL or high F (F-NLR 1 $n = 25$; 49%), and low NRL and low F (F-NLR 0 $n = 11$; 21.6%).

Patients' clinical characteristics grouped by inflammatory-related parameters—NLR, F, and F-NLR score—are listed in Table 2. Interestingly, patients with T1–2 disease were more likely to have NLR levels below the cut-off when compared with T3–4 disease ($p = 0.02$).

Overall survival and prognostic factors Median follow-up was 36 months and 11 patients had died during the course of follow-up. The 5-year OS rate for the entire population was 68.6% (95% CI 0.482–0.823). The 5-year OS was 80.7% (95% CI 0.511–0.934) for patients with low NLR and 48.6% (95% CI 0.156–0.755) with high NLR ($p = 0.02$) (Fig. 1). Similarly, patients with low F level have longer OS compared with those with high F level (5-year OS 83.3% versus 59.4%, respectively; $p = 0.05$) (Fig. 2). Kaplan-Meier survival analysis showed that patients with high F-NLR had significantly shorter OS ($p = 0.002$) compared with patients with low F-NLR. The 5-year OS rate was 80.0% (95% CI 0.204–0.969), 83.6% (95% CI 0.480–0.957), and 34.3% (95% CI 0.068–0.652) for F-NLR 0, F-NLR 1, and F-NLR 2, respectively. Details are shown in Fig. 3.

Disease-free survival The overall 5-year DFS rate was 59.7% (95% CI 0.411–0.742). The F-NLR score was robustly correlated with the risk of recurrent disease, as showed in Fig. 4. The 5-year DFS was 72% for low F-NLR cases, 65.5% (95% CI 0.359–0.840) for F-NLR 1, and 38.6% (95% CI 0.120–0.652) for those patients with high F-NLR.

Discussion

Our study demonstrated that inflammatory-related parameters, including NLR, F, and the derived F-NLR score, provided prognostic information in anal canal cancer patients treated with definitive concurrent CRT. Pre-treatment high levels of NLR, F, and F-NLR were associated with worse OS and DFS rates. According to inflammatory-related parameters, we found that patients with advanced stage disease had statistically higher levels of NLR when compared with patients with a T1–2 lesion. We did not find any difference in terms of nodal involvement and inappropriate surgery at diagnosis.

To the best of our knowledge, this is the first study aimed to evaluate the possible prognostic role of NLR, F value, and F-NLR score in anal canal carcinoma. Therefore, a direct comparative analysis was not possible. Actually, Toh et al. [14] explored the NLR in anal cancer in a retrospective cohort of 92 patients. In order to perform the survival analysis, they dichotomized patients in two groups based on NLR value of 4.75 (< 4.75 versus ≥ 4.75). In accordance with our results, high NLR was significantly associated with worse OS (HR 6.38, 95% CI 1.742–23.372). No other inflammatory markers were used.

The relationship between inflammatory-related parameters and carcinogenesis is complex. In recent years, different inflammatory-related biomarkers were found to be prognostic

Table 2 Baseline patients' characteristics and inflammatory-related parameters

Characteristic	NLR			F *			F-NLR *			
	≤ 2.5	> 2.5	*p* value	≤ 3	> 3	*p* value	0	1	2	*p* value
Age (years), mean (range)	60 (44–75)	64 (38–91)		61 (44–74)	65 (44–91)		58 (44–65)	63 (44–75)	68 (57–91)	
Gender										
Male	5	7	0.74	6	5	0.49	2	7	2	0.62
Female	22	24		16	24		9	18	13	
ECOG performance status										
0	24	25	0.47	20	24	0.90	11	21	12	0.33
≥ 1	3	6		2	5		0	4	3	
Body mass index										
;≤ 25	14	20	0.59	15	15	0.26	8	12	10	0.39
> 25	13	11		7	14		3	13	5	
HPV infection										
No	22	27	0.72	15	27	0.13	7	21	14	0.24
Yes	5	4		6	3		4	4	1	
HIV infection										
No	23	29	0.41	19	26	0.84	9	22	14	0.69
Yes	4	2		3	3		2	3	1	
Clinical T stage										
T1–2	19	11	0.02	12	14	0.67	8	13	5	0.18
T3–4	8	20		10	15		3	12	10	
Clinical N status										
Negative	14	9	0.17	10	10	0.48	6	10	4	0.42
Positive	13	22		12	19		5	15	11	
Inappropriate surgery before CRT										
No	17	23	0.57	15	22	0.64	6	19	12	0.31
Yes	10	8		7	7		5	6	3	

*Missing values in seven cases

NLR, neutrophil to lymphocyte ratio; *F*, fibrinogen; *F-NLR*, fibrinogen-neutrophil to lymphocyte ratio score; *HPV*, human papillomavirus; *HIV*, human immunodeficiency virus; *T*, tumor; *N*, node; *CRT*, chemoradiotherapy

