Head and Neck

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Journal:	*Head & Neck*
Manuscript ID:	HED-15-0196
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	17-Feb-2015
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Prognostic value of the neutrophil to lymphocyte ratio in laryngeal squamous cell carcinoma patients

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Brief running title: NLR and laryngeal cancer

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Abstract

Background. The neutrophil to lymphocyte ratio (NLR) has been found to be predictive of survival outcome in a range of tumours. The purpose of this study was to investigate the prognostic value of pre-treatment (NLR) in laryngeal squamous cell carcinoma (LSCC) patients.

Methods. A retrospective analysis of 140 LSCC patients treated between 2005 and 2010 in the Hull and East Yorkshire Hospitals NHS Trust was carried out. Patient records were evaluated and both pretreatment neutrophil and lymphocyte counts were documented together with survival data, gender, smoking status, nodal status and disease staging.

Results. An elevated NLR was significantly associated with advanced disease stage, e.g. node positive and tumours Stage III & IV. In addition a high NLR was significantly associated with poor overall survival but not disease free survival on multivariate analysis, with the greatest significance seen in patients with the highest NLR.

Conclusion. Pretreatment NLR may serve as a useful prognostic marker in LSCC, however a large prospective study is required to determine an optimal NLR cut-off value.

Introduction

In England, the incidence of laryngeal cancer has fallen by 20% since 1990[1]. Similar findings have also been observed in Europe with a fall from 45,900 new cases in 2006 to 39,900 cases in 2012[2,3]. Better public health promotion on the adverse effects of smoking and drinking alcohol is likely to be a major contribution to the observed decrease. However, the survival rate for patients with laryngeal cancer has remained unchanged for the last two decades with a 5-year relative survival of 65% in England[1]. In contrast, the survival rates for other head and neck cancer sites such as oral cavity, salivary gland, oropharynx and nasopharynx have shown some improvement[1]. In order to improve the survival outcome of laryngeal cancer patients, alternative ways of assessing the best treatment plan are necessary.

Understanding tumour biology is essential in developing optimal treatment and management for cancer patients. In recent years, there have been a number of studies investigating the role of inflammation and the immune system in tumorigenisis.[4,5] Cancer cells are known to evoke an immune response against tumour specific antigens, causing an influx of lymphocytes into the tumour microenvironment[6] and the attempted destruction of the cancer and surrounding area lead subsequently to an inflammatory response[6]. It is now widely recognised that inflammatory cells, such as neutrophils, have a role in cancer development and progression by promoting tumour cell growth and invasion via the production of pro-angiogenic vascular endothelial growth factor (VEGF) [7,36,8,9], as well as remodelling the extracellular matrix via the release of

multiple cytokines and chemokines, such as IL-1β, IL-8 and IL12[7]. Tumour cells of various types have been demonstrated to escape immunosurveillance, one of the hallmarks of cancer development[10], by lymphocyte suppression using a number of direct cell:cell mechanisms as well as the release of soluble factors. These include the promotion, by the tumour, of myeloid-derived suppressor cells (MDSC) which have the ability to inhibit both innate and adaptive immunity.[7] Furthermore neutrophils inhibit the suppression of the cytolytic activity of T lymphocytes with the magnitude of this suppression being proportional to the number of neutrophils present.[11] Therefore, an elevated neutrophil/lymphocyte ratio (NLR) as a result of neutrophilia and lymphocytopenia could be an identifier of poor prognosis in cancer patients.

A number of studies have investigated the relationship between NLR and survival of cancer patients including those with renal cell cancer[12], sacrcoma[13] , colorectal [14,15] and oesophageal [16] cancer, which all found patients to have poorer outcomes with elevated NLR. Ikeguchi *et al.* have even suggested incorporating NLR in the prognostic scoring system in colorectal cancer[17]. Despite the positive data from other tumours and the fact that results are readily available, at a low cost, as part of the routine pre-treatment investigation only a few studies on the prognostic significance of NLR in head and neck cancer have been reported[18–24], with none of these examining ~~the~~ cancer of the larynx. The aim of this study therefore was to evaluate the prognostic value of NLR in patients with laryngeal (SCC).

