



Granulocyte Colony-Stimulating Factor Treatment During Radiotherapy Is Associated With Survival Benefit in Patients With Lung Cancer

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Abstract

Objectives: Granulocyte colony-stimulating factor, an agent commonly used for neutropenia treatment, plays an important role in cancer treatment. However, the effect of granulocyte colony-stimulating factor treatment on patient's survival during radiation therapy in lung cancer remains unknown. **Materials and Methods:** A retrospective study of patients with lung cancer who underwent radiation therapy from 2012 to 2015 at Shandong Provincial Qianfoshan Hospital was performed. Granulocyte colony-stimulating factor was administered when grade 3 or 4 leukopenia and/or neutropenia occurred during radiation therapy, and no prophylactic granulocyte colony-stimulating factor was used in this study. Patients were classified into high and low granulocyte colony-stimulating factor group according the dosage of granulocyte colony-stimulating factor use during radiation therapy. The influence of granulocyte colony-stimulating factor on survival was investigated. In addition, the predict value of granulocyte colony-stimulating factor in concurrent chemoradiotherapy group and radiation therapy alone group was also evaluated, respectively. **Results:** A total of 231 patients were enrolled, with 56 in the high granulocyte colony-stimulating factor group and 175 in the low granulocyte colony-stimulating factor group. High dose of granulocyte colony-stimulating factor for the entire population group was associated with a favorable overall survival (hazard ratio [95% confidence interval] = 1.798 [1.260-2.568]; $P = .001$) and a longer progression-free survival (hazard ratio = 1.550 [1.127-2.132]; $P = .002$). However, compared with a lower granulocyte colony-stimulating factor, a higher granulocyte colony-stimulating factor was associated with significant better overall survival and progression-free survival in radiation therapy group, not in concurrent chemoradiotherapy group. Although there was no statistical significance in concurrent chemoradiotherapy group, the median overall survival and progression-free survival of patients in the higher granulocyte colony-stimulating factor group were longer than those in the lower group. Furthermore, the treatment strategy was also associated with the overall survival, not the progression-free survival. **Conclusion:** This study suggests that granulocyte colony-stimulating factor treatment during radiation therapy has favorable impact on outcome in patients with lung cancer. Besides, results showed that patients treated with concurrent chemoradiotherapy had better prognosis than those treated with radiation therapy alone.

Keywords

G-CSF, lung cancer, radiation therapy, concurrent chemoradiotherapy, survival

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Abbreviations

CCRT, concurrent chemoradiotherapy; CI, confidence interval; G-CSF, granulocyte colony-stimulating factor; HR, hazard ratio; IMRT, intensity-modulated radiation therapy; IV, intravenously; LC, lung cancer; OS, overall survival; PFS, progression-free survival; ROC, receiver operating characteristic; RT, radiation therapy.

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Introduction

Lung cancer (LC) is the most frequently diagnosed chest solid tumors and the leading cause of cancer death in human.¹ Despite the tremendous development of the diagnosis and treatment in recent years, the prognosis of LC remains extremely poor.² To improve the treatment outcome of these patients further, novel therapeutic strategies are very necessary.

Granulocyte colony-stimulating factor (G-CSF) is a cytokine, produced by endothelium, macrophages, and several other immune cells.³ The G-CSF is known to reduce the incidence and minimize the duration of neutropenia in adult patients with solid tumors.⁴ Specific performance is that G-CSF stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. Recently, some studies reported that G-CSF supported the accumulation of regulatory T cells,^{5,6} which could promote tumor growth and migration. In addition, G-CSF stimulates tumor growth, metastasis, and invasion through paracrine activation of a tumor-supportive stroma.^{7,8} Taken together, these data indicate an important role of G-CSF in tumor growth and progression.

On the other hand, G-CSF stimulates the survival, recruitment, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils; therefore, it is commonly used in cancer therapy to ameliorate neutropenia. In addition, G-CSF was found to be effective in reducing the risk of potentially fatal febrile neutropenia in a meta-analysis.⁹ The beneficial effect on G-CSF is shown in several clinical trials. Alexander Meisel *et al* proved G-CSF could ameliorate chemotherapy-induced neutropenia and prolong the survival time of metastatic castration-resistant prostate cancer, especially for those with grade ≥ 3 neutropenia.¹⁰ Moreover, a recent *in vivo* study demonstrated that concurrent use of G-CSF and radiation therapy (RT) enhanced RT-mediated anti-tumor activity.¹¹ Thus, G-CSF may play a significant role in antitumor therapy. Nevertheless, the survival benefit for patients with LC who underwent G-CSF therapy during RT has not been elucidated.

