Bone Marrow Dosimetric Analysis of Lymphopenia in Patients With Esophageal Squamous Cell Carcinoma Treated With Chemoradiotherapy

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Abstract

Background: Lymphocytes as a marker of immune function are essential to the immune response. Sternum and vertebra bone marrow (BM) exposed to radiation may affect lymphocytes during radiotherapy (RT) for esophageal carcinoma (EC). We analyzed the relationship among peripheral blood lymphocytes, exposed sternum and vertebra body BM, and overall survival (OS) to find BM dosimetric parameters of lymphopenia during chemoradiotherapy (CRT) for patients with esophageal squamous cell carcinoma (ESCC).

Methods: We examined 476 ESCC patients from January 2012 to January 2015, all of whom received concurrent or sequential CRT. Absolute lymphocyte counts (ALC) during RT of each patient were collected from the routine workup at the following RT times: pretreatment ALC (ALC0), at 1–5, 6–10, 11–15, 16–20, and 21–25, and more than 26 sessions (called ALC1–6, respectively). The sternum and vertebral body BM were delineated in accordance with uniform standards, and the irradiated volumes were calculated by dose-volume histograms (DVH). The Kaplan–Meier method and Cox proportional hazards regression were used to analyze the survival of the patients. Comparisons of DVH were performed using the Mann–Whitney U test or two-sample t-test where appropriate.

Results: A relative volume of sternum BM irradiated by more than 20 Gy could clearly affect the peripheral blood lymphocytes. The V20 of sternum BM and V50 of vertebra body BM were related to the OS of the patients, and the level of ALC2(at 6–10 times of RT) could predict the patients' outcomes. The Cox regression analyses showed that the 218 patients with ALC2 $\geq 0.8\times10^9$/L had a significantly longer OS (47.0 vs. 30.9, $p<0.0001$) than the 258 patients with ALC2 $< 0.8\times10^9$/L.

Conclusion: In patients with ESCC, the relative volume of sternum BM irradiated by more than 20 Gy was associated with lymphocyte. The V20 of the sternum BM and the V50 of the vertebra body BM were related to the OS of the patients. The level of ALC2 is a significant prognostic factor in esophageal carcinoma patients.

Background

Esophageal cancer (EC) is the eighth most common cancer worldwide and is the sixth most common cause of death from cancer[1]. At present, neoadjuvant chemoradiotherapy (CRT) and radical concurrent CRT are the standard treatment of patients with advanced EC. Although these treatment methods have improved the overall survival (OS) rate relative to traditional treatments, they have also increased the occurrence of acute toxic effects[2], and the 5-year survival rate is still less than 30–40%.

Studies of immunotherapy, such as KEYNOTE-181 and ATTRACTION-3, have shown it to be superior to chemotherapy in advanced EC, so research on the immune system is the key to further prolonging the survival rate of the patients. Lymphocytes as the primary mediators of cellular immunity in the human body are effective indicators in clinical work-ups that reflect the immune function of patients with cancer. The occurrence, development and prognosis of tumors are closely related to the immune function. When the immune function declines, tumor cells will escape the immune surveillance of the immune system and form solid tumors. However, ionizing radiation during radiotherapy (RT) will not only kill tumor cells, but will also have a killing and inhibitory effect on normal lymphocytes, which indirectly interferes with the anti-tumor process and affects the prognosis of the patients.

Previous studies have shown that lymphopenia induced by radiation (LIR) can be used as a predictor of a poor outcome for many types of tumors, such as non-small-cell lung cancer, pancreatic cancer, breast cancer, glioma, and head and neck cancer[3-7]. As we all know, LIR is related to bone marrow suppression. Bone marrow (BM), as the main site of lymphocyte generation, is very sensitive to radiation. In the process of treating EC patients with RT, the sternum and thoracic vertebra BM will inevitably be exposed to different doses of radiation as the target area changes. The hematopoiesis of the sternum and vertebral body BM accounts for 30% of the entire amount, so the generation of lymphocytes will be affected. In similar studies in the past, patients with cervical cancer who received low doses of pelvic BM (V10≥90%) had a higher incidence of leukopenia and neutropenia than those who received BM V10 <90%[8]. In addition, the mean dose and low-dose radiation parameters (V5, V10, V15, V20) of whole bone or bone cavities of the lumbosacral spine were found to be correlated most significantly with HT3+ for squamous cell carcinoma of the anal canal[9].

Based on the previous findings described above, this study aimed to explore the relationships between the exposure of the sternum and vertebra body BM to radiation to the lymphocytes and OS of EC patients to find dosimetric predictors of lymphopenia during CRT for patients with ESCC.