Materials and Methods

Patients

A retrospective review of 218 patients with newly diagnosed SCC of the larynx admitted between January 2005 to December 2010 from Hull and East Yorkshire Hospital NHS Trusts, UK was carried out following ethical approval (REC:14/SW/0107; NHS R&D approval R1650). Patients with concurrent infection, chronic inflammatory conditions, long-term immunosuppressive medications, synchronous cancer, previous history of cancer, palliative treatment and those with no preoperative blood test were excluded from analysis. Medical records of all patients were reviewed and clinical information was collected, i.e. age at diagnosis, gender, TNM staging, treatment modalities, pre-treatment absolute neutrophil and absolute lymphocyte counts, time to recurrence and death. NLR was calculated as absolute neutrophil count divided by absolute lymphocyte count.[14,18] All patients were followed up for 5 years or until December 2013 or death.

Statistical analysis

Patient demographics (age, gender, smoking status) and clinical characteristics (nodal status, clinical stage, recurrence status) were displayed as frequency counts and percentages. Continuous data were expressed as medians with interquartile ranges and clinical stage was categorised into early (stage I and II) and late (stage III and IV) stage based on TNM classification using the seventh edition of the American Joint Committee on Cancer (AJCC) staging system. Continuous variables were analysed using the Mann-Whitney U or Kruskal-Wallis test as appropriate.

For survival analysis, patients were categorised into 4 groups on the basis of NLR quartiles where overall survival (OS) and disease-free survival (DFS) were the endpoints being assessed. Kaplan-Meier analysis was employed and compared by the log-rank test to determine any significance of NLR on survival. The Cox proportional hazard model was used to evaluate the potential prognostic variables and multivariate analysis was carried out on variables which were found to be significant on the initial univariate analysis. A p value <0.05 was considered to be statistically significant with all statistics being tested two sided. SPSS software v20 (SPSS, Chicago, IL) was used to perform all statistical analyses throughout.

Results

Patient demographics

Out of the initial 218 patients identified, 140 patients were eligible for further analysis after excluding 78 patients for reasons indicated in Table 1. Of the140 patients, there were 121 males and 19 females with a median age of 66 years (range 36-92). Demographic details and clinical characteristics of the patients along with NLR median and quartiles are presented in Table 2. Laryngeal cancer patients with advanced stage disease (III/IV) had a significantly higher NLR (2.46) than early stage disease (I/II; 2.07;p=0.006). No significant differences in the NLR were observed for any of the other characteristics.

The effect of NLR on survival

Using the upper and lower quartile and the median NLR from the whole patient cohort as divisions, Kaplan-Meier survival analysis showed that the presence of a high NLR in the peripheral circulation (greater than the upper quartile 3.10) was associated with poorer OS (p =0.014) but not DFS (p = 0.351;Figure 1 and 2). Univariate Cox regression analysis was performed for demographic and clinical variables (Table 3) and showed that female gender, nodal positive status and advanced clinical stage were all associated with poorer OS and DFS. NLR was significantly associated with OS (HR, 3.0 95% CI 1.28 – 6.97) but not DFS. Subsequently, a multivariate Cox regression analysis was carried out and revealed that patients with the highest quartile of NLR had a significantly poorer OS (HR, 3.06; 95% CI 1.08 – 8.67) compared to the lowest quartile (Table 4). Advanced clinical stage is significantly associated with a poorer OS [HR, 2.46; 95%CI 1.03 – 5.91) but not DFS. Patients with node positive disease as well as

being a female had significant poorer prognosis for both OS (HR, 2.58; 95% CI 1.38 – 4.81; HR 2.78 95% CI 1.39 – 5.57, respectively) and DFS (HR, 2.82; 95% CI 1.55 – 5.11; HR 2.29 95% CI 1.23 – 4.26, respectively) when compared with their counterparts.

Discussion

In the current study LSCC patients with a high pretreatment NLR were associated with poorer OS. Advanced disease (stage III/IV) was found to be associated with high NLR when compared with early stage disease (stage I/II). However, NLR was found to have no predictive value for DFS.

