CANCER RESEARCH AND TREATMENT (CRT)

Original Article

Prognostic Significance of Bulky Nodal Disease in Anal Cancer Management: A Multi Institutional Study

Seok-Joo Chun[1,2], Eunji Kim[3], Won Il Jang[4], Mi-Sook Kim[4], Hyun-Cheol Kang[1], Byoung Hyuck Kim[1,3], Eui Kyu Chie[1,5]

[1]Department of Radiation Oncology, Seoul National University College of Medicine, Seoul, [2]Department of Radiation Oncology, Dongguk University Ilsan Hospital, Dongguk University College of Medicine, Goyang, [3]Department of Radiation Oncology, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul, [4]Department of Radiation Oncology, Korea Institute of Radiological and Medical Sciences, Seoul, [5]Institute of Radiation Medicine, Medical Research Center, Seoul National University, Seoul, Korea

*Seok-Joo Chun and Eunji Kim contributed equally to this work.

Correspondence: Byoung Hyuck Kim

Department of Radiation Oncology, Seoul Metropolitan Government Seoul National University Boramae Medical Center 20, Boramae-ro 5-gil, Dongjak-gu, Seoul 07061, Korea

Tel : 82-2-870-1683 Fax : 82-2-870-1689 E-mail: karlly71@snu.ac.kr

Co-correspondence: Eui Kyu Chie

Department of Radiation Oncology, Seoul National University College of Medicine 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea

Tel : 82-2-2072-3705 Fax : 82-2-765-3317 E-mail: ekchie93@snu.ac.kr

*Part of the manuscript in abstract form was presented at the 2024 ASCO Gastrointestinal Cancers Symposium (J Clin Oncol 2024;42(3 Suppl):abstr 1). License agreement for not-for-profit publication was made between Professor Eui Kyu Chie, MD, PhD, the corresponding author, and Wolters Kluwer Health Inc., the publisher of the Journal of Clinical Oncology.

Abstract

Purpose

This study aimed to assess the prognostic significance of bulky nodal involvement in patients with anal squamous cell carcinoma treated with definitive chemoradiotherapy.

Materials and Methods

We retrospectively analyzed medical records of patients diagnosed with anal squamous cell carcinoma who underwent definitive chemoradiotherapy at three medical centers between 2004 and 2021. Exclusion criteria included distant metastasis at diagnosis, 2D radiotherapy, and salvage treatment for local relapse. Bulky N+ was defined as nodes with a long diameter of 2 cm or greater.

Results

A total of 104 patients were included, comprising 51 with N0, 46 with non-bulky N+, and 7 with bulky N+. The median follow-up duration was 54.0 months (range, 6.4-162.2 months). Estimated 5-year progression-free survival (PFS), loco-regional recurrence-free survival (LRRFS), and overall survival (OS) rates for patients with bulky N+ were 42.9%, 42.9%, and 47.6%, respectively. Bulky N+ was significantly associated with inferior PFS, LRRFS and OS compared to patients without or with non-bulky N+, even after multivariate analysis. We proposed a new staging system incorporating bulky N+ as N2 stage, with estimated 5-year LRRFS, PFS, and OS rates of 81.1%, 80.6%, and 86.2% for stage I, 67.7%, 60.9%, and 93.3% for stage II, and 42.9%, 42.9%, and 47.6% for stage III disease, enhancing the predictability of prognosis.

Conclusion

Patients with bulky nodal disease treated with standard chemoradiotherapy experienced poor survival outcomes, indicating the potential necessity for further treatment intensification.

Key words Anus neoplasms, Bulky nodes, Chemoradiotherapy

Introduction

Anal cancer is a rare malignancy, representing approximately 0.1% of all newly diagnosed cancers [1,2]. The predominant histologic subtype is squamous cell carcinoma, accounting for 70-90% of anal cancers [2]. Definitive chemoradiotherapy is the current standard of care for localized squamous cell carcinoma of the anus, with a 5-year overall survival rate of approximately 80% [3,4].

Factors that are widely known to affect survival are tumor size, nodal metastasis and sex [5]. Additionally, as etiology of anal cancer is closely related with human papilloma virus (HPV) or human immunodeficiency virus (HIV) infection, immunologic factors such as p16 expression, leukocytopenia, neutropenia, and lymphocytosis have been related with improved survival [6-11].

Nodal staging for anal cancer was divided into 4 groups; N0, N1a, N1b and N1c in previous American Joint Committee on Cancer (AJCC) 8th staging system [12]. However, current AJCC staging system 9th edition incorporate nodal staging as either N0 and N1. Meanwhile, there have been several reports that the size of nodal metastasis affects survival and treatment outcomes in squamous cell carcinoma of uterine cervix and oropharynx [13-15]. Moreover, the size of lymph node metastasis is incorporated in oropharyngeal cancer staging system [15]. As cancer of uterine cervix, oropharyngeal cancer and anal cancer share biologic similarities due to association with squamous entity and chronic HPV infection, we hypothesized that the nodal size may also be a prognosticator for anal cancer. Additionally, we aimed to incorporate bulky nodes into nodal staging to better predict treatment outcomes. Of note, preliminary analysis on the potential impact of bulky nodes in anal cancer was recently reported in abstract form as detailed in the declaration section [16].

Materials and Methods

1. Patients

Medical records of patients with anal squamous cell cancer diagnosed from 2004 to 2021 at three institutions were retrospectively reviewed. All patients were pathologically confirmed by biopsy and had either computed tomography (CT) or magnetic resonance imaging (MRI) for anal cancer staging work up. Positron emission tomography (PET) was selectively performed for patients with advanced disease to rule out any distant metastasis. Patients who had distant metastasis or patients with past medical history of double primary cancer within 5 years from the diagnosis of anal cancer were excluded from analysis. Patients treated with abdominoperineal resection or patients undergoing salvage treatment for recurrence were also excluded. Factors such as T stage, N stage according to AJCC 8th edition, nodal size, age, sex, p16 presentation, baseline and post-treatment complete blood count test were collected. This study was approved by Institutional Review Board at participating institutions.

2. Treatment

All patients underwent definitive chemoradiotherapy. Patients who were treated with conventional radiotherapy technique was excluded for treatment homogeneity, leaving either 3D-conformal radiotherapy (3D-CRT) or intensity modulated radiotherapy (IMRT) as employed radiotherapy technique. Prescribed dose to the pelvis was 40-50Gy followed by sequential boost of 5.4-10.8Gy, depending on the extent or residuum of disease for 3D-CRT. Simultaneous integrated boost (SIB) of 50-55 Gy to gross primary and nodal disease and 40-45 Gy to elective pelvic nodes were common prescription for IMRT. Nodal size was measured as the longest axis in pretreatment CT or MRI. Bulky N+ was defined as longest axis nodal size of 2cm or greater. For patients with lymphocyte less than 500/μL was defined as having lymphopenia.