In the current study, we investigated the effects of G-CSF treatment on patient's survival with LC who underwent RT. In addition, we evaluated the prognostic value of G-CSF in concurrent chemoradiotherapy (CCRT) group and RT alone group, respectively.

Materials and Methods

Patients

The retrospective study included consecutive patients with pathologically confirmed LC who received intensity-modulated radiation therapy (IMRT) from January 2012 to December 2015 at Shandong Provincial Qianfoshan Hospital. This study was approved by the ethical committee of Qianfoshan Hospital affiliated to Shandong University. Each patient or patient's parent/carer signed written informed consent. All the patients were medically inoperable or who refused to have surgery. Clinical characteristics of these patients, including sex, age, pathologic diagnosis, TNM stage, performance status, chemotherapy regimens, dose of G-CSF treatment during RT, pretreatment lymphocyte, and neutrophil count, were obtained from medical records. The tumor TNM stage was classified according to American Joint Committee on Cancer TNM Classification (7th Edition, 2010).

Overall survival (OS) was calculated from the time when the beginning of RT to death or last follow-up. Progression-free survival (PFS) was defined as the time from RT to disease progression or death of any cause. The follow-up data were obtained by a review of medical records and telephone, and the latest follow-up was carried out on August 13, 2016.

Treatment Plan

All patients received IMRT in the current study. Radiation therapy was administrated with a daily fraction of 2Gy. Concurrent chemoradiotherapy was performed using weekly docetaxel plus cisplatin in some patients. When grade 3 or 4 leukopenia and/or neutropenia occurred, the administration of G-CSF was permitted. It is worth noting that the prophylactic G-CSF is not allowed. Once patients who had grade 3 or 4 leukopenia and/or neutropenia, G-CSF was subcutaneously injected in those patients. Injection of G-CSF was done one time for 3 consecutive days. Generally speaking, once the G-CSF has been used for 3 consecutive days, the patient's hematological indicators will be retested, and then the G-CSF will be cancelled when the white blood cell count $\geq 10 \times 10^9/L$.

The dose for docetaxel was 30 mg/m² per week administered intravenously (IV) on day 1. Docetaxel was infused IV in 500 mL 5% glucose over 60 minutes. Treatment consisted of docetaxel followed by cisplatin (CDDP) on day 2. CDDP (30 mg/m²) was diluted with 500 mL of 0.9% saline and

administered as a 2-hour infusion on day 2. Anti-allergic prophylaxis was given with dexamethasone (10 mg IV) 2 to 4 hours before docetaxel. An antiemetic was also given with granisetron or ondansetron (3 mg IV) 30 minutes before docetaxel administration. The administration of cisplatin and docetaxel was cancelled if the leukocyte count was $\leq 2 \times 10^9/L$.

Determination of the Optimal Cutoff Value of the G-CSF

The hematological variables including neutrophil and lymphocyte count were obtained from blood tests routinely performed 1 to 3 days before radiotherapy. Receiver operating characteristic (ROC) curve was used to define the optimal cutoff value of G-CSF as prognostic factor. Based on this, patients were divided into high group and low group for G-CSF, respectively.

Statistical Analysis

Survival analyses were performed by using the Kaplan-Meier method with the logrank test. All *P* values were 2-sided and *P* < .05 was considered as statistically significant. Multivariate analysis was carried out by using the Cox proportional hazards model, hazard ratios (HRs) and 95% confidence intervals (95% CIs). Multivariate Cox proportional analyses were performed in a step-forward logistic regression approach. All statistical analyses were performed by using SPSS 19.0 software program (SPSS Inc, Chicago, Illinois).

Results

Patient Characteristics

A total of 231 patients were finally enrolled in our study. The enrolled 231 patients had a median age of 65 years (range: 27-85 years), with 188 (81.4%) males and 43 (18.6%) females. The basic characteristics of the enrolled patients are detailed in Table 1. During the follow-up period, 184 patients died and 211 patients progressed.

All patients received IMRT in this research. Radiation therapy was administered to a total dose of 50 to 70 Gy. The median time for radiotherapy was 6 weeks, ranged from 5 to 8 weeks. One hundred twenty (51.9%) patients received G-CSF treatment during RT. One hundred sixteen (50.2%) patients received CCRT.