Methods

Patients and clinical data

This article is a retrospective study. Patients with histologically confirmed ESCC who received CRT at Shandong Cancer Hospital Affiliated to Shandong First Medical University between January 2012 and January 2015 were considered to be eligible. Considering the EC staging criteria have slight differences in recent years, we reclassified the disease stage of the patients according to the eighth edition of the American Joint Committee on Cancer's Cancer Staging Manual. Inclusion criteria: 1. KPS≥70, pathologically confirmed patients with ESCC who received CRT; 2. No other tumors, no previous history of chemotherapy or RT; 3. Survival period greater than 6 months; 4. Normal blood cell level before treatment, no other immune diseases, no immune related drugs (suppression or enhancement). Exclusion criteria: 1. Patients with incomplete case data who could not be reclassified as to disease stage; 2. The total dose of RT did not reach the established radical dose; 3. A lack of blood routine results during RT ≥2 times; 4. Poor physical condition with severe underlying disease; 5. Chronic inflammatory or autoimmune disease; 6. Blood transfusion within the last 3 months.

We collected the following information from all of the patients: age, sex, KPS, tumor location, tumor length, differentiation, and TNM stage. The absolute value of lymphocytes (ALC) was collected within 1 week of starting RT, and then at1–5, 6–10, 11–15, 16–20, 21–25, and more than 26 sessions of RT (referred to as ALC0, ALC1–6, respectively). One missing value was allowed during the whole treatment. Meanwhile, we also obtained the maximum, minimum, rate of decline, and the average value of lymphocytes. After the entire treatment was completed, the patients were followed-up every 2 months for half a year. If their condition was stable, the follow-up time was prolonged to every 3–6 months, and the overall follow-up was for at least 5 years. The study endpoints were overall survival (OS) (time from the end of the treatment to death from any cause or the last follow-up time).

Radiotherapy and Chemotherapy

The target of RT was delineated on the eclipse system as follows. Gross Tumor Volume(GTV): Combined with CT/PET-CT or titanium clip markers under the endoscope to identify the primary tumors and positive lymph node areas;clinical Target Volume(CTV): Add the high-risk areas of lymphatic drainage on the basis of GTV; Planning Target Volume(PTV): 0.8 cm was added in the horizontal directions and 3 cm expansion in the axial direction to account for setup uncertainty and organ motion. Radiation dose: 50–60 Gy (delivered in 25–33 fractions of 1.8–2.0 Gy/fraction). Normally endangered organs: total lung V20 ≤35%, V5 ≤65%, Dmean ≤20 Gy; heart Dmean ≤35 Gy; spinal cord Dmax ≤45 Gy. In addition, all patients received platinum-based chemotherapy with paclitaxel or fluorouracil every 21–28 days. The chemotherapy drugs were given according to the standard dose. Concurrent CRT means chemotherapy drugs at intervals of 21–28 days were given at the beginning of RT.Nonconcurrent CRT was mainly sequential and induction CRT.

Delineation of sternum and vertebra body BM

We transferred the CT images of all of the patients to the MIM(6.8.2) system, and the delineation process was completed on mediastinal window. Considering that the hematopoietic site of adults is mainly in the BM cavity, we declined the BM according to the following criteria shown in (Fig. 1). Sternum BM: 1. upper boundary is the sternum notch, lower boundary is the xiphoid process, the circumference is the junction with the bone; 2. due to the sternum angle being indistinguishable from the surrounding costal cartilage, the delineation of this area was done under the guidance of two imaging physicians. Vertebral body BM: 1. upper and lower bounds are the projection range of the 10% isodose line of PTV on the vertebrae, and the circumference is the junction with the bone; 2. avoid delineation of intervertebral discs and pyramidal attachments based on the continuity and anatomical structure of the spine.

Statistical analysis

The characteristics of the patients were described by descriptive statistics. Survival was estimated by the Kaplan–Meier method and survival curve comparisons were performed using the log-rank test. The relationship between clinical characteristics of the patients and OS were tested by Cox proportional hazards regression. Propensity score matching was used to compensate for the differences in baseline characteristics. The cutoff value of the lymphocytes was obtained by X-tile software. Chi-square tests were used to analyze the differences between different groups. Comparisons of DVH were performed using the Mann–Whitney U test or two-sample t-test, where appropriate. All statistical analyses were performed using Statistical Package for the Social Sciences, SPSS software 22.0, and $P < 0.05$ was considered to be significant.

