Original article

The influence of systemic inflammation on treatment response and survival in anal squamous cell cancer.

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Abstract

Background: The incidence of anal squamous cell cancer (SCCA) is rising. While chemoradiotherapy (CRT) provides a chance of cure, a proportion of patients have an incomplete response or develop recurrence. This study assessed the value of inflammation-based prognostic indicators including the modified Glasgow Prognostic Score (mGPS) and neutrophil to lymphocyte ratio (NLR) in patients with SCCA treated by CRT with curative intent.

Material and methods: Patients with histologically confirmed SCCA were identified from pathology records. Medical records were retrospectively reviewed and clinical, pathological and treatment characteristics were abstracted. The mGPS (0 = normal CRP and albumin, 1 = CRP >10mg/L and 2 = CRP >10mg/L and albumin <35mg/L) and NLR were calculated from routine blood tests obtained prior to CRT.

Results: In total, 118 patients underwent CRT for SCCA between December 2007 and February 2018. Of these, 99 patients had appropriate pre-treatment blood results available. Systemic inflammation as indicated by NLR>3 and mGPS>0 was present in 41% and 39% of patients respectively. Most patients had T2 or larger tumours (n=85, 86%) without nodal involvement (n=64, 65%). An elevated mGPS was associated with more advanced T-stage (56% vs 35%, p=0.036). NLR>5 was associated with nodal positivity (56% vs 31%, p=0.047). On multivariate analysis, more advanced T-stage (OR 7.49, 95%CI 1.51 – 37.20, p=0.014) and a raised mGPS (OR 5.13, 95%CI 1.25 – 21.14, p=0.024) were independently related to incomplete CRT response. An elevated mGPS was prognostic of inferior survival (HR 3.09, 95% CI 1.47 – 6.50,

p=0.003) and cancer-specific survival (HR 4.32, 95% CI 1.54 – 12.15, p=0.006), independent of TNM stage.

Conclusion: Systemic inflammation, as measured by the mGPS, is associated with incomplete CRT response and is independently prognostic of inferior survival in patients with SCCA. The mGPS may offer a simple marker of inferior outcome which could be used to identify high risk patients.

Keywords: anal cancer, chemoradiotherapy, systemic inflammation, prognosis, survival.

Abbreviations:

cCR complete clinical response

CRP c-reactive protein

CRT chemoradiotherapy

CSS cancer-specific survival

HPV human papillomavirus

mGPS modified Glasgow Prognostic Score

MDT multidisciplinary team

NLR neutrophil: lymphocyte ratio

OS overall survival

RFS recurrence-free survival

SCCA anal squamous cell cancer

SIR systemic inflammatory response

Introduction

Anal squamous cell cancer (SCCA) is rare, with a global annual incidence of approximately 1 per 100,000[1]. However, incidence rates have risen substantially in the last 3 decades and are projected to increase by over 40% in the UK in the next 15 years[2]. Five-year survival is around 80% in patients who present with locoregional disease but only 13% in patients with distant metastases at the time of diagnosis[3].

Radiation with concurrent chemotherapy remains the standard of care for SCCA[4-7], with recent randomised controlled trials providing refinements to the cytotoxic components of treatment[8,9]. Local recurrence remains a problem, with rates of over 20% reported in the largest recent UK phase III study[9]. Beyond recognised clinicopathological characteristics including increasing tumour size[9-11] and nodal positivity[12], routinely available prognostic indicators are lacking.

The presence of a host systemic inflammatory response (SIR) prior to treatment is a well-established indicator of poor prognosis in cancers of the gastrointestinal tract[13]. The SIR has been evaluated most commonly by ratios of white cell differentials such as the neutrophil:lymphocyte ratio (NLR) and by composite scores assessing acute phase proteins albumin and C-reactive protein (CRP), such as the modified Glasgow Prognostic Score (mGPS)[14]. Cancer-associated systemic inflammation constitutes a complex phenomenon. Inflammatory cytokines stimulate haematopoiesis resulting in alterations in circulating neutrophil and lymphocytes levels[15] while acute phase proteins, the downstream effectors of the interleukin-6 pathway, induce hepatocyte CRP production. Impaired cell-mediated

immunity, both in the systemic circulation and the local tumour microenvironment, is associated with a raised CRP[16,17]. Alterations in albumin levels reflect changes in energy metabolism and loss of lean muscle as a result of cancer-associated systemic inflammation[18,19]. The mGPS therefore represents the impact of systemic inflammation on multiple organs and integrates both the innate and adaptive immune response to cancer. It has prognostic value in a range of solid tumours independent of tumour factors including TNM stage and host factors such as comorbidity[13,14]. Critically, it is associated with negative characteristics such as obesity and advanced age and recognised negative pathologic prognostic indicators[14], reflecting their combined adverse effect.