A growing number of studies investigating the association between NLR and cancer from different anatomical sites have emerged in recent years.[13–16,25–27]. Studies on sarcoma, colorectal, renal and gastric cancer found that high NLR is associated with poor OS[13,14,28,29], DSS[15] , PFS [12,18], or DFS[30] as well as being a positive indicator of cancer recurrence [13,15]. Meanwhile, studies on oral cavity, gastric, colorectal, bladder and oesophageal cancers reported NLR had no prognostic values in predicting OS [25–27,31], DSS[24] or recurrence[29]. However, only seven studies have examined the relationship of NLR with cancer in the head and neck region, of which three were on the nasopharynx [18–20], one on the thyroid [21] and three on the oral cavity[22–24]. Again different outcomes have been reported from these studies in part because different survival endpoints have been used, i.e. OS, disease-specific survival (DSS) / cancer-specific survival (CSS) and progression-free survival (PFS).

Our findings are comparable with the study by Jankova *et al.*[29] on colorectal cancer in which they found a link between high NLR and poor OS but no association was found between NLR and recurrence or cancer specific death. Within the head and neck studies, He *et al.,*[18] An *et al.,*[19] and Jin *et al.*[20] examined patients with newly presenting nasopharyngeal carcinoma (NPC) with 1410, 363 and 229 patients respectively and all concluded that high NLR was associated

with poor survival. Similar to the current study Jin *et al.,*[20] divided patients into groups based on the NLR quartile for comparison, with the same conclusion of poor OS being associated with high NLR. The current study additionally included DFS as a further endpoint although no association with NLR was found. Fang *et al*. and Perisanidis *et al.* also reported an association of high NLR with poor survival for oral cavity cancer patients (n=226 and 97 respectively). The only previous head and neck study examining DFS was by Fang *et al.,* and they also observed an association of poor DFS and high NLR. The thyroid study by Liu *et al*., reported an association between high NLR and tumour volume as well as the risk of recurrence but they did not investigate the association between NLR and survival. In contrast to the other head and neck studies, a recent study on oral cavity cancer patients by Tsai *et al.,* which included 202 patients, reported NLR had no predictive value on survival, however, they did report an association between high monocyte count and poor survival.

Given the conflicting results of these studies on NLR and cancer, a systematic review and meta-analysis[32] was carried out recently by Templeton *et al.* on 100 studies including 40,559 patients. Colorectal, gastroesophageal and hepatoceullar carcinoma patients made up the majority of the cohorts in this review with 22, 14 and 8 studies respectively. OS was the most common end point studied (n=79), follow by DFS (n= 28), PFS (n = 16) and CSS (n = 10). The overall conclusion was that high NLR is associated with an adverse OS in many solid tumours.

One of the reasons for the differences observed in the relationship of NLR to survival may be down to the different methods which have been used to identify and categorise the NLR cut-off values. The two methods most commonly used are receiver operating characteristic (ROC) curves, and comparison of medians and quartiles. An NLR of 5 has commonly been used as a cut-off value in colorectal[33] and mesothelioma studies[25]. However, the cut-off value used by many studies is often not clearly stated as documented by Templeton *et al*. in their meta analysis[32]. Furthermore, the underlying aetiology of cancer development is different between different cancer sites, for example, inflammatory bowel disease has a strong link with colorectal cancer [34], whereas cancer of the larynx does not share a similar inflammatory aetiology instead smoking-related irritations are the major risk factor, therefore the NLR cut-off could be very different between tumours. Within head and neck cancer subsites, our group has previously demonstrated that the prevalence of tumour infiltrating immune cells (CD4, CD8 and Foxp3) were subsite dependent with a higher number found in cancer of the oropharynx compared with the larynx[35]. Given the relationship between site specific inflammation and immune response, this further supports the idea that site-specific NLR cut-offs are essential.

In the present study, high NLR was found to relate significantly with a poorer OS but not DFS. Further investigations are not only needed to examine NLR and its clinical value but also the underlying mechanisms. Determination of NLR post treatment would also be of value as it might provide an insight into the response of the immune system following treatment, these data were not available as this was a retrospective analysis and the post treatment bloods were not analysed

routinely. If NLR normalised after treatment, this could mean that NLR is not only a prognostic marker but also a possible marker for monitoring disease progression. With these data available, it might allow patients to be further categorised based on the NLR in addition to the current TNM staging system and thus enable more tailored treatments to be administered. The retrospective nature of the current study meant that a proportion of patients were excluded due to the unavailability of pretreatment blood tests results. A large well designed prospective study is needed to address these clinical and basic science questions to provide definitive clinical prognostic information on patient outcome.