3. Statistics

R project version 4.2.3 was used for all statistical analysis. For comparing variables between multiple groups, one-way ANOVA was used for continuous variables while chi-square test was used for categorical variables. Kaplan-Meier method was used for survival plots. Loco-regional recurrence free survival (LRRFS), progression-free survival (PFS), and overall survival (OS) were defined as time between the day of diagnosis to the corresponding event. Log-rank test was used to compare survival and Cox model was used for univariate analysis. Factors with p value less than 0.10 in univariate analysis was selected for multivariate cox regression model with backward elimination method. Factors with p value less than 0.05 were considered as statistically significant.

Results

One hundred four patients meeting inclusion criteria were accrued. Median age was 60 year and 76% were female. T1/T2 disease consisted of 77.7% of the patients and approximately half of patients (51.0%) had nodal involvement. Average prescription radiation dose was 54.1Gy (range, 40.0 - 64.8Gy) for primary and gross nodal diseases and 44.1Gy (range, 32.0 - 54.0Gy) for elective nodal station, respectively. Most patients (80.4%) received concurrent chemoradiotherapy with two cycles of 5-FU and mitomycin C. (Table 1) Among 104 patients, 83 patients received elective inguinal radiotherapy, with a median dose of 45Gy ranging from 30.6Gy to 54.0Gy.

With a median follow-up of 54.0 months (range, 6.4-162.2 months), 18 (17.3%) relapses were observed. There were 15 loco-regional failure, with 7 local, 2 local & regional and 6 regional relapses. Among regional relapse, 4 were in pelvic, 3 were in inguinal, and 1 in both pelvic and inguinal region. Eleven patients (10.6%) experienced distant metastasis with 3

in liver, 5 in lung and 3 in non-regional lymph node such as para-aortic and mediastinal stations. Three patients had distant metastasis without loco-regional relapse. During the follow-up, 19 patients (18.3%) expired, of which 9 were cancer related. Estimated 5-year LRRFS, PFS, and OS of entire study population were 76.2%, 74.8%, and 84.5%, respectively.

In univariate analysis, performance status, tumor grade, advanced T stage and bulky N+ were related with poor LRRFS. Performance status and bulky N+ remained statistically significant after multivariate analysis. For PFS, performance status, tumor grade, advanced T stage, and bulky N+ were poor prognosticators. T stage remained statistically significance after multivariate analysis, while bulky N+ was marginally significant (p=0.089). Bulky N+ and performance status was related with OS in both univariate and multivariate analysis. When compared to the combined entity of N0 and non-bulky N+ disease, bulky N+ exhibited hazard ratios of 4.11 for LRRFS (p=0.007), 2.60 for PFS (p=0.089), and 4.51 for OS (p=0.029) (Table 2). However, no significant LRRFS or PFS difference was observed between the N0 and non-bulky N+ groups (S1 Table).

There were 51 patients as N0, 46 with non-bulky N+, and 7 with bulky N+. Between three groups, there were more patients with T3/T4 disease in node positive groups compared to N0 group (p=0.002). Additionally, higher dose was given to primary site and gross nodes in bulky N+ group. (Table 3) Detailed patient characteristics of 7 patients with bulky N+ is separately shown in S2 Table. Five patients had bulky nodes at the inguinal station, while two patients had bulky nodes at the internal iliac station. Estimated 5-year LRRFS, PFS, and OS of patients with bulky N+ were 42.9%, 42.9%, and 47.6%, respectively (Fig. 1).

In current study, both AJCC 8th and 9th edition staging system failed to demonstrate a significant survival difference between stages (S3 Fig.). As bulky N+ was closely related with poor prognosis compared to the remaining patients, bulky N+ was designated as new N2 entity.

Patient groups were divided into five categories, incorporating bulky nodal component to current staging system: T1-2N0, T3-4N0, T1-2N1, T3-4N1, and any N2. Interestingly, similar outcomes between the T1-2N0 and T1-2N1 groups, as well as between the T3-4N0 and T3-4N1 groups were observed (S4 Fig.). Thus, a new staging system was revised, categorizing T1-2N0-1 as stage I, T3-4N0-1 as stage II, and any N2 disease as stage III. Estimated LRRFS, PFS, and OS at 5 years were as follows: 81.1%, 80.6%, and 86.2%, 67.7%, 60.9%, and 93.3%, and 42.9%, 42.9%, and 47.6% for patients with stage I, II, and III disease, respectively (p=0.0052 for LRRFS, 0.0052 for PFS, 0.099 for OS, Fig. 2), demonstrating improved predictive value of newly constructed staging system over others.

Acute adverse event of grade 2 or greater were observed in 38 patients (36.5%), from which all patients recovered. Eleven patients (10.6%) experienced delayed adverse event of grade 2 or greater. Specifically, 7 patients had grade 2 toxicity, including 3 cases of proctitis, 2 of anal fibrosis and 2 of vagina fistula. Three patients suffered grade 3 toxicities: 2 proctitis and 1 anal perforation. One patient succumbed to mitomycin C-induced thrombotic microangiopathy within 5 months from undergoing treatment.

Discussion

In this study, bulky N+ was a poor prognosticator in patients with anal cancer. Additionally, new staging system was proposed with bulky N+ designated as N2 disease, which clearly corresponds better to treatment outcomes in current analysis.

Among 7 patients with bulky N+, 4 patients experienced loco-regional recurrence and 2 patients with distant metastasis eventually succumbed to disease. Even though relatively high dose radiotherapy with median 58.9 Gy (range 54.0-60.4 Gy) was prescribed to both primary and gross lymph nodes, more than half of patients have failed loco-regionally. It is noteworthy

that though radiation dose and chemotherapy regimen failed to demonstrate prognostic value in all patients, only one patient out of four with bulky N+ treated with radiation dose higher than 55Gy and concurrent chemotherapy experienced disease relapse. This suggests the possibility that higher radiation dose may be required for effective cancer control. Although ACCORD 03 failed to demonstrate the benefit of boost dose escalation, post-hoc analysis of ACT II suggested possible radiation dose response relationship [17,18]. Currently on-going PLATO ACT5 trial is investigating dose intensification, comparing standard arm 53.2Gy against escalated arm 58.8Gy or 61.6Gy, in patients with advanced anal cancer, which may help to elucidate the role of dose intensification in advanced anal cancer [19].

As aforementioned, etiology of uterine cervical cancer, oropharyngeal cancer and anal cancer are quite similar. First, they are all related to HPV infection. Population based cohort from Surveillance, Epidemiology and End Results have reported that uterine cervical cancer survivors had 4.36 increased risk of oropharyngeal cancer and 2.20 of anal cancer diagnosis [20]. Additionally, meta-analysis have reported that each of HPV-associated tumors have increased incidence of other HPV-associated tumors [20]. Moreover, p16 expression was related with improved survival in not only oropharyngeal cancer, but also in anal cancer [21]. There have been multiple reports regarding nodal size in oropharyngeal and uterine cervical cancer, but not in anal cancer [13-15,22-24] (S5Table). In uterine cervical cancer, nodal size more than 1.5-2.0cm have been reported to have more recurrences, compared to others [13,14,24]. Additionally, bulky lymph nodes have been related with poor overall survival or disease-free survival in oropharyngeal or oral cavity cancer, though definition of bulky nodes in oropharyngeal cancer was quite large at 6cm or larger [15,22,23].