The NLR has previously been described as a potential predictor of recurrence and death from SCCA[20,21]. However, the prognostic value of the mGPS in SCCA has yet to be investigated. The aim of this study was to examine the prognostic value of the mGPS and NLR in a retrospective cohort of patients with primary SCCA undergoing chemoradiotherapy (CRT) with curative intent.

Material and methods

All patients with histologically confirmed SCCA treated by primary CRT between December 2007 and February 2018 were identified from a prospectively maintained pathology database using keyword searches (anal cancer, anal squamous cell carcinoma). Clinical data, including treatment modality, response to CRT and survival were abstracted retrospectively from patient records. Imaging and pathology reports combined with the records of multidisciplinary team (MDT) discussions were used to determine local recurrence and distant metastatic disease. Local recurrence was defined as biopsy- or imaging-proven SCCA more than 6 months following completion of CRT in a patient with previously achieving a complete clinical response (cCR). Local recurrence included new local disease with or without involvement of perirectal, internal pudendal, iliac or inguinal nodes. Distant recurrence was defined as extra-pelvic disease, including para-aortic nodal involvement. Persistent disease was defined as clinical, radiological and/or histopathological evidence of SCCA identified within 6 months of completion of primary CRT. Cause of death was determined from the clinical record.

Pre-treatment values of neutrophils, lymphocytes, albumin and C-reactive protein (CRP) from venous blood samples obtained at the pre-assessment visit to the treating cancer centre immediately prior to starting CRT were extracted from the electronic medical record. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. Previous literature has most commonly used a NLR threshold of 3 or 5 to define systemic inflammation, with the optimal threshold remaining controversial[22]. For this reason, a NLR greater than 3 and 5 were used. The mGPS was recorded as 0 for those patients with an albumin greater than 35 g/L and

CRP less than 10mg/L, 1 for those with CRP greater than 10mg/L and 2 for those with an albumin value of less than 35 g/L and CRP greater than 10mg/L. A mGPS score of greater than 0 was regarded as evidence of systemic inflammation.

Overall survival (OS) was measured from the date of first treatment to date of death from any cause. Cancer-specific survival (CSS) was measured from the date of first treatment to date of death due to SCCA. Recurrence-free survival was calculated from date of first treatment until the date of documented recurrence. Survival data were censored in May 2019.

Ethical approval for this study was granted by the XX Research Ethics Committee.

Statistical analysis

Descriptive statistics were used to summarise the patient population. Univariate Cox regression was performed to assess the relationship between preoperative clinicopathological features and cancer-specific and overall survival respectively. Variables with a p-value of 0.05 or less were entered into a multivariate Cox regression model using a backward conditional method. A p-value of less than 0.05 was considered significant.

Results

During the study period, 207 patients received a histological diagnosis of SCCA within one health board. Of these, 59 patients had surgery as their primary treatment, 20 patients had CRT with palliative intent and 10 patients received no treatment. In total, 118 proceeded to CRT with curative intent. The study population consisted of 99 patients who had serum inflammatory markers measured prior to commencing CRT.

Patients received external beam radiation therapy using either a shrinking-field technique or Volumetric arc therapies. A dose of 50 – 53.2 Gy was delivered to the primary tumour over 5 or 5 and a half weeks with elective nodal areas receiving approximately 3000cGy. Radiation therapy was combined with systemic chemotherapy, generally with mitomycin-C and a fluoropyrimidine.

Baseline characteristics are presented in table 1. The majority of patients were female (n=69, 70%) and younger than 65 years old (n=76, 77%). Two patients were receiving treatment for HIV and two patients were immunocompromised as a result of long-term steroids or immunosuppressant therapy. Most patients had T2 or larger tumours (n=85, 86%) without nodal involvement (n=64, 65%).