References

1. Profile of Head and Neck Cancers in England : Incidence, Mortality and Survival. 2010; at <www.ncin.org.uk/view?rid=69> [Accessed 17 Feburary 2015]

2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013; 49**:** 1374–403.

3. Ferlay J, Autier P, Boniol M et al. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2007; 18**:** 581–92.

4. Balkwill F & Mantovani a. Inflammation and cancer: back to Virchow? *Lancet* 2001; 357**:** 539–45.

5. Coussens L, Werb Z. Inflammation and cancer. *Nature* 2002; 420**:** 860–867.

6. Nagtegaal ID, Marijnen CAM, Kranenbarg EK et al. Local and distant recurrences in rectal cancer patients are predicted by the nonspecific immune response ; specific immune response has only a systemic effect - a histopathological and immunohistochemical study. *BMC Cancer* 2001; 1:7

7. Lança T, Silva-Santos B. The split nature of tumor-infiltrating leukocytes: Implications for cancer surveillance and immunotherapy. *Oncoimmunology* 2012; 1**:** 717–725.

8. Teramukai S, Kitano T, Kishida Y et al. Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: an analysis of Japan Multinational Trial Organisation LC00-03. *Eur J Cancer* 2009; 45**:** 1950–8.

9. Kusumanto YH, Dam WA, Hospers GAP, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003; 6**:** 283–7.

10. Hanahan D & Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144**:** 646–74.

11. Petrie HT, Klassen LW, Kay HD. Inhibition of human cytotoxic T lymphocyte activity in vitro by autologous peripheral blood granulocytes. *J Immunol* 1985; 134**:** 230–234.

12. Santoni M, De Giorgi U, Iacovelli R et al. Pre-treatment neutrophil-to-lymphocyte ratio may be associated with the outcome in patients treated with everolimus for metastatic renal cell carcinoma. *Br J Cancer* 2013; 109**:** 1755–9.

13. Szkandera J, Absenger G, Liegl-Atzwanger B et al. Elevated preoperative neutrophil/lymphocyte ratio is associated with poor prognosis in soft-tissue sarcoma patients. *Br J Cancer* 2013; 108**:** 1677–83.

14. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol* 2005; 91**:** 181–4.

15. Toh E, Wilson J, Sebag-Montefiore D, Botterill I. Neutrophil:lymphocyte ratio as a simple and novel biomarker for prediction of locoregional recurrence after chemoradiotherapy for squamous cell carcinoma of the anus. *Colorectal Dis* 2014; 16**:** O90–7.

16. Sato H, Tsubosa Y, Kawano T. Correlation between the pretherapeutic neutrophil to lymphocyte ratio and the pathologic response to neoadjuvant chemotherapy in patients with advanced esophageal cancer. *World J Surg* 2012; 36**:** 617–22.

17. Ikeguchi M, Urushibara SI, Shimoda R et al. Inflammation-based prognostic scores and nutritional prognostic index in patients with locally-advanced unresectable colorectal cancer. *World J Surg Oncol* 2014; 12**:** 210.

18. He J, Shen G, Ren Z, Qin H, Cui C. Pretreatment levels of peripheral neutrophils and lymphocytes as independent prognostic factors in patients with nasopharyngeal carcinoma. *Head Neck* 2012; 1769–1776.

19. An X, Ding PR, Wang FH, Jiang WQ, Li YH. Elevated neutrophil to lymphocyte ratio predicts poor prognosis in nasopharyngeal carcinoma. *Tumour Biol* 2011; 32**:** 317–24.

20. Jin Y, Ye X, He C, Zhang B, Zhang Y. Pretreatment neutrophil-to-lymphocyte ratio as predictor of survival for patients with metastatic nasopharyngeal carcinoma. *Head Neck* 2013; 1–7

21. Liu CL, Lee JJ, Liu TP et al. Blood neutrophil-to-lymphocyte ratio correlates with tumor size in patients with differentiated thyroid cancer. *J Surg Oncol* 2013; 107**:** 493–497.