Although HPV infection or p16 expression were closely related with nodal involvement in both oral cavity cancer and oropharyngeal cancer [15,25,26], p16 expression is

related with superior treatment outcomes in oropharyngeal cancer [15,27]. In anal cancer, two studies have reported on HPV and p16 status, where HPV expression was related with improved local control [6,7]. In current study, p16 or HPV infection were not routinely tested, thus relationship between p16 status and bulky nodal involvement was not explored. Considering findings related with p16 expression in anal cancer and head and neck cancers, it would be necessary to evaluate relationship between p16 status and bulky nodal disease in future studies.

In addition to HPV infection, immune system is known to play a role in treatment outcomes for anal cancer. Previous studies have suggested that leukocyte related factors, such as neutrophilia, higher neutrophil-lymphocyte ratio (NLR), leukocytosis, and lymphopenia, are associated with deteriorated treatment outcomes [5,8-10]. Although current study showed increase of hazard ratio related with higher NLR or lymphopenia, statistical significance was not found (Table 2). It would be important to note that both current study and previous studies had a small proportion of patients with neutrophilia or lymphopenia. To validate the impact of leukocyte related factors in anal cancer, further research with larger cohort would be required.

As previously mentioned, currently employed AJCC 9th edition staging system for anal cancer employs nodal involvement without further substaging. As a result, current staging system lacks the ability to account for the diversity of nodal involvement. Furthermore, nodal disease was downstaged in the AJCC staging system 9th edition compared to the 8th edition, for example, T1-2N1, which was stage IIIA in AJCC 8th edition, is now IIB in AJCC 9th edition. However, in current cohort, we identified three cases of bulky nodes with T stage 1-2, and among them, two patients experienced treatment failure. This suggests that the current staging system would underestimate the poor prognosis associated with bulky N+.

Nodal status failed to demonstrate statistically significance in terms of LRRFS and PFS. Additionally, when patients with nodal disease was subdivided to non-bulky N+ against bulky

N+, no significant difference was observed between patients with N0 and non-bulky N+. As a result, we proposed a 3-tier staging system stressing the bulky nodal disease, which accurately predict survival outcomes in the studied cohort. While nodal status is recognized as one of the key prognostic factors, we observed that the distinction between N0 and non-bulky N+ disease becomes less pronounced, while that of bulky node disease retained the impact.

This study is not free from the limitations. Limitations of this study stems from retrospective design. Several cofounding factors that are known to affect treatment outcomes were not evenly distributed. However, many factors were not closely related in this study, and multivariate analysis showed that nodal size retained its impact on both LRRFS and PFS. Additionally, as the number of patients with bulky nodes was relatively small, statistical significance of analyzed parameters including predictive value of current staging system may have been underscored and further analysis such as propensity score matching was not possible. Lastly, suggested new staging model was not validated externally. Considering the limited number of patients in both the overall cohort and the bulky N+ group, there is a possibility of selection bias, which should be externally validated. Nevertheless, bulky N+ in patients with anal cancer carries distinctive prognosis and thus may be incorporated into tumor staging, as suggested in current analysis, for improved predictability.

Bulky nodal disease was distinctive prognosticator for patients with squamous cell carcinoma of anus undergoing standard of care chemoradiotherapy, which calls for further treatment intensification. Additionally, newly suggested staging system incorporating bulky nodal disease as N2 disease showed promise in improving patient outcomes prediction.

Ethical Statement

This study was approved by Institutional Review Board at participating institutions. Informed consent was waived according to institutional policies.

Author Contributions

Conceived and designed the analysis: Chun SJ, Kim E, Kim BH, Chie EK.

Collected the data: Chun SJ, Kim E, Jang WI, Kim MS, Kang HC.

Contributed data or analysis tools: Chun SJ, Jang WI, Kim MS, Kang HC, Kim BH, Chie EK.

Performed the analysis: Chun SJ.

Wrote the paper: Chun SJ, Kim E, Jang WI, Kim MS, Kang HC, Kim BH, Chie EK.

ORCID iDs

Seok-Joo Chun: https://orcid.org/0009-0004-2118-9671

Eunji Kim: https://orcid.org/0000-0002-0565-9301

Byoung Hyuck Kim: https://orcid.org/0000-0002-6156-0744

Eui Kyu Chie: https://orcid.org/0000-0003-2027-7472

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

References

1. Kang MJ, Won YJ, Lee JJ, Jung KW, Kim HJ, Kong HJ, et al. Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2019. Cancer Res Treat. 2022;54:330–44.
2. Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. Anal cancer incidence and survival: The Surveilance, epidemiology, and end results experience, 1973-2000. Cancer. 2004;101:281–8.
3. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Anal Carcinoma Version 2. 2023.
4. Rao S, Guren MG, Khan K, Brown G, Renehan AG, Steigen SE, et al. Anal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up☆. Ann Oncol. 2021;32:1087–100.
5. Theophanous S, Samuel R, Lilley J, Henry A, Sebag-Montefiore D, Gilbert A, et al. Prognostic factors for patients with anal cancer treated with conformal radiotherapy—a systematic review. BMC Cancer. 2022;22:607.
6. Rödel F, Steinhäuser K, Kreis NN, Friemel A, Martin D, Wieland U, et al. Prognostic impact of RITA expression in patients with anal squamous cell carcinoma treated with chemoradiotherapy. Radiother Oncol. 2018;126:214–21.
7. Balermpas P, Martin D, Wieland U, Rave-Fränk M, Strebhardt K, Rödel C, et al. Human papilloma virus load and PD-1/PD-L1, CD8+ and FOXP3 in anal cancer patients treated with chemoradiotherapy: Rationale for immunotherapy. Oncoimmunology. 2017;6:e1288331.
8. Schernberg A, Escande A, Rivin Del Campo E, Ducreux M, Nguyen F, Goere D, et al. Leukocytosis and neutrophilia predicts outcome in anal cancer. Radiother Oncol. 2017;122:137–45.
9. Schernberg A, Huguet F, Moureau-Zabotto L, Chargari C, Rivin Del Campo E, Schlienger M, et al. External validation of leukocytosis and neutrophilia as a prognostic marker in anal carcinoma treated with definitive chemoradiation. Radiother Oncol. 2017;124:110–7.
10. Kim E, Kim TH, Jung W, Kim K, Chang AR, Park HJ, et al. Prognostic impact of neutrophilia and lymphopenia on survival in anal cancer treated with definitive concurrent chemoradiotherapy: a retrospective multicenter study. Int J Clin Oncol. 2022;27:553–62.
11. Shakir R, Adams R, Cooper R, Downing A, Geh I, Gilbert D, et al. Patterns and Predictors

of Relapse Following Radical Chemoradiation Therapy Delivered Using Intensity Modulated Radiation Therapy With a Simultaneous Integrated Boost in Anal Squamous Cell Carcinoma. Int J Radiat Oncol Biol Phys. 2020;106:329–39.