A complete clinical response (cCR) was found in 86 patients (87%) while 8 patients had persistent disease and 5 patients progressed on treatment. Of the patients with persistent disease post-CRT, 4 underwent salvage surgery, two had palliative chemotherapy and two were managed with supportive care. Of those with a cCR, 14 patients (16%) developed a

recurrence more than 6 months following completion of CRT. Local recurrence developed in 9 patients (11%), distant in 2 patients (2%) and synchronous local and distant recurrence in 3 patients (3%). Treatment for local recurrence was salvage surgery in 4 patients, palliative chemotherapy in 4 patients and supportive care in 1 patient.

Median follow-up for survivors was 58 (15 to 130) months. During this period, 29 deaths occurred: 17 deaths (17%) due to SCCA and 12 deaths (12%) attributable to other causes. In patients with a cCR (n=86), 8 (9%) died due to recurrent disease and 10 (12%) died due to other causes. In patients who had an incomplete response (n=13), 9 deaths due to SCCA occurred and 2 deaths occurred which were not related to SCCA. Two patients who had an incomplete response were alive at the time of last follow-up.

Based on pre-treatment laboratory tests, 39 patients (39%) had an elevated mGPS while 41 patients (41%) had a NLR greater than 3 and 18 patients (18%) had a NLR above 5. The associations between pre-treatment systemic inflammatory markers (mGPS, NLR>3 and NLR>5) and clinicopathological characteristics are shown in table 2. Patients with pre-treatment elevation of the mGPS were more likely to have higher T stage tumours. A trend towards significance was noted in patients with a NLR >3 when comparing T stage: 54% patients had a NLR >3 had T3 or greater tumours compared with 36% of patients with T3 or greater tumours who had NLR <3 (p=0.084). Patients with a NLR>5 were more likely to have node-positive disease (56% vs 31%, p=0.047).

Associations between clinicopathological characteristics and response to CRT are shown in table 3. Incomplete response was associated with a higher T stage (85% T3 or greater vs 15%

T1-2 tumours, p<0.001), nodal disease (69% node positive vs 31% node negative, p=0.006) and a raised mGPS (77% mGPS 1-2 versus 23% mGPS 0, p=0.003). No significant differences were noted when examining the NLR in relation to CRT response regardless of threshold used. On multivariate binary logistic regression, more advanced T stage (OR 7.49, 95%CI 1.51 – 37.20, p=0.014) and the presence of a raised mGPS (OR 5.13, 95%CI 1.25 – 21.14, p=0.024) were independently related to incomplete response to CRT (data not shown).

The relationship between pre-treatment factors and survival was studied using Cox-proportional regression (table 4). Inferior overall survival was related to increasing TNM stage (HR 3.75, 95% CI 1.77 - 7.93, p=0.001) and a raised mGPS (HR 3.24, 95% CI 1.54 – 6.81, p=0.002) on univariate analysis. The NLR was not significantly associated with overall survival on univariate analysis using a threshold of 3 (HR 1.66, 95% CI 0.81– 3.41, p=0.166) or 5 (HR 1.07, 95% CI 0.44 - 2.62, p=0.886). On multivariate analysis, higher TNM stage (HR 3.59, 95% CI 1.69 – 7.64, p=0.001) and a raised mGPS (HR 3.09, 95% CI 1.47 – 6.50, p=0.003) were independently prognostic of inferior overall survival.

For cancer-specific survival, univariate analysis demonstrated that more advanced T stage (HR 3.17, 95% CI 1.80 – 5.61, p<0.001), higher nodal stage (HR 1.99, 95%CI 1.40 – 2.83, p<0.001) and raised mGPS (HR 4.80, 95% CI 1.71 – 13.47, p=0.001) were associated with inferior survival. Male gender (HR 0.78, 95%CI 0.28 – 2.19, p=0.639) and a low pre-treatment haemoglobin (HR 0.82, 95%CI 0.31 – 2.20, p=0.698, data not shown) were not associated with CSS. On multivariate analysis, a raised mGPS (HR 4.32, 95% CI 1.54 – 12.15, p=0.006) was prognostic of inferior cancer-specific survival, independent of TNM stage (HR 5.00, 95% CI 1.82 – 13.69, p=0.002). There were no significant relationships between NLR and overall or

cancer-specific survival on univariate analysis, regardless of threshold value used. Kaplan Meier curves demonstrating the relationship between cancer-specific survival and TNM stage and mGPS are displayed in figures 1a and 1b.