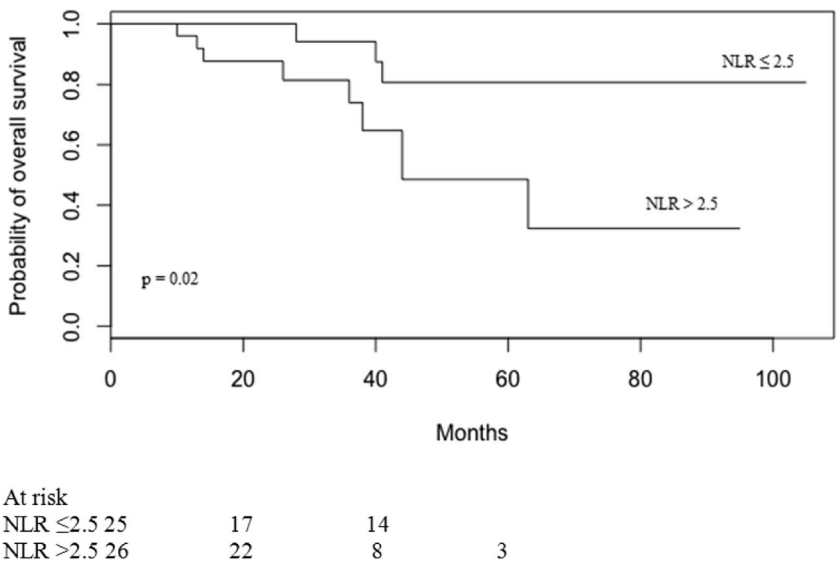

Fig. 1 Overall survival according to neutrophil to lymphocyte ratio (NLR)

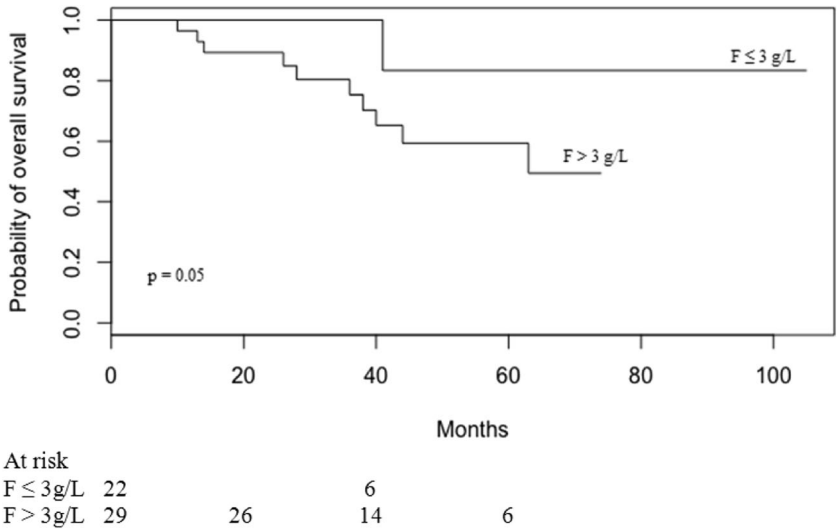

Fig. 2 Overall survival according to fibrinogen (F) level

in various malignancies [2, 5–9]. This growing body of evidence indicated that neutrophils and lymphocytes count in peripheral blood and plasma F level are key factors in cancer progression, mainly due to their role in systemic inflammatory changes and tumor-associated neo-angiogenesis. But the exact biological background of this process has not been fully elucidated. One assumption is that neutrophil cells and fibrinogen are known to produce inflammatory cytokines that could also induce growth signals and angiogenesis, promoting cancer development and progression. Both NLR and F have been demonstrated as representative prognostic markers in a variety of solid tumors [2, 5–7, 15, 16]. Globally, the higher the NLR and/or F levels, the worse prognosis was found, but no optimal NLR and F cut-off points can be standardized. There is a relatively wide range of NLR and F cut-offs.

Studies reported NLR and F cut-offs ranging from 1.9 to 7.2 and from 2.345 to 4 g/L, respectively [2, 5–7, 15, 16]. Discrepancies reported in the literature regarding cut-off values might be explained by several factors including differences in method of cut-off determination and variation in treatment and patients' population characteristics. We performed the ROC curve analysis to determine the optimal cut-off points of inflammatory-related parameters in order to reduce bias toward overestimation and recognize the most favorable values able to guide the stratification for our cohort.