Results

Basic characteristics

After 5 years of follow-up, 476 patients were recruited into this study, including 367 men and 109 women. As shown in Table 1 the median age of all patients was 63, and 64.5% were over 60 years. Phase I and II, III, and IV were administered to 11.3, 61.6, and 27.1% of the patients, respectively. Phase I and II patients were given CRT treatment due to the following reasons: contraindicated for surgery due to a poor physical condition (11

patients), cervical EC (16 patients) and refusal of surgery (27 patients). Only 12.2% of patients were treated with concurrent CRT, while 87.8% had nonconcurrent CRT. The average cycle of chemotherapy was 4.2, and the average dose of RT was 59.3 Gy. Most patients (65.8%) were treated with intensity-modulated radiation therapy (IMRT). The median OS of the patients was 28.4 months, and the 1-year, 3-year, and 5-year OS rates were 82.1%, 32.8% and 3.8%, respectively.

Correlation between basic clinicopathological characteristics and OS

The correlations between the basic clinicopathological characteristics and OS were analyzed by COX regression model, and (Karnofsky Performance Status)KPS ($p=0.011$), TNM staging ($p<0.0001$), ALC2 (6–10 sessions of RT) ($p=0.025$), and the pretreatment cyfra level ($p=0.002$) had a statistical correlation with OS (Table 1). On the other hand, there was no significant correlation between OS and chemotherapy, minimum value of lymphocytes, the maximum value of ALC, the drop rate, ALC0,1,3–6, sex, age, RT dose, or RT technique ($p>0.05$). We obtained the ALC2 cutoff value (0.8×10^9/L) using X-tile. All patients were divided into two groups: less than 0.8×10^9/L group (group 1) and the greater than or equal to 0.8×10^9/L group (group 2). The patients in group 1 were matched 1:1 to the patients in group 2 according to their propensity score using the global optimum method; 55 pairs of patients were matched finally.

Correlation between the sternum and vertebral body BM and OS

The dose-relative/absolute volume histograms of all patients could be obtained after drawing the sternum and vertebral body BM according to the criteria mentioned above. The results shown in Table 2 were obtained by using Cox proportional hazard model: the relative and absolute V20 of the sternum BM were both statistically correlated with OS ($p=0.044$; $p=0.027$), and the relative and absolute V50 of the vertebra body BM were also statistically correlated with OS ($p=0.027$; $p=0.024$).

Comparison of the differences between the two groups

Group 1 showed a significantly inferior overall median survival compared with group 2 (30.9 vs. 47.0 months, $p<0.0001$) (Fig. 2). After matching for the other confounding factors, the median OS of the two groups were 35.5 vs. 49.8 months ($p = 0.001$). In Table 3, we can see there were significant differences between the two groups for tumor location, RT pattern, chemotherapy, and cyfra level before treatment ($p<0.05$). On further analysis of the tumor location, we found that middle and lower thoracic EC were more likely to be associated with a decrease in peripheral blood lymphocytes than cervical EC (Fig. 3).

Relationship between the sternum and vertebra body BM and lymphocytes

To analyze the relationship between the sternum and vertebra body BM and lymphocytes, we compared the differences of exposed BM between the two groups of patients divided according to the cutoff value of the lymphocytes. After propensity score matching (Fig. 4), it was concluded that there was a significant difference in the relative volume of the sternum BM irradiated by more than 20 Gy between the two groups ($p<0.05$), but there was no significant difference for vertebral body BM ($p>0.05$)(Table 4).

Discussion

The results of this study indicated that the relative volume of sternum BM irradiated by more than 20 Gy could obviously affect the peripheral blood lymphocytes. The V20 of the sternum BM and the V50 of the vertebra body BM were related to the OS of the patients, and the level of ALC2 could predict the outcomes of the patients. Based on these findings, we suggest that in clinical work, while ensuring the target treatment dose of patients with ESCC, the V20 of the sternum BM and the V50 of the vertebra body BM should be reduced as much as possible, so that the peripheral blood lymphocytes of the patients are less affected by ionizing radiation, which may be ultimately beneficial to improve the therapeutic efficacy of the RT and the survival of the patients.

During this study, 350 patients suffered leukopenia during treatment, clinically treated with Recombinant Human Granulocyte Colony Stimulating Factor (rhG-CSF)Injection.Considering the half-life of rhG-CSF is 3.5 hours, and its effect was mainly on myeloid system but weaker effect on lymphatic system,so we collected ALC after injecting rhG-CSF 24 hours to avoid this influence. Some previous studies have reported lymphocytes are correlated with the prognosis of patients. Deng et al. demonstrated that significant G4 lymphopenia (<200 cells/mL) during CRT was an independent predictor of survival outcomes