Survival Analyses

According to ROC curve, the optimal cutoff value of G-CSF was 500 μg . Thus, patients were divided into a high-dose G-CSF group ($\geq 500 \mu\text{g}$) and a low-dose G-CSF group ($< 500 \mu\text{g}$). The number of patients who were treated with low- and high-dose G-CSF was 175 (75.8%) and 56 (24.2%), respectively. Kaplan-Meier curves showed that the entire population group with high G-CSF had a significantly better OS and PFS than low group (Figure 1), with all *P* < .05 by logrank test. In addition, compared with a lower G-CSF, a higher G-CSF were associated with significant better OS and PFS in RT group

Table 1. Patient Characteristics and Treatment.

Characteristics	Low Dose G-CSF, no. (%)	High Dose G-CSF, no. (%)	<i>P</i> Value
Total	175 (100)	56 (100)	
Sex			
Male	140 (80%)	48 (85.7%)	<i>P</i> = .339
Female	35 (20%)	8 (14.3%)	
Age			
<65	94 (53.7%)	34 (60.7%)	.359
≥ 65	81 (46.3%)	22 (39.3%)	
TNM			
II, III	86 (49.1%)	28 (50%)	.911
IV	89 (50.9%)	28 (50%)	
Treatment strategy			
RT	100 (57.1%)	16 (28.6%)	<.001
CCRT	75 (42.9%)	40 (71.4%)	
Performance status			
1, 2	108 (61.7%)	25 (44.6%)	.196
3	67 (38.3%)	31 (55.4%)	

Abbreviations: CCRT, concurrent chemoradiotherapy; G-CSF, granulocyte colony-stimulating factor; IV, intravenously; RT, radiation therapy; TNM, tumor node metastasis.

(HR = 1.783, 95% CI: 1.232-2.584; *P* = .06 for OS; and HR = 1.515, 95% CI: 1.089-2.109; *P* = .025 for PFS), not in CCRT group (*P* = .075 for OS and *P* = .135 for PFS). Although there was no statistical significance in CCRT group, the median OS and PFS of patients in the high-dose G-CSF group were longer than those in the low-dose group (high group vs low group = 18 months vs 15 months for OS; high group vs low group = 9 months vs 5 months for PFS; Figure 2).

In the univariate analysis about OS, G-CSF was proved to be a significant factor, with HR = 1.798 (95% CI: 1.260-2.568; *P* = .001). Additional, high G-CSF (HR = 1.550, 95% CI: 1.127-2.132; *P* = .007) was also associated with PFS. Other identified prognostic factors identified by univariate analysis included treatment strategy and TNM stage for outcome. Noteworthy, CCRT was an independent prognostic predictor for OS (*P* = .03) but it had no significant impact on PFS (*P* = .1). The results of univariate survival analyses were shown in Table 2.

Granulocyte colony-stimulating factor was brought into the model with all other significant factors in univariate survival analyses. These variables were selected for multivariate analysis to use the Cox proportional hazards model. The multivariate Cox proportional regression was performed to examine independent factors for OS and PFS. The multivariate survival analyses demonstrated G-CSF (HR = 1.528; *P* = .034 for OS; and HR = 1.548; *P* = .017 for PFS) was still independent prognostic factor (Table 3).

Adverse Events

No patient died of G-CSF-related toxicities in this study. There was no obvious adverse reaction when using low doses of G-CSF, and 8 patients occasionally cause flu-like symptoms