Recurrence-free survival (RFS) at 3 years was also assessed in those patients with a complete response to CRT (Table 4b). Aside from higher T-stage, there was no clinical or pathological characteristic which related to inferior RFS at 3 years. Specifically, neither the pre-treatment NLR nor mGPS was associated with RFS.

Discussion

This study highlights the prognostic value of the SIR in patients with SCCA treated by CRT. In this cohort, an elevated pre-treatment mGPS was associated with a lower incidence of complete response and inferior overall and cancer-specific survival, independent of traditional predictors such as TNM stage. Recurrence-free survival was not associated with the presence of a preoperative SIR when assessed by the mGPS or NLR. It was notable that, contrary to other series[20,21], we did not find that the pre-treatment NLR was associated with treatment outcome or survival regardless of the threshold value used. On this basis, the mGPS may represent a useful prognostic indicator in patients with SCCA treated by CRT.

The search for biomarkers in SCCA is driven by the need to improve patient selection for CRT and enable disease surveillance thereafter[23]. The lack of progress in identifying such biomarkers highlights the ongoing need for greater appreciation of the underlying molecular biology, but also the key role of co-factors. While it is well recognised that immunosuppression plays an important role in facilitating HPV-induced carcinogenesis, the potential facilitative role of systemic inflammation in enabling progression to invasive SCCA has received little attention[24].

A higher incidence of chronic HPV infection is reported in patients with systemic inflammatory conditions[25-28]. Inflammatory mechanisms are implicated in various key steps in the development of SCCA. At the cellular level, HPV infection results in deregulation of inflammatory responses via E6 and E7 oncoprotein-mediated suppression of NF-KB signalling, interrupting the normal regulatory function of tumour suppressor p53[29]. Up-regulation of

anti-apoptotic gene expression results in suppression of cell death and stimulation of tumour cell proliferation. HPV-positive tumours display upregulation of the pro-inflammatory cytokine IL -6 and higher levels of tumour necrosis than HPV-negative tumours[30]. Both intra-tumoral IL-6 levels and tumour necrosis have been found to stimulate systemic inflammation in colorectal cancer[31], with such mechanisms potentially underpinning the immune-inflammatory interaction in SCCA. Similarly, studies of immunotherapies in melanoma have highlighted the role of inflammation in stimulating secretion of interferon-γ which subsequently activates immune checkpoints, resulting in acquired resistance[32,33]. Trials of immune checkpoint blockade in patients with metastatic SCCA have demonstrated durable responses in selected cases[34,35]. Combining anti-inflammatory therapies with immunotherapy in a manner similar to that currently being investigated in patients with recurrent cervical cancer[36] may hold future therapeutic promise.

A subset of SCCAs have been shown to develop via HPV-independent mechanisms, with co-factors such as smoking and inflammation proposed as key mediators of this pathway[37]. Importantly, inferior prognosis is associated with such HPV-negative tumours[37]. The major inducers of systemic inflammation in otherwise healthy individuals include obesity and comorbidity as well as smoking, with elevated CRP levels well described in population-based studies of such individuals[38,39]. It is conceivable that a pro-inflammatory host environment may be an important factor in the pathogenesis of SCCA in the absence of HPV.

In established SCCA, it has also been shown that circulating markers of systemic inflammation correlate inversely with the intra-tumoral inflammatory infiltrate. Martin and colleagues demonstrated that peripheral leucocytosis was associated with reduced density of cytotoxic

T lymphocytes within the tumour microenvironment, which translated to inferior disease-free and overall survival in a cohort of 79 patients with SCCA[40]. However, the effect of peripheral leucocytosis on oncologic outcome was mainly attributable to distant metastases in this study, suggesting abnormalities in white cell count alone are not sufficient to highlight patients at risk of locoregional relapse. Using the CRP to albumin ratio (CAR), the same group recently reported weaker cytotoxic lymphocyte infiltration in patients with a higher pre-treatment CAR but acknowledged lack of concordance in the existing literature regarding the optimal threshold value of the CAR used to define systemic inflammation[41].