To date, only few studies have specifically assessed F-NLR score, and outcomes are conflicting [8, 17–19]. F-NLR is a novel inflammation-based grading system and appeared to have a promising prognostic value in non-small-cell lung cancer, ovarian cancer, esophageal carcinoma, and gastric cancer. At present, the NLR and F have never been simultaneously assessed as markers of prognosis in patients with anal canal carcinoma. In our study, we developed the F-NLR score and we noted significant differences in OS and DFS, between high

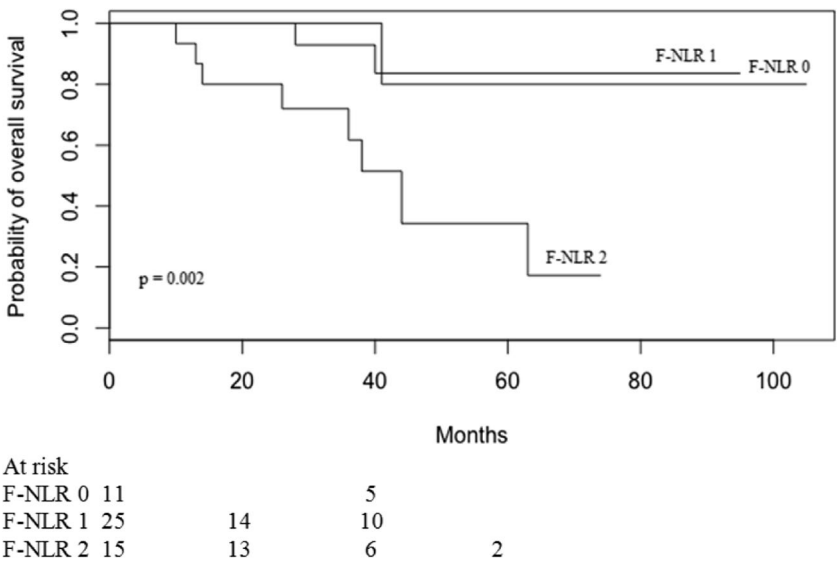

Fig. 3 Overall survival according to fibrinogen-neutrophil to lymphocyte ratio (F-NLR) score

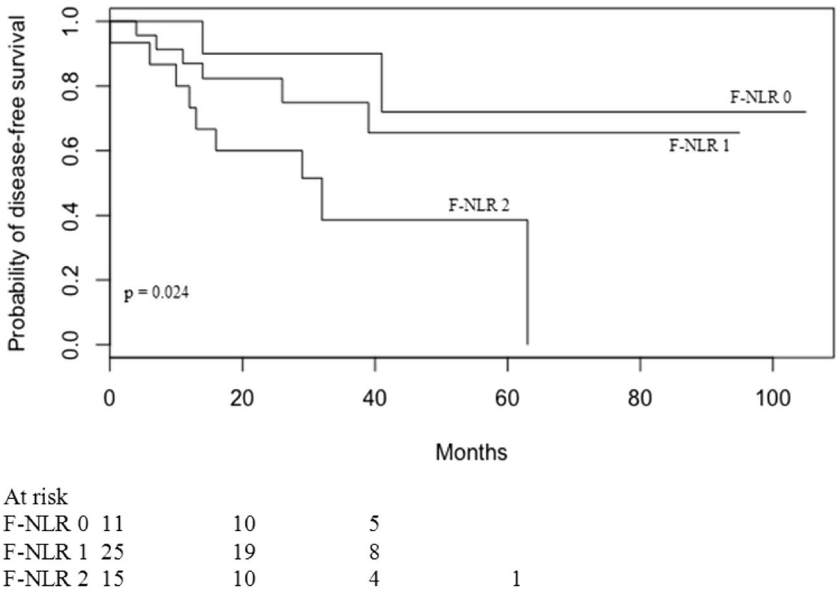

Fig. 4 Disease-free survival according to fibrinogen-neutrophil to lymphocyte ratio (F-NLR) score

and low value. Although this score has not been specifically validated for survival risk assessment, further studies, ideally designed as prospective trials, should be recommended to propose F-NLR score as a novel blood prognostic factor in the clinical management of anal cancer patients.

For sure, a better knowledge on prognostic factors in anal canal cancer could have a strong impact on the clinical practice. Pre-treatment NLR, F, and derived F-NLR could be easily integrated into routine daily clinical practice because of their low economic costs and large accessibility even in primary hospitals. These inflammatory-related parameters could represent simply reproducible biomarkers at diagnosis able to potentially personalize patient care and identify a subgroup of anal canal cancer patients requiring more intensive therapy.

There are several limitations of this study. It was based on a retrospective analysis designed by a single institution. The relatively small sample sizes compromised statistical analysis power and its quality of information. But, the homogeneity in both patients' population and treatment approach is the main analysis force. Results are hypothesis generating rather than confirmatory and only an association between inflammatory-related parameters and worse survival can be inferred and not a cause-effect relationship. A larger population size and a longer follow-up period are necessary to confirm the potential prognostic significance of inflammatory-related parameters.