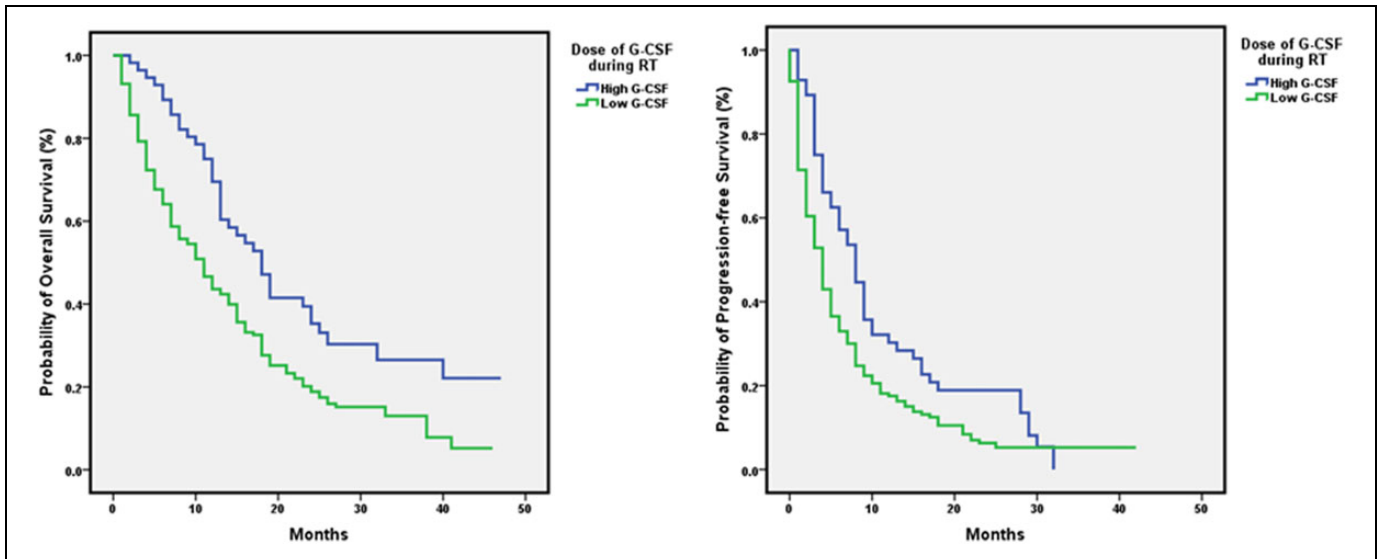


Figure 1. Kaplan-Meier curves showed that the entire population group patients with high granulocyte colony-stimulating factor (G-CSF) had a significantly better overall survival (OS) and progression-free survival (PFS) than low group, with all $P < .05$ by logrank test.