While the prognostic role of the NLR in various cancers has been widely described, it similarly remains a problematic measure due to the lack of concordance around the optimal threshold used to define systemic inflammation[22]. In this cohort, it was notable that less than half of those who had systemic inflammation as evidenced by a raised mGPS had a NLR greater than 5. Toh and colleagues used ROC analysis to derive a cut-off for NLR of 4.75 in a cohort of 92 patients, with NLR values above this threshold reported to be independently prognostic of recurrence as well as overall and cancer-specific survival[20]. However, multivariate analysis was carried out in 18 patients who developed recurrence within the 30-month follow-up period. Data on patient characteristics was also absent, with only age and gender used to describe the cohort. De Felice and co-workers reported the NLR to hold prognostic value for overall survival in 58 patients with SCCA treated with primary CRT[42]. In this study, a threshold of 2.5 was derived on ROC analysis, with a corresponding area under the curve of 0.55. The lack of concordance between these studies highlights the difficulty in clinical application of the NLR as a measure of the systemic inflammatory response. Recent studies in patients with oropharyngeal SCC demonstrated a relationship between HPV-positive tumours and low NLR

values[43,44], with the authors suggesting that HPV status may interact with the prognostic value of NLR.

The prognostic utility of the mGPS has, by comparison, been consistently demonstrated in other squamous HPV-related cancers. In cervical cancer, several studies have confirmed an elevated mGPS to be an independent predictor of survival in patients undergoing CRT and surgery[45-47], while in a single study in patients with vulval cancer[48], it was associated with inferior disease-free and overall survival. Attempts have been made to reduce progression to invasive cancer in patients with cervical intra-epithelial neoplasia through regular administration of non-steroidal anti-inflammatory drugs. However, there are currently insufficient data to support their routine use[49].

Beyond prognostication, the mGPS may also provide a useful risk-stratification measure. Validation is first required, ideally in a prospective setting. Subsequent assessment of the SIR throughout treatment would better define its role in tailoring treatment or follow-up in conjunction with the existing prognostic information provided by TNM staging. Platforms such as the PLATO trial[50] offer a vehicle for validation as well as assessment of response to radiotherapy with respect to pre-treatment markers of systemic inflammation. If validated, the mGPS benefits from being a simple and cost-effective indicator of adverse outcome that would require little in terms of additional resource for clinical application in this setting.

The use of selective or non-selective agents targeting the inflammatory response[51,52] represent important areas of future study in patients with SCCA. Systemic inflammation has long been recognised as a component of the cancer-cachexia syndrome and as such, therapeutic manipulation of the SIR is best evidenced in trials of anti-inflammatory therapies

in advanced cancer. Use of non-steroidal anti-inflammatory drugs has been associated with both slowing of weight loss and an improvement in SIR status in several trials, although often relatively small and non-randomised by design[53-57]. In colorectal cancer, reduced recurrence and improved survival have been attributed to the anti-inflammatory properties of aspirin and statins[58,59]. Indeed, the use of aspirin as an adjuvant therapy in patients with resected solid tumours is the subject of ongoing trial[60]. Similar trials in patients with SCCA treated with CRT would enable understanding of whether those with a pre-treatment SIR could benefit from such a strategy.

This study has several limitations. The retrospective design and limited number of recurrence events mandate external validation of our findings. The lack of association between the mGPS and recurrence-free survival at 3 years is an unexpected finding given its status as an independent predictor of cancer-specific survival in this cohort. It may be related to the limited number of recurrence events but warrants further investigation in a larger cohort. It was not possible to characterise the prevalence of HPV infection within this cohort due to a lack of formal HPV testing and variability in the pathological reporting of features associated with HPV. Furthermore, comorbidity and BMI are known to influence systemic inflammation but were not included in the analysis as a consequence of missing data in a significant number of patients.

Despite the limitations, these data suggest that the pre-treatment mGPS provides a simple, reliable and readily available prognostic indicator in patients with SCCA. Confirmatory studies which include assessment of serum inflammatory markers before, during and after CRT are required in order to evaluate further their role in prognostication and surveillance.

Conclusions

The modified Glasgow Prognostic Score appears to be a clinically relevant biomarker in patients with anal squamous cell cancer treated with primary chemoradiotherapy. Systemic inflammation may be useful as an adjunctive measure in the pre-treatment identification of patients at risk of inferior oncologic outcome.