22. Perisanidis C, Kornek G, Pöschl PW et al. High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. *Med Oncol* 2013; 30**:** 334.

23. Fang HY, Huang XY, Chien HT et al. Refining the role of preoperative C-reactive protein by neutrophil/lymphocyte ratio in oral cavity squamous cell carcinoma. *Laryngoscope* 2013; 123**:** 2690–9.

24. Tsai YD, Wang CP, Chen CY et al. Pretreatment circulating monocyte count associated with poor prognosis in patients with oral cavity cancer. *Head Neck* 2014; 36**:** 947–53.

25. Meniawy TM, Creaney J, Lake RA, Nowak AK. Existing models, but not neutrophil-to-lymphocyte ratio, are prognostic in malignant mesothelioma. *Br J Cancer* 2013; 109**:** 1813–20.

26. Lee DY, Hong SW, Chang YG, Lee WY, Lee B. Clinical significance of preoperative inflammatory parameters in gastric cancer patients. *J Gastric Cancer* 2013; 13**:** 111–6.

27. Demirtaş A, Sabur V, Akinsal EC et al. Can Neutrophil-Lymphocyte Ratio and Lymph Node Density Be Used as Prognostic Factors in Patients Undergoing Radical Cystectomy? Scientific World Journal 2013; 2013**:**703579

28. Wang S, Zhang Z, Fang F et al. The neutrophil/lymphocyte ratio is an independent prognostic indicator in patients with bone metastasis. *Oncol Lett* 2011; 2**:** 735–740.

29. Jankova L, Dent OF, Chan C, Chapuis P, Clarke SJ. Preoperative neutrophil/lymphocyte ratio predicts overall survival but does not predict recurrence or cancer-specific survival after curative resection of node-positive colorectal cancer. *BMC Cancer* 2013; 13**:** 442.

30. Jung MR, Park YK, Jeong O et al. Elevated preoperative neutrophil to lymphocyte ratio predicts poor survival following resection in late stage gastric cancer. *J Surg Oncol* 2011; 104**:** 504–10.

31. Rashid F, Waraich N, Bhatti I et al. A pre-operative elevated neutrophil: lymphocyte ratio does not predict survival from oesophageal cancer resection. *World J Surg Oncol* 2010; 8**:** 1.

32. Templeton AJ, McNamara MG, Šeruga B et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014; 106**:** dju124.

33. Li MX, Liu XM, Zhang XF et al. Prognostic role of neutrophil-to-lymphocyte ratio in colorectal cancer: a systematic review and meta-analysis. *Int J Cancer* 2014; 134**:** 2403–13.

34. Jess T, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin Gastroenterol Hepatol* 2012; 10**:** 639–45.

35. Green VL, Michno A, Stafford ND, Greenman J. Increased prevalence of tumour infiltrating immune cells in oropharyngeal tumours in comparison to other subsites: relationship to peripheral immunity. *Cancer Immunol Immunother* 2013; 62**:** 863–73.

Table 1 - Indications for exclusion of patients from the study

Indications	*n*
No pre-treatment blood tests	25
Previous history of cancer	21
Concurrent infection	10
Synchronous cancer	8
Palliative care	7
Long term immunosuppressant medications / chronic inflammatory conditions	6
Declined treatment	1
Total	78

Table 2 Patient demographics and clinical characteristics

Characteristics	No. (%)	NLR median (25^{th} , 75^{th})
Total	140	2.41 (1.78, 3.10)
Age, yr		***p* = 0.357**
≤60	48 (34.3%)	2.50 (1.85, 3.27)
>60	92 (65.7%)	2.35 (1.75, 2.98)
Gender		***p* = 0.324**
Male	121 (86.4%)	2.45 (1.82, 3.16)
Female	19 (14.6%)	2.06 (1.68, 2.77)
Smoking status		***p* =0 .768**
Non or Ex-smoker	60 (42.9%)	2.37 (1.85, 2.88)
Smoker	74 (52.9%)	2.46 (1.76, 3.28)
Missing data	6 (4.3%)	-
Nodal status		***p* =0 .447**
N0	105 (75%)	2.44 (1.74, 2.95)
N+	35 (25%)	2.36 (1.95, 3.38)
Disease stage		***p* =0 .006**
Stage I/II	57 (40.7%)	2.07 (1.60, 2.81)
Stage III/IV	83 (59.3%)	2.46 (2.03, 3.38)
Recurrence		***p* = 0.186**
Yes	41 (29.3%)	2.47 (2.02, 3.35)
No	99 (70.7%)	2.36 (1.75, 2.93)