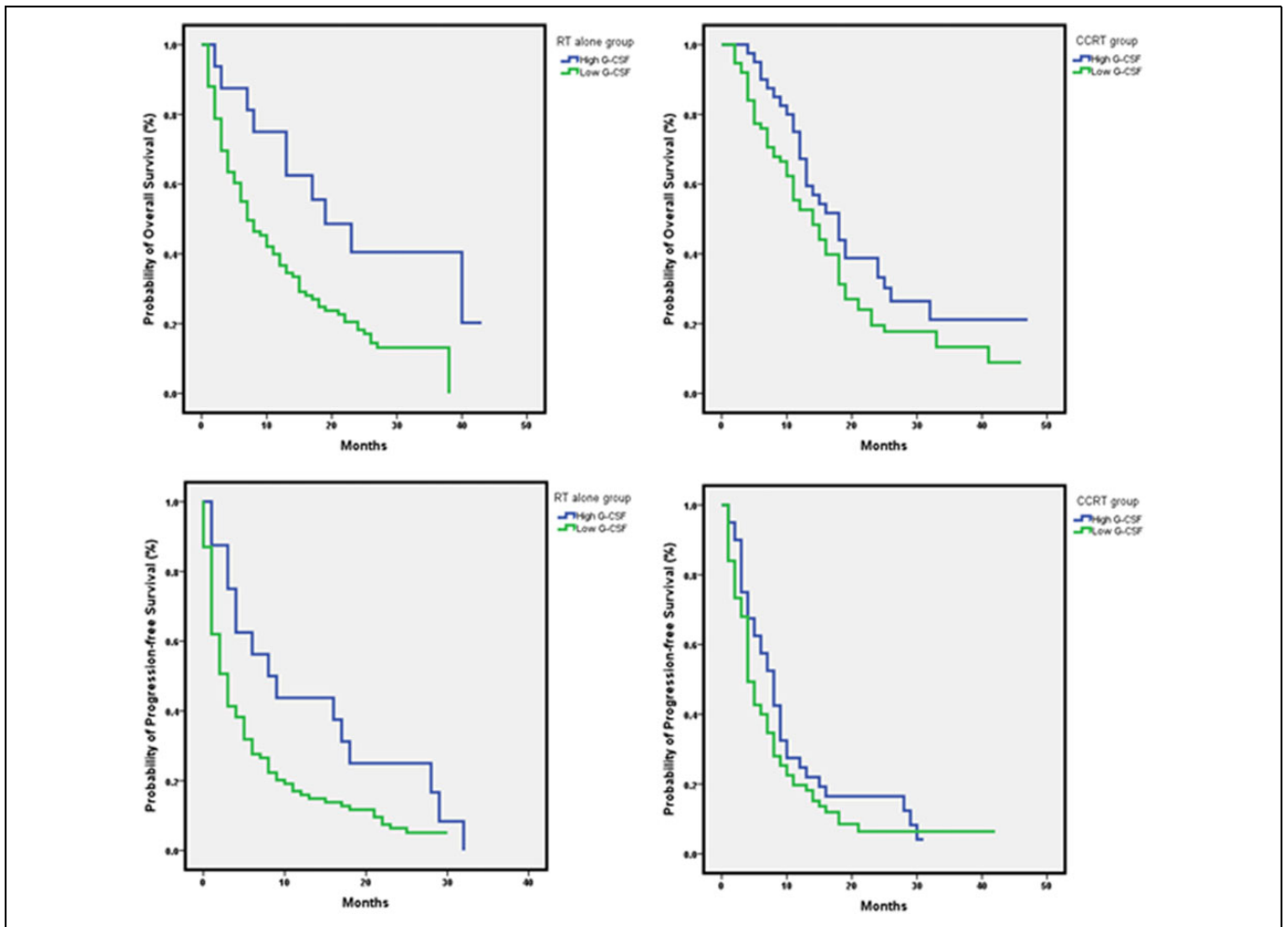


Figure 2. Kaplan-Meier curves showed that there was a different survival in 2 groups for overall survival (OS) and progression-free survival (PFS). However, compared with a lower granulocyte colony-stimulating factor (G-CSF), a higher G-CSF was associated with significant better OS and PFS in radiation therapy (RT) group, not in concurrent chemoradiotherapy (CCRT) group.

Table 2. Univariate Analyses.

Variables	OS		PFS	
	HR (95% CI)	P Value	HR (95% CI)	P Value
G-CSF				
High dose	1		1	
Low dose	1.798 (1.260-2.568)	<i>P</i> = .001	1.550 (1.127-2.132)	.007
Sex				
Male	1		1 ^a	
Female	^a	<i>P</i> = .634	^a	.848
Age				
<65 years	1		1	
≥65 years	^a	<i>P</i> = .97	^a	.922
TNM stage				
II, III	1		1	
IV	2.365 (1.734-3.226)	<i>P</i> < .001	2.012 (1.503-2.694)	<.001
Treatment strategy				
CCRT	1		1 ^a	
RT	1.380 (1.032-1.844)	<i>P</i> = .03	^a	.1

Abbreviations: CI, confidence interval; CCRT, concurrent chemoradiotherapy; G-CSF, granulocyte colony-stimulating factor; HR, hazard ratio; IV, intravenously; OS, overall survival; PFS, progression-free survival; RT, radiation therapy; TNM, tumor node metastasis.

^aNo significant impact.

Table 3. Multivariate Analyses.

Variables	OS		PFS	
	HR (95% CI)	P Value	HR (95% CI)	P Value
G-CSF				
High dose	1		1	
Low dose	1.528 (1.032-2.263)	<i>P</i> = .034	1.548 (1.081-2.216)	<i>P</i> = .017
Sex				
Male	1		1 ^a	
Female	^a	<i>P</i> = .455	^a	.44
Age				
<65 years	1		1	
≥65 years	^a	<i>P</i> = .399	^a	.844
TNM stage				
II, III	1		1	
IV	2.209 (1.564-3.120)	<i>P</i> < .001	2.027 (1.458-2.817)	<.001
Treatment strategy				
CCRT	1		1 ^a	
RT	1.516 (1.115-2.061)	<i>P</i> = .008	^a	.077

Abbreviations: CI, confidence interval; CCRT, concurrent chemoradiotherapy; G-CSF, granulocyte colony-stimulating factor; HR, hazard ratio; IV, intravenously; OS, overall survival; PFS, progression-free survival; RT, radiation therapy; TNM, tumor node metastasis.

^aNot in the final step of multivariate analyses.

who received high-dose G-CSF administration, such as fever and muscle soreness. In addition, occasionally platelet reductions occurred in very few patients. But these symptoms are not statistically significant.