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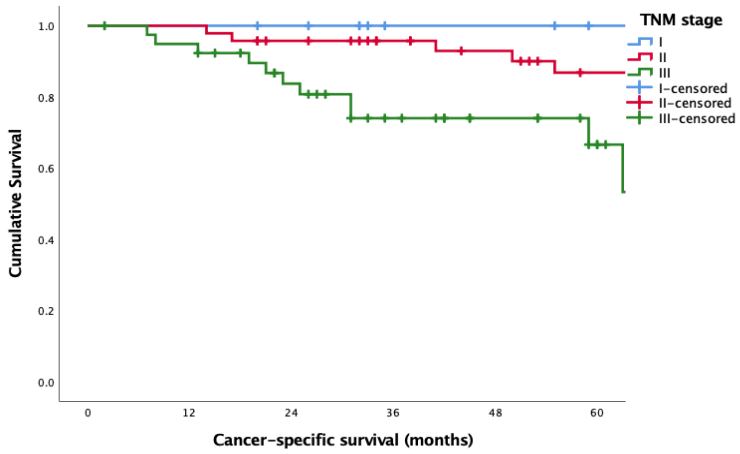
Figure 1. Kaplan–Meier curves showing the relationship between cancer-specific survival and (a) TNM stage (Log-rank test P = 0.003) and (b) modified Glasgow Prognostic Score (mGPS) (P = 0.003).

1a.



1b.

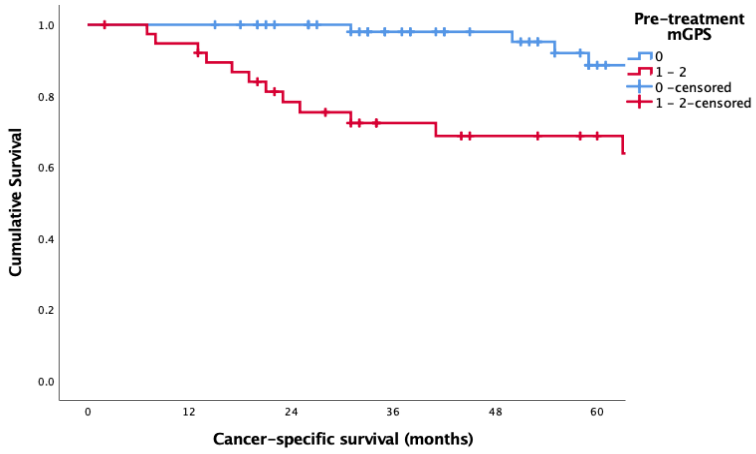


Table 1: Baseline characteristics of patients with SCCA undergoing CRT with curative intent (n=99).

		n (%)
Age (years)	<65	76 (77)
	65-75	15 (15)
	>75	8 (8)
Gender	Male	30 (30)
	Female	69 (70)
HIV status	Negative	97 (98)
	Positive	2 (2)
Immunosuppression	No	97 (98)
	Yes	2 (2)
Smoking History	No	36 (36)
	Yes	63 (64)
T-stage	1 - 2	56 (57)
	3 - 4	43 (43)
N-stage	Negative	64 (65)
	Positive	35 (35)
mGPS	0	60 (61)
	1	13 (13)
	2	26 (26)
NLR	<3	58 (59)
	>3	41 (41)
NLR	< 5	81 (82)
	> 5	18 (18)

Table 2: Associations between systemic inflammatory measures and clinicopathological characteristics in patients with SCCA undergoing primary CRT (n=99).

		mGPS = 0	mGPS = 1 or 2	p-value	NLR <3	NLR >3	p-value	NLR <5	NLR >5	p-value
Age (years)	<65	46 (76)	30 (81)	0.738	48 (83)	28 (68)	0.169	63 (78)	13 (72)	0.565
	65 - 74	10 (17)	5 (14)		6 (10)	9 (22)		12 (14)	3 (17)	
	>75	4 (7)	2 (5)		4 (7)	4 (10)		6 (8)	2 (11)	
Gender	Female	45 (75)	24 (83)	0.154	42 (72)	27 (66)	0.485	57 (70)	12 (67)	0.757
	Male	15 (25)	5 (17)		16 (28)	14 (44)		24 (30)	6 (33)	
Smoking history*	No	33 (56)	17 (44)	0.232	30 (52)	20 (50)	0.867	42 (53)	8 (44)	0.537
	Yes	26 (44)	22 (56)		28 (48)	20 (50)		38 (47)	10 (56)	
T-stage	1 – 2	39 (65)	17 (44)	0.036	37 (64)	19 (46)	0.084	47 (58)	9 (50)	0.534
	3+	21 (35)	22 (56)		21 (36)	22 (54)		34 (42)	9 (50)	
Nodal status	Negative	42 (70)	22 (56)	0.332	41 (71)	23 (56)	0.136	56 (69)	8 (44)	0.047
	Positive	18 (30)	17 (44)		17 (29)	18 (44)		25 (31)	10 (56)	

mGPS, modified Glasgow Prognostic Score; NLR, neutrophil:lymphocyte ratio.
*Missing cases - 1

Table 3: Associations between clinicopathological characteristics and CRT response in patients with SCCA undergoing primary CRT (n=99).