12. Janczewski LM, Faski J, Heidi MS, Gollub MJ, Eng C, Brierley JD, et al. Survival outcomes used to generate version 9 American Joint Committee on Cancer staging system for anal cancer. CA Cancer J Clin. 2023;73:516–23.
13. Tiwari R, Narayanan GS, Reddy VP, Vishwanathan B, Narayanan S, Venugopal R. Impact of nodal boost irradiation and MR-based brachytherapy on oncologic outcomes in node-positive cervical cancer. Gynecol Oncol. 2021;163:110–6.
14. Grigsby PW, Singh AK, Siegel BA, Dehdashti F, Rader J, Zoberi I. Lymph node control in cervical cancer. Int J Radiat Oncol Biol Phys. 2004;59:706–12.
15. O'Sullivan B, Huang SH, Su J, Garden AS, Sturgis EM, Dahlstrom K, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. Lancet Oncol. 2016;17:440–51.
16. Chie EK, Chun S-J, Kim E, Jang W Il, Kim M-S, Kang H-C, et al. Bulky nodal disease as distinctive prognosticator in anal cancer management. J Clin Oncol. 2024;42:3_Suppl.
17. Peiffert D, Tournier-Rangeard L, Gérard JP, Lemanski C, François E, Giovannini M, et al. Induction chemotherapy and dose intensification of the radiation boost in locally advanced anal canal carcinoma: Final analysis of the randomized UNICANCER ACCORD 03 trial. J Clin Oncol. 2012;30:1941–8.
18. Glynne-Jones R, Meadows HM, Lopes A, Muirhead R, Sebag-Montefiore D, Adams R. Impact of compliance to chemoradiation on long-term outcomes in squamous cell carcinoma of the anus: results of a post hoc analysis from the randomised phase III ACT II trial. Ann Oncol. 2020;31:1376–85.
19. Gilbert A, McParland L, Webster J, Bell S, Copeland J, Adams RA, et al. Pre-specified pilot analysis of a randomised pilot/phase II/III trial comparing standard dose vs dose-escalated concurrent chemoradiotherapy (CRT) in anal cancer (PLATO-ACT5). Ann Oncol. 2019;30 Supplement 5:v203–4.
20. Gilbert DC, Wakeham K, Langley RE, Vale CL. Increased risk of second cancers at sites associated with HPV after a prior HPV-associated malignancy, a systematic review and meta-analysis. Br J Cancer. 2019;120:256–68.

21. Sun G, Dong X, Tang X, Qu H, Zhang H, Zhao E. The prognostic value of HPV combined p16 status in patients with anal squamous cell carcinoma: A meta-analysis. Oncotarget. 2018;9:8081–8.
22. Spector ME, Gallagher KK, Bellile E, Chinn SB, Ibrahim M, Byrd S, et al. Patterns of nodal metastasis and prognosis in human papillomavirus–positive oropharyngeal squamous cell carcinoma. Head Neck. 2014;36:1233–40.
23. Ho AS, Kim S, Tighiouart M, Gudino C, Mita A, Scher KS, et al. Metastatic lymph node burden and survival in oral cavity cancer. J Clin Oncol. 2017;35:3601–9.
24. Olthof EP, Wenzel H, Van Der Velden J, Spijkerboer AM, Bekkers R, Beltman JJ, et al. Treatment of bulky lymph nodes in locally advanced cervical cancer: boosting versus debulking. Int J Gynecol Cancer. 2022;32:861–8.
25. Li P, Fang Q, Yang Y, Chen D, Du W, Liu F, et al. Survival Significance of Number of Positive Lymph Nodes in Oral Squamous Cell Carcinoma Stratified by p16. Front Oncol. 2021;11:545433.
26. Bauwens L, Baltres A, Fiani DJ, Zrounba P, Buiret G, Fleury B, et al. Prevalence and distribution of cervical lymph node metastases in HPV-positive and HPV-negative oropharyngeal squamous cell carcinoma. Radiother Oncol. 2021;157:122–9.
27. Porceddu S V., Milne R, Brown E, Bernard A, Rahbari R, Cartmill B, et al. Validation of the ICON-S staging for HPV-associated oropharyngeal carcinoma using a pre-defined treatment policy. Oral Oncol. 2017;66:81–6.

Table 1. Patient, tumor, treatment characteristics of anal cancer patients

Variables	No. of patients (N=104)
Age*	61.4±10.3 year
≤60	52 (50.0%)
>60	52 (50.0%)
Sex	
Female	79 (76.0%)
Male	25 (24.0%)
HIV**	
Yes	3 (3.8%)
No	76 (96.2%)
ECOG PS	
0-1	101 (97.1%)
2-3	3 (2.9%)
Local excision	
Yes	20 (19.2%)
No	84 (80.8%)
p16 expression	
Yes	14 (13.5%)
No	0 (0.0%)
N/A	90 (86.5%)
Tumor grade	
W/D or M/D	47 (45.2%)
P/D	21 (20.2%)
N/A	36 (34.6%)
T stage	
T1/T2	81 (77.9%)
T3/T4	23 (22.1%)
N stage	
N0	51 (49.0%)
N1	53 (51.0%)
Stage (AJCC 8th)	
I	16 (15.4%)

II	33 (31.7%)
III	55 (52.9%)
Baseline NLR**	
<2.5	74 (75.5%)
≥2.5	24 (24.5%)
Baseline lymphopenia** (500/μL)	
No	97 (99.0%)
Yes	1 (1.0%)
Post-treatment NLR	
<2.5	55 (52.9%)
≥2.5	49 (47.1%)
Post-treatment lymphopenia (500/μL)	
No	90 (86.5%)
Yes	14 (13.5%)
Radiation technique	
3D-CRT	60 (57.7%)
IMRT	44 (42.3%)
Radiation dose (primary tumor)*	54.0 ± 4.5Gy
45≤, <50 Gy	11 (10.6%)
50≤, <55 Gy	49 (47.1%)
55≤ Gy	44 (42.3%)
Radiation dose (gross nodes)*,**	53.9 ± 5.2Gy
Radiation dose (elective nodes)*	44.1 ± 3.7Gy
Radiotherapy to inguinal nodes	
Yes	83 (79.8%)
No	21 (20.2%)
Chemotherapy regimen	
MMC included	84 (80.8%)
MMC excluded	20 (19.2%)

*Average ± SD, **Analysis of available data. HIV, human immunodeficiency virus; ECOG PS, European Cooperative Oncology Group Performance Status; w/d, well differentiated; m/d, moderately differentiated; p/d, poorly differentiated; n/a: not available; AJCC: American Joint Committee on Cancer; NLR, neutrophil-lymphocyte ratio; 3D-CRT: 3-dimensional-conformal radiotherapy; IMRT: intensity modulated radiotherapy; MMC, Mitomycin C.