Discussion

Granulocyte colony-stimulating factor, a hemopoietic growth factor established in inducing mobilization of neutrophils from bone marrow to circulation, is often used for increasing the number of neutrophils following chemotherapy or RT. Rapid

infiltration of neutrophils into the irradiated site is observed in radiation-induced inflammation.^{12,13} Growing evidence proves that in the context of cancer, neutrophils exhibit functional plasticity, with both tumor-promoting and tumor-inhibiting phenotype.¹⁴ Recently, Takeshima *et al* demonstrated that RT-induced infiltration of neutrophils inhibited tumor growth and concurrent G-CSF treatment enhanced RT-mediated anti-tumor activity in preclinical models.¹¹ Besides, Meisel *et al* found that G-CSF could ameliorate chemotherapy-induced neutropenia and prolong the survival of metastatic castration-resistant prostate cancer.¹⁰ These indicate that combination

therapy with RT and G-CSF may yield synergistic therapeutic effects. However, the clinical settings of G-CSF treatment on patient's survival during RT in chest solid tumors remain unknown. Our study investigated the relationship between G-CSF treatment and the prognosis in chest solid tumor and suggested that G-CSF treatment during RT had favorable impact on outcome for the first time.

Despite the well-known effect of G-CSF on neutropenia, the other roles of G-CSF in cancer therapy remain poorly understood. Altundag *et al* hypothesized that G-CSF treatment in dose-dense therapies improve the sensitivity of chemotherapy and OS in breast cancer by activating breast cancer stem cells, based on the INT C9741 trial.^{15,16}

The patients included in the present study all received IMRT with or without G-CSF. We confirmed that high-dose G-CSF treatment during RT correlated with longer PFS and OS in patients with LC, and it was identified as an independent favorable prognostic factor for outcome.

These observations do not provide data regarding whether G-CSF is directly responsible for prevent or delay tumor progression and death, or whether it affects the immune system's response to radiation. Further, the specific mechanisms as to the relationship between G-CSF and survival of cancer have yet to be identified. There are some reasons that can be used for explaining this result. A possible explanation is that G-CSF induced the production of neutrophil which may prevent the progression of those patients. It is well known that tumor-associated neutrophils can be converted into an antitumor (N1) phenotype or a protumor (N2) phenotype.¹⁷ Adjuvant therapy with G-CSF regulates recruitment and differentiation of neutrophil granulocytes and makes them modulated toward an N1 phenotype during radiotherapy, which further mediate oxidative damage, apoptosis of tumor cells, and resulting enhance the antitumor activity of RT.¹¹ Besides, Morris *et al* reported that macrophages and T cell phenotypes were transformed to anti-tumorigenic phenotypes in colons of G-CSF treated mice.¹⁸

On the other hand, overexpressed G-CSF might result in enhanced metastasis. Indeed, G-CSF overexpression has been correlated with a poor prognosis in many types of tumor.^{19,20} Specifically, G-CSF has been associated with tumor progression and increased invasiveness.²¹ Moreover, G-CSF can either activate or increase the recruitment and activity of protumor neutrophils and other myeloid-derived suppressor cells, enhancing tumor growth via angiogenesis.²² Besides, it was reported that prophylactic G-CSF administration was associated with poor prognosis in advanced head and neck cancer.²³

The clinical value of G-CSF treatment on survival of patients with LC remained controversial. The G-CSF may exert both antitumor and protumor activities. Based on the limitation of the retrospective nature and small simple size of our study, the results should to be interpreted cautiously. The effects of G-CSF treatment under RT need further investigation to be determined.

It is worth explaining that some patients with stage IV LC in this study. Now the main strategy for stage IV patients is

systemic chemotherapy and/or local radiotherapy. For patients who with poor physical condition, combined with other medical diseases, and significant weight loss, we recommend simple palliative radiotherapy. Treatment strategy for each patient was individualized according to the integrated condition of patient and his/her willing to accept the recommended treatment. On this account, it was hard to unify the treatment protocols and doses delivered to patients in this research. But we separated the whole patients into radiotherapy group and CCRT group for statistical analysis hoping to reduce relevant error.

Conclusions

In conclusion, this study confirmed that G-CSF treatment during RT could positively impact patient outcome in patients with LC. Furthermore, compared with a lower G-CSF, a higher G-CSF was associated with significant better OS and PFS in RT group, not in CCRT group. Although there was no statistical significance in CCRT group, the median OS and PFS of patients in the higher G-CSF group were longer than those in the lower group. In addition, compared with CCRT group, the patients who treated with RT alone had a poor OS and PFS. However, the results of current study need to be validated in larger prospective studies in the near future.

Authors' Note

All authors have contributed equally to this work. This study was approved by the Ethical Committee of Qianfoshan Hospital affiliated to Shandong University (number S017).

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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