Conclusion

This study gave the first evidence that inflammatory-related status, reflected by high NLR, elevated F and high F-NLR score, could be a negative prognostic factor in patients undergoing definitive CRT for anal canal carcinoma. Further validation studies with larger patient cohorts in a multicenter setting are warranted to verify our results.

Compliance with ethical standards

The protocol was reviewed and approved by the institutional review board and the scientific review committee. Written informed consent was obtained from all patients before the initiation of therapy.

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Anal carcinoma version 2.2018. http://www.nccn.org/. Accessed June 2018
2. Ji R, Ren Q, Bai S, Wang Y, Zhou Y (2018) Prognostic significance of pretreatment plasma fibrinogen level in patients with digestive system tumors: a meta-analysis. Int J Biol Markers 1724600818773627
3. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68(1):7–30
4. Surveillance, Epidemiology, and End Results (SEER) Program. Cancer stat facts: anal cancer https://seer.cancer.gov/statfacts/html/anus.html. Accessed June 2018
5. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, Tannock IF, Amir E (2014) Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 106(6):dju124
6. Peng B, Wang YH, Liu YM, Ma LX (2015) Prognostic significance of the neutrophil to lymphocyte ratio in patients with non-small cell lung cancer: a systemic review and meta-analysis. Int J Clin Exp Med 8(3):3098–3106

7. Hu K, Lou L, Ye J, Zhang S (2015) Prognostic role of the neutrophil-lymphocyte ratio in renal cell carcinoma: a meta-analysis. BMJ Open 5(4):e006404
8. Marchetti C, Romito A, Musella A, Santo G, Palaia I, Perniola G, Di Donato V, Muzii L, Benedetti Panici P (2018) Combined plasma fibrinogen and neutrophil lymphocyte ratio in ovarian cancer prognosis may play a role? Int J Gynecol Cancer 28(5):939–944
9. Perisanidis C, Psyrri A, Cohen EE, Engelmann J, Heinze G, Perisanidis B, Stift A, Filipits M, Kornek G, Nkenke E (2015) Prognostic role of pretreatment plasma fibrinogen in patients with solid tumors: a systematic review and meta-analysis. Cancer Treat Rev 41(10):960–970
10. Amin MB (2017) AJCC cancer staging manual. Eight Edition. https://doi.org/10.1007/978-3-319-40618-3_21
11. De Felice F, Martinetti MT, Orelli S, Bulzonetti N, Musio D, Tombolini V (2018) Definitive chemoradiotherapy for anal carcinoma: long-term results based on consistent time-to-event endpoints. Oncology 94(1):25–30
12. Musio D, De Felice F, Raffetto N, Tombolini V (2014) Management of persistent anal canal carcinoma after combined-modality therapy: a clinical review. Radiat Oncol 9:39
13. Clauss A (1957) Rapid physiological coagulation method in determination of fibrinogen. Acta Haematol 17(4):237–246
14. Toh E, Wilson J, Sebag-Montefiore D, Botterill I (2014) Neutrophil:lymphocyte ratio as a simple and novel biomarker for prediction of locoregional recurrence after chemoradiotherapy for squamous cell carcinoma of the anus. Color Dis 16(3):O90–O97
15. Luo Y, She DL, Xiong H, Fu SJ, Yang L (2015) Pretreatment neutrophil to lymphocyte ratio as a prognostic predictor of urologic tumors: a systematic review and meta-analysis. Medicine (Baltimore) 94(40):e1670
16. Sun W, Zhang L, Luo M, Hu G, Mei Q, Liu D, Long G, Hu G (2016) Pretreatment hematologic markers as prognostic factors in patients with nasopharyngeal carcinoma: neutrophil-lymphocyte ratio and platelet-lymphocyte ratio. Head Neck 38(Suppl 1):E1332–E1340
17. Wang H, Zhao J, Zhang M, Han L, Wang M, Xingde L (2018) The combination of plasma fibrinogen and neutrophil lymphocyte ratio (F-NLR) is a predictive factor in patients with resectable non small cell lung cancer. J Cell Physiol 233(5):4216–4224
18. Kijima T, Arigami T, Uchikado Y, Uenosono Y, Kita Y, Owaki T, Mori S, Kurahara H, Kijima Y, Okumura H, Maemura K, Ishigami S, Natsugoe S (2017) Combined fibrinogen and neutrophil-lymphocyte ratio as a prognostic marker of advanced esophageal squamous cell carcinoma. Cancer Sci 108(2):193–199
19. Arigami T, Uenosono Y, Ishigami S, Okubo K, Kijima T, Yanagita S, Okumura H, Uchikado Y, Kijima Y, Nakajo A, Kurahara H, Kita Y, Mori S, Maemura K, Natsugoe S (2016) A novel scoring system based on fibrinogen and the neutrophil-lymphocyte ratio as a predictor of chemotherapy response and prognosis in patients with advanced gastric cancer. Oncology 90(4):186–192