		Complete response	Incomplete response	p-value
Age (years)	<65	67 (79)	9 (70)	0.352
	65 - 74	13 (15)	2 (15)	
	>75	6 (6)	2 (15)	
Gender	Female	60 (70)	9 (69)	0.969
	Male	26 (30)	4 (31)	
Smoking history*	No	44 (52)	6 (46)	0.706
	Yes	41 (48)	7 (54)	
T-stage	1 – 2	54 (63)	2 (15)	0.001
	3	32 (37)	11 (85)	
N-stage	Negative	60 (70)	4 (31)	0.006
	Positive	26 (30)	9 (69)	
mGPS	0	57 (66)	3 (23)	0.003
	1 - 2	29 (34)	10 (77)	
NLR >3	No	53 (61)	5 (38)	0.114
	Yes	33 (39)	8 (62)	
NLR >5	No	72 (84)	9 (69)	0.207
	Yes	14 (16)	4 (31)	

mGPS, modified Glasgow Prognostic Score; NLR, neutrophil:lymphocyte ratio.
*Missing cases – 1

Table 4: Cox regression analysis of the relationship between pre-treatment factors and survival in patients with SCCA undergoing primary CRT (n=99).

	Overall Survival				**Cancer-Specific Survival**			
	Univariate analysis	**p-value**	**Multivariate analysis**	**p-value**	**Univariate analysis**	**p-value**	**Multivariate analysis**	**p-value**
Age (<65/65-74/>75)	1.48 (0.93 - 2.38)	0.102	-	-	0.97 (0.45 - 2.09)	0.941	-	-
Gender (Female/Male)	0.59 (0.25 - 1.38)	0.224	-	-	0.78 (0.28 - 2.19)	0.639	-	-
T-stage (1-2/3+)	5.14 (2.28 - 11.61)	0.001	-	-	3.17 (1.80 - 5.61)	0.001	-	-
N-stage (negative/positive)	3.52 (1.69 - 7.33)	0.001	-	-	1.99 (1.40 - 2.83)	0.001	-	-
TNM stage (I – II/III)	3.75 (1.77 - 7.93)	0.001	3.59 (1.69 – 7.64)	0.001	5.17 (1.92 – 13.95)	0.001	5.00 (1.82 – 13.69)	0.002
Smoking history (No/Yes)	0.84 (0.41 - 1.73)	0.640	-	-	0.70 (0.27, 1.81)	0.464	-	-
NLR (<3/>3)	1.66 (0.81– 3.41)	0.166	-	-	1.78 (0.70 – 4.51)	0.228	-	-
NLR (<5/>5)	1.07 (0.44 - 2.62)	0.886	-	-	1.64 (0.58, 4.62)	0.350	-	-
mGPS (0/1-2)	3.24 (1.54 – 6.81)	0.002	3.09 (1.47 – 6.50)	0.003	4.80 (1.71 – 13.47)	0.003	4.32 (1.54 – 12.15)	0.006

Table 5: The relationship between pre-treatment factors and recurrence-free survival at 3 years in patients with complete response to primary CRT (n=86)

Recurrence-Free Survival	**Univariate analysis (HR (95%CI))**	**p-value**
Age (<65/65-74/>75)	0.61 (0.17 - 2.11)	0.606
Gender (Female/Male)	0.425 (0.09 - 1.95)	0.270
T-stage (1-2/3+)	3.68 (1.11 - 12.24)	0.033
N-stage (Negative/Positive)	2.55 (0.82 - 7.91)	0.105
Smoking history (No/Yes)	0.367 (0.10 - 1.36)	0.133
NLR (<3/>3)	1.15 (0.37– 3.63)	0.809
NLR (<5/>5)	0.44 (0.06 – 3.43)	0.435
mGPS (0/1-2)	1.57 (0.50 – 4.94)	0.444

mGPS, modified Glasgow Prognostic Score; NLR, neutrophil:lymphocyte ratio.