Table 2. Univariate and multivariate analysis of factors associated with anal cancer

Factor	LRRFS				PFS				OS			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Age (≤60 vs. >60)	0.9 (0.41-1.99)	0.791			0.9 (0.42-1.93)	0.785			1.64 (0.60-4.52)	0.335		
Sex (Female vs. Male)	1.58 (0.68-3.70)	0.291			1.38 (0.60-3.18)	0.446			**2.64 (0.98-7.09)**	**0.055**	**2.72 (0.92-8.01)**	**0.069**
ECOG PS (Continuous)	**2.40 (1.12-5.13)**	**0.024**	**2.47 (1.17-5.21)**	**0.018**	1.94 (0.89-4.22)	**0.093**	-	-	**3.75 (1.50-9.35)**	**0.005**	**3.51 (1.20-10.25)**	**0.022**
Local excision (No vs. Yes)	0.67 (0.23-1.98)	0.47			0.85 (0.32-2.27)	0.745			1.08 (0.34-3.37)	0.897		
P16 (N/A vs. Yes)	1.45 (0.49-4.27)	0.499			1.28 (0.44-3.72)	0.654			1.1 (0.25-4.89)	0.903		
Tumor grade (Continuous)	**0.69 (0.49-0.98)**	**0.04**	-	-	0.74 (0.54-1.03)	**0.075**	**0.76 (0.55-1.05)**	**0.095**	0.88 (0.61-1.29)	0.518		
T stage (T1/2 vs. T3/4)	**2.68 (1.17-6.16)**	**0.02**	-	-	**2.83 (1.28-6.27)**	**0.01**	**2.51 (1.11-5.66)**	**0.026**	2.32 (0.84-6.45)	0.106		
N stage (N0 vs. N1)	1.24 (0.56-2.75)	0.59			1.47 (0.68-3.17)	0.33			0.92 (0.35-2.39)	0.858		
Bulky Nodal status (N0, non-bulky N+ vs. bulky N+)	**4.11 (1.37-12.35)**	**0.012**	**4.6 (1.51-14.06)**	**0.007**	**3.41 (1.15-10.09)**	**0.026**	**2.6 (0.86-7.89)**	**0.089**	**3.67 (1.28-10.98)**	**0.045**	**4.51 (1.17-17.4)**	**0.029**
Baseline NLR (<2.5 vs. ≥2.5)	1.58 (0.66-3.80)	0.302			1.7 (0.74-3.90)	0.211			**3.75 (1.28-10.98)**	**0.016**	**2.8 (0.90-8.77)**	**0.077**
Post-treatment NLR (<2.5 vs. ≥2.5)	1.37 (0.63-2.97)	0.424			1.32 (0.63-2.79)	0.46			1.1 (0.44-2.79)	0.834		
Post-treatment lymphopenia	0.71	0.534			0.57	0.268			0.6	0.427		

(No vs. Yes)	(0.24-2.09)		(0.22-1.53)		(0.17-2.13)	
RT technique (3D-CRT vs. IMRT)	1.01 (0.43-2.35)	0.981	1.04 (0.47-2.34)	0.919	1.76 (0.63-4.92)	0.281
RT dose (≤54 vs. >54)	1.08 (0.48-2.42)	0.846	1.11 (0.51-2.40)	0.795	0.85 (0.32-2.27)	0.75
Chemotherapy (MMC vs. non-MMC)	1.5 (0.63-3.56)	0.356	1.34 (0.57-3.11)	0.504	0.99 (0.34-2.91)	0.988

PFS, progression free survival; LRRFS, loco-regional recurrence free survival; OS, overall survival; HR, hazard ratio; ECOG PS, European Cooperative Oncology Group Performance Status; NLR, neutrophil-lymphocyte ratio; MMC, Mitomycin-C.

Table 3. Patient characteristics by treatment groups classified by nodal size

Variables	N0 (N=51)	Non-bulky (N=46)	Bulky N+ (N=7)	p value
Age	62.2 ± 10.7	60.8 ± 9.5	60.6 ± 13.4	0.816
≤60	27 (52.9%)	22 (47.8%)	3 (42.9%)	
>60	24 (47.1%)	24 (52.2%)	4 (57.1%)	
Sex				0.584
Female	41 (80.4%)	33 (71.7%)	5 (71.4%)	
Male	10 (19.6%)	13 (28.3%)	2 (28.6%)	
HIV				0.127
Yes	3 (8.8%)	0 (0.0%)	0 (0.0%)	
No	31 (91.2%)	38 (100.0%)	7 (100.0%)	
Local excision				0.180
Yes	13 (25.5%)	7 (15.2%)	0 (0.0%)	
No	38 (74.5%)	39 (84.8%)	7 (100.0%)	
p16				0.519
Yes	8 (15.7%)	6 (13.0%)	0 (0.0%)	
No	0 (0.0%)	0 (0.0%)	0 (0.0%)	
N/A	43 (84.3%)	40 (87.0%)	7 (100.0%)	
Tumor grade				0.927
W/D or M/D	21 (41.2%)	22 (47.8%)	4 (57.1%)	
P/D	11 (21.6%)	9 (19.6%)	1 (14.3%)	
N/A	19 (37.3%)	15 (32.6%)	2 (28.6%)	
T stage				0.002
T1/T2	47 (92.2%)	30 (65.2%)	4 (57.1%)	
T3/T4	4 (7.8%)	16 (34.8%)	3 (42.9%)	
N stage				<0.001
N0	51	0 (0.0%)	0 (0.0%)	
N1	0 (0.0%)	46 (100.0%)	7 (100.0%)	
Stage (AJCC 8th)				<0.001
I	16 (31.4%)	0 (0.0%)	0 (0.0%)	
II	33 (64.7%)	0 (0.0%)	0 (0.0%)	
III	2 (3.9%)	46 (100.0%)	7 (100.0%)	
Baseline NLR				0.036
<2.5	39 (81.2%)	33 (75.0%)	2 (33.3%)	
≥2.5	9 (18.8%)	11 (25.0%)	4 (66.7%)	
Baseline lymphopenia (500/μL)				0.538
No	48	43 (97.7%)	6 (100.0%)	
Yes	0 (0.0%)	1 (2.3%)	0 (0.0%)	
Post-treatment NLR				0.066
<2.5	26 (51.0%)	28 (60.9%)	1 (14.3%)	
≥2.5	25 (49.0%)	18 (39.1%)	6 (85.7%)	
Post-treatment lymphopenia (500/μL)				0.240
No	47 (92.2%)	37 (80.4%)	6 (85.7%)	