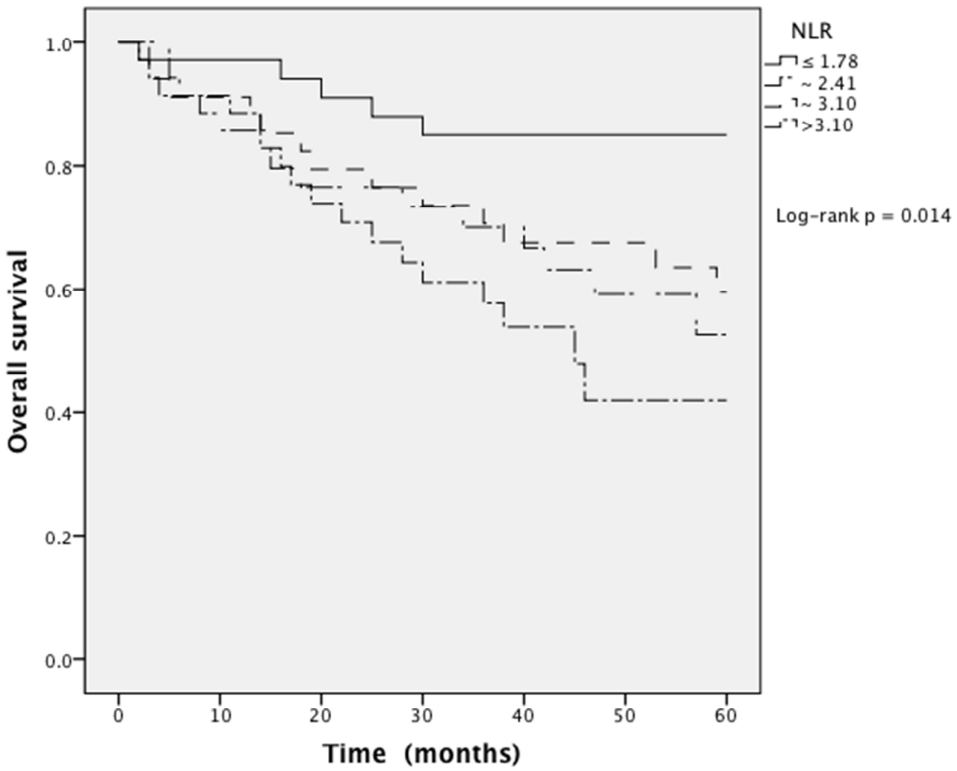

Figure 1 Kaplan-Meier survival analysis compared the four NLR groups based on NLR quartiles showing significant association between high NLR and poorer OS