Yes	4 (7.8%)	9 (19.6%)	1 (14.3%)	
Radiation Technique				0.678
3D-CRT	30 (58.8%)	25 (54.3%)	5 (71.4%)	
IMRT	21 (41.2%)	21 (45.7%)	2 (28.6%)	
Radiation dose (primary tumor)	52.1 ± 4.5	55.5 ± 3.8	57.4 ± 2.9	<0.001
45≤, <50 Gy	9 (17.6%)	2 (4.3%)	0 (0.0%)	0.001
50≤, <55 Gy	31 (60.8%)	16 (34.8%)	2 (28.6%)	
55≤ Gy	11 (21.6%)	28 (60.9%)	5 (71.4%)	
Radiation dose (gross nodes)		53.1 ± 5.0	58.9 ± 3.5	0.005
Radiation dose (elective nodes)	43.6 ± 3.6	44.1 ± 3.5	47.4 ± 5.0	0.041
Radiotherapy to inguinal nodes				0.001
Yes	33 (64.7%)	43 (93.5%)	7 (100.0%)	
No	18 (35.3%)	3 (6.5%)	0 (0.0%)	
Chemotherapy regimen				0.361
MMC included	44 (86.3%)	35 (76.1%)	5 (71.4%)	
MMC excluded	7 (13.7%)	11 (23.9%)	2 (28.6%)	

HIV, human immunodeficiency virus; ECOG PS, European Cooperative Oncology Group Performance Status; w/d, well differentiated; m/d, moderately differentiated; p/d, poorly differentiated; n/a: not available; AJCC: American Joint Committee on Cancer; NLR, neutrophil-lymphocyte ratio; 3D-CRT: 3-dimensional-conformal radiotherapy; IMRT: intensity modulated radiotherapy; MMC, Mitomycin C.

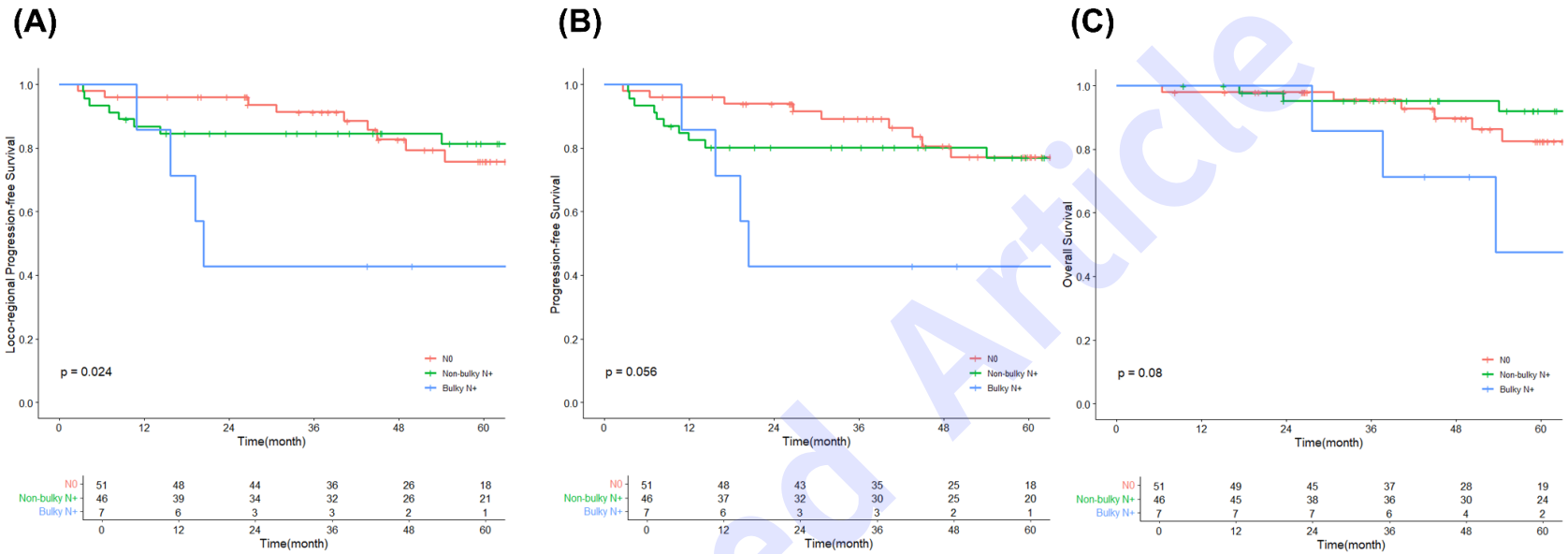


Fig. 1. (A) Loco-regional recurrence-free survival (B) progression-free survival, and (C) overall survival of patients with N0, non-bulky N+, and bulky N+.

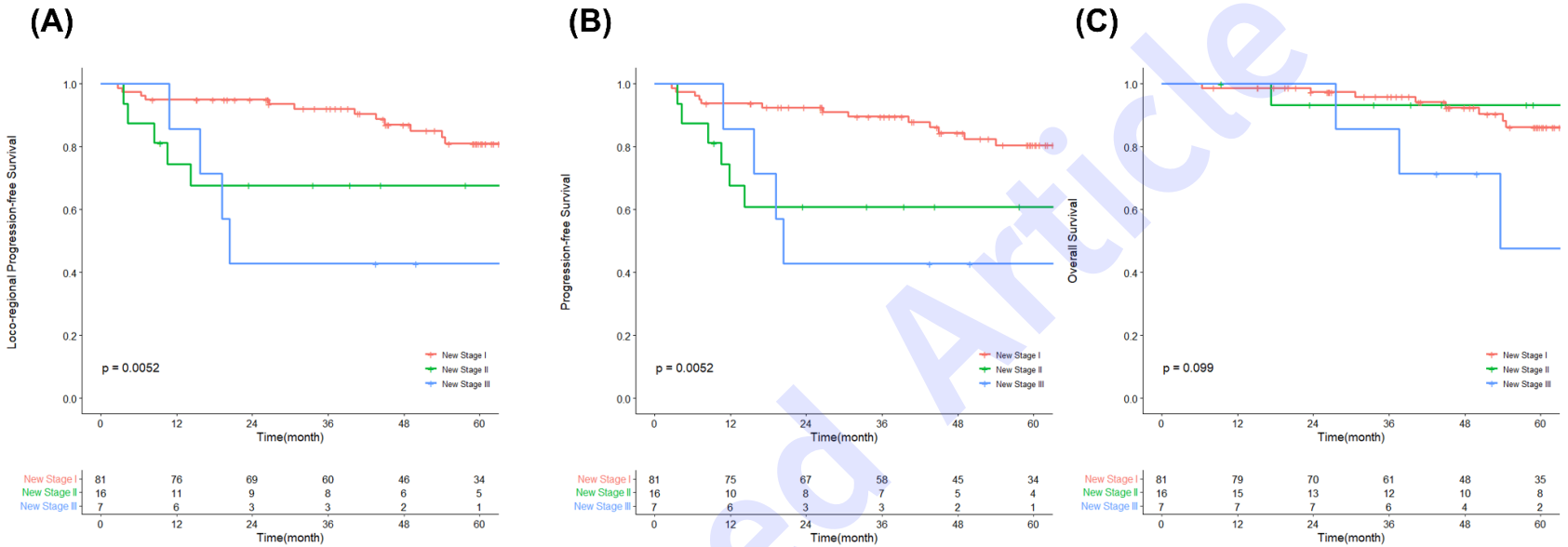


Fig. 2. (A) Loco-regional recurrence-free survival (B) progression-free survival, and (C) overall survival of patients according to newly suggested stage.