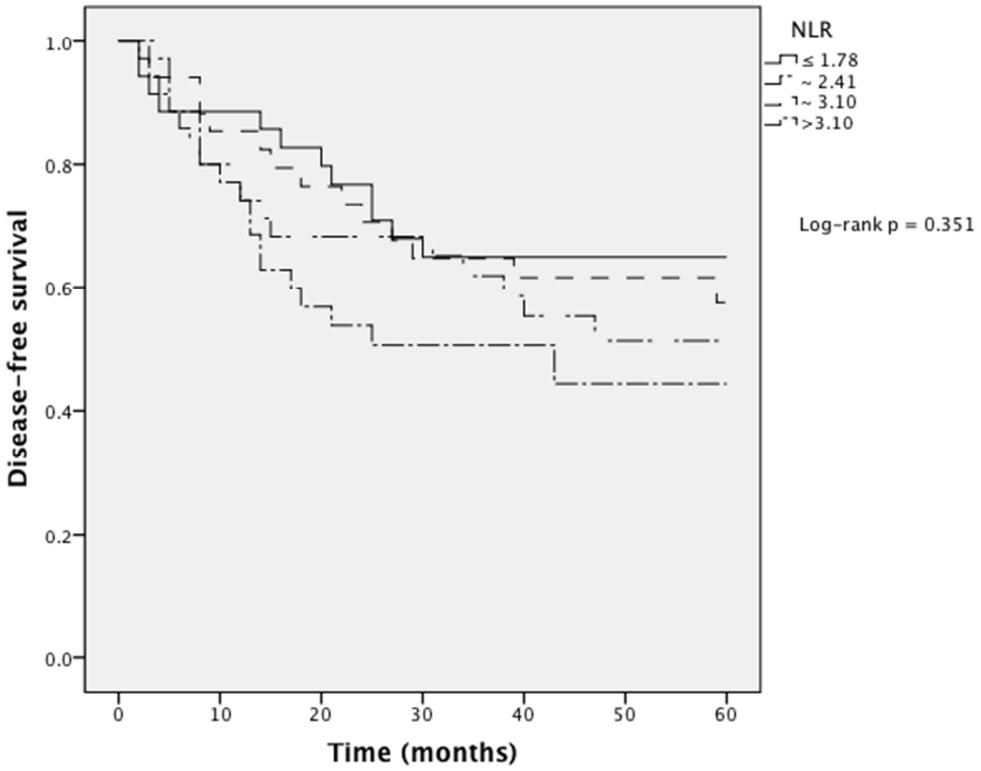

Figure 2 Kaplan-Meier survival analysis compared the four NLR groups based on NLR quartiles, showing no association between high NLR and DFS

Table 3 Univariate Cox regression analysis for OS and DFS in patients with laryngeal SCC

Variables	No. of patients	OS			DFS		
		No. of events	HR (95% CI)	*p*	No. of events	HR (95% CI)	*p*
Gender				**<0.01**			**<0.01**
Male	121	41			47		
Female	19	19	2.81 (1.42 – 5.54)		13	2.53 (1.36 – 4.69)	
Age				**0.24**			**0.87**
≤60	48	21			22		
>60	92	28	0.72 (0.41 – 1.26)		38	0.957 (0.57 – 1.62)	
Smoking				**0.75**			**0.72**
Non or ex-smoker	60	29			24		
Smoker	74	22	1.10 (0.62 – 1.96)		32	1.10 (0.65 – 1.87)	
Nodal Status				**<0.01**			**<0.01**
N0	105	26			34		
N+	35	23	4.26 (2.41 – 7.51)		26	3.65 (2.18 – 6.13)	
Clinical stage				**<0.01**			**0.01**
Stage I and II	57	10			15		
Stage III and IV	83	43	4.75 (2.22 – 10.17)		45	2.61 (1.45 – 4.68)	
NLR				**0.03**			**0.37**
≤1.78	35	8			12		
~2.41	35	15	3.33 (0.97 – 5.45)	**0.02**	16	1.45 (0.69 – 3.07)	0.33
~3.10	35	13	2.80 (0.72 – 4.21)	**0.05**	14	1.17 (0.54 – 2.53)	0.69
>3.10	35	17	4.64 (1.28 – 6.97)	**<0.01**	18	1.85 (0.89 – 3.84)	0.10

Table 4 Multivariate Cox regression analysis for OS and DFS in patients with laryngeal SCC

		OS		DFS	
		HR (95% CI)	***p***	HR (95% CI)	***p***
Gender					
Male	121		**<0.01**		**<0.01**
Female	19	2.78 (1.39 – 5.57)		2.29 (1.23 – 4.26)	
Nodal Status					
N0			**<0.01**		**<0.01**
N+		2.58 (1.38 – 4.81)		2.82 (1.55 – 5.11)	
Staging					
Stage I and II	57		**0.04**		**0.18**
Stage III and IV	83	2.46 (1.03 – 5.91)		1.59 (0.81 – 3.14)	
NLR			**0.18**		
≤1.78	35			-	-
~2.41	35	2.01 (0.70 – 5.76)	**0.19**	-	-
~3.10	35	2.6 (0.91 – 7.31)	**0.08**	-	-
>3.10	35	3.06 (1.08 – 8.67)	**0.04**	-	-