S1 Table. Univariate and multivariate analysis of factors associated with anal cancer (bulky nodal status as N0 vs non-bulky N+ vs bulky N+)

Factor	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Age (≤60 vs >60)	0.9 (0.41-1.99)	0.791			1.64 (0.60-4.52)	0.335		
Sex (Female vs Male)	1.58 (0.68-3.70)	0.291			**2.64 (0.98-7.09)**	**0.055**	2.68 (0.91-7.89)	0.074
ECOG PS (Continuous)	**2.4 (1.12-5.13)**	**0.024**	**2.46 (1.16-5.20)**	**0.018**	**3.75 (1.50-9.35)**	**0.005**	**3.22 (1.13-9.19)**	**0.029**
Local excision (No vs Yes)	0.67 (0.23-1.98)	0.47			1.08 (0.34-3.37)	0.897		
P16 (N/A vs Yes)	1.45 (0.49-4.27)	0.499			1.10 (0.25-4.89)	0.903		
Tumor grade (Continuous)	**0.69 (0.49-0.98)**	**0.04**	-	-	0.88 (0.61-1.29)	0.518		
T stage (T1/2 vs T3/4)	**2.68 (1.17-6.16)**	**0.02**	-	-	2.32 (0.84-6.45)	0.106		
N stage (N0 vs N1)	1.24 (0.56-2.75)	0.59			0.92 (0.35-2.39)	0.858		
Bulky nodal status								
(N0 vs non-bulky N+)	0.97 (0.41-2.29)	0.94	1.04 (0.44-2.46)	0.938	0.92 (0.35-2.39)	0.48	0.55 (0.17-1.73)	0.302
(N0 vs bulky N+)	**4.05 (1.26-13.03)**	**0.019**	**4.69 (1.40-15.73)**	**0.012**	**4.48 (1.10-18.3)**	**0.037**	3.40 (0.82-14.1)	0.091
(non-bulky N+ vs bulky N+)	**4.19 (1.27-13.82)**	**0.019**	**4.69 (1.40-15.73)**	**0.012**	**4.48 (1.10-18.3)**	**0.037**	**6.25 (1.37-28.49)**	**0.018**
Baseline NLR (<2.5 vs ≥2.5)	1.58 (0.66-3.80)	0.302			**3.75 (1.28-10.98)**	**0.016**	2.87 (0.91-9.04)	0.072
Post-treatment NLR (<2.5 vs ≥2.5)	1.37 (0.63-2.97)	0.424			1.1 (0.44-2.79)	0.834		

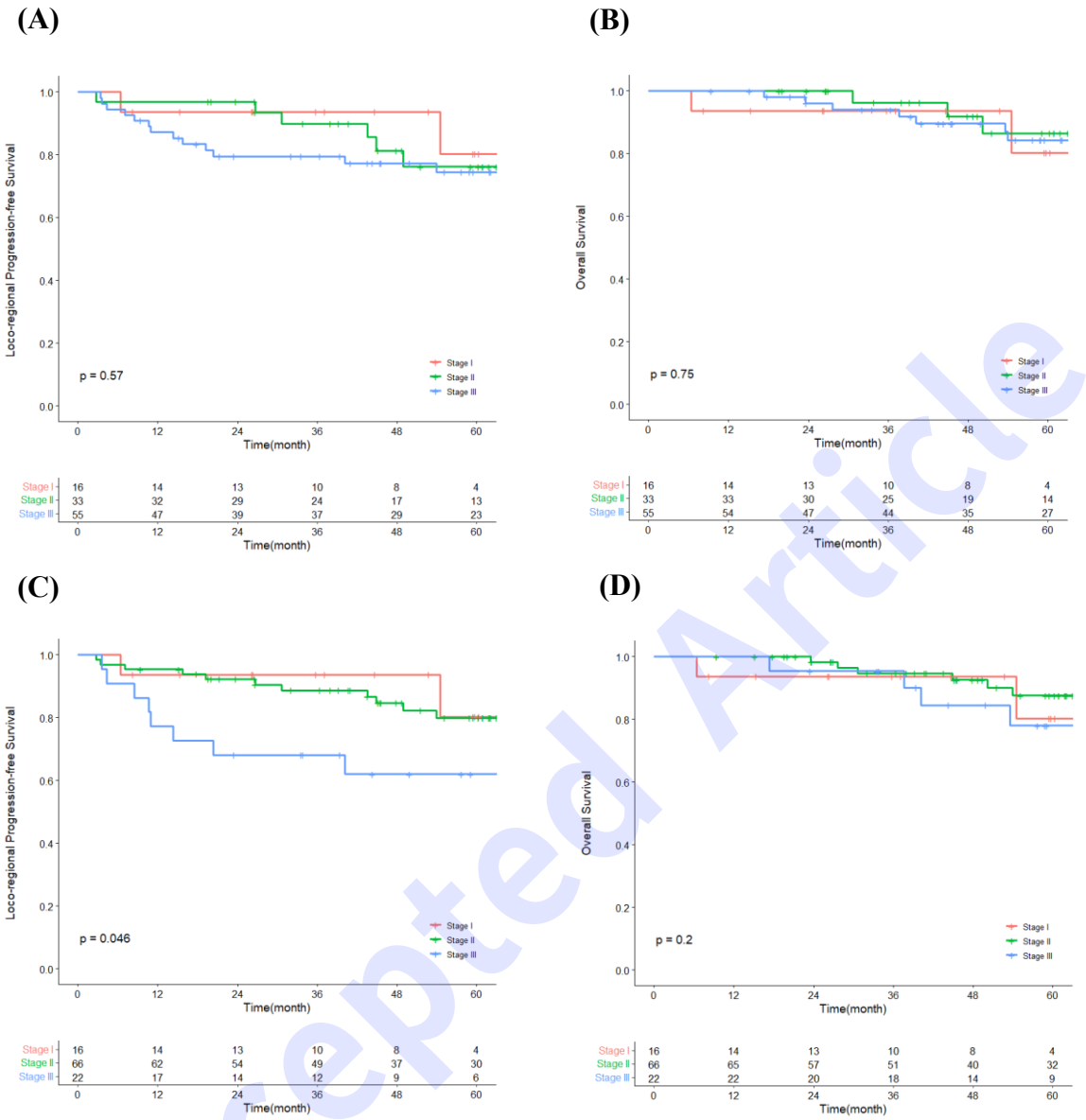
Post-treatment lymphopenia (<0.5 vs ≥0.5)	0.71 (0.24-2.09)	0.534	0.60 (0.17-2.13)	0.427
RT technique (3D-CRT vs IMRT)	1.01 (0.43-2.35)	0.981	1.76 (0.63-4.92)	0.281
RT dose (≤54 vs >54)	1.08 (0.48-2.42)	0.846	0.85 (0.32-2.27)	0.75
Chemotherapy (MMC vs non-MMC)	1.50 (0.63-3.56)	0.356	0.99 (0.34-2.91)	0.988

LRRFS, loco-regional recurrence-free survival; OS, overall survival; HR, hazard ratio; ECOG PS, European Cooperative Oncology Group Performance Status; NLR, neutrophil-lymphocyte ratio; MMC, Mitomycin-C.

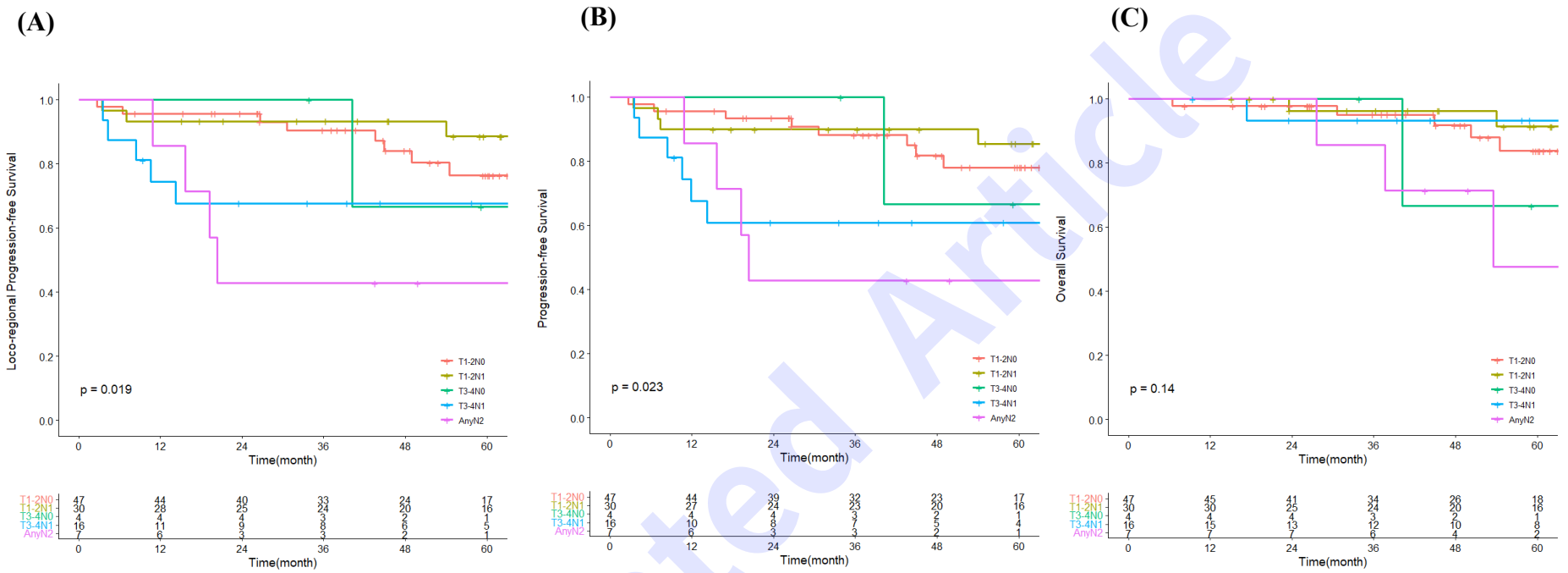
S2 Table. Detailed characteristics of patients with bulky node involvement

No.	Age	Sex	T stage	N stage	Largest Node (cm, location)	Nodal disease station (PR/II/EI/IN)	RT dose (primary, Gy)	RT dose (gross node, Gy)	RT dose (elective, Gy)	CRx	Failure pattern	DFI (months)
1	54	F	1	N1a	2.5, IN	-/-/-/+	60.4	60.4	50.4	FC	Local	19.2
2	74	F	2	N1a	2.4, II	+/+/-/-	55.0	55.0	50.0	FM	None	43.6
3	56	F	3	N1a	5.5, IN	+/+/-/+	59.4	59.4	41.4	FM	Regional (IN)	20.4
4	62	M	3	N1a	3.1, IN	-/+/-/+	59.4	59.4	50.4	FM	None	49.9
5	69	F	3	N1c	2.9, IN	-/+/+/+	54.0	64.8	54.0	XP	Local & distant	10.9
6	71	M	2	N1c	5.2, IN	+/+/+/+	54.0	54.0	44.0	FM	LR (EI, IN) & DM	15.7
7	35	F	2	N1a	2.4, II	+/+/-/-	59.4	59.4	50.4	FM	None	99.7

PR, perirectal; II, internal iliac; EI, external iliac; IN, inguinal; RT, radiotherapy; CRx, chemotherapy; LR, loco-regional; DM, distant metastasis; DFI, disease free interval; FC, 5-FU + Carboplatin; FM, 5-FU + Mitomycin-C; XP, Capecitabine + Cisplatin.



S3 Fig. Loco-regional recurrence-free survival of patients staged by (A) AJCC 8th edition and (C) AJCC 9th edition, and overall survival of patients staged by (B) AJCC 8th edition and (D) AJCC 9th edition.



S4 Fig. (A) Loco-regional recurrence-free survival (B) progression-free survival, and (C) overall survival of patients with T1-T2N0, T1-2N1, T3-4N0, T3-4N1 and N2 disease. T1-2N0 (red) and T1-2N1 (yellow) indicate newly staged I. T3-4N0 (green) and T3-4N1 (blue) indicate newly staged II. Any N2 (or bulky N+ in purple) indicate newly staged III.

S5 Table. Outcomes of reported studies regarding bulky nodal disease in uterine cervix, oral cavity and oropharyngeal cancer

Type of cancer	No. of patients	No. of patients with bulky nodal disease (criteria for bulky disease)	Radiation dose	Outcomes
Uterine cervix [13]	161	70 (>2cm)	46-60Gy	Increased regional recur (HR 1.9)
Uterine cervix [14]	208	22 (>2cm)	66.9-74.1Gy	Pelvic recur 2/22 (total 5/208)
Uterine cervix [24]	151	151 (≥1.5cm)		Survival 45% in median follow-up of 45 months
Oral cavity [23]	14,554	2665 (>2cm)	N/A	Poor OS of HR 2.14-2.35 but not in MVA
Oropharynx [15]	1,907	133 (>6cm)	N/A	5-year OS 59% (compared to 80%-83%)
Oropharynx [22]	156	79 (>6cm)	N/A	3-year DFS 88% (100% for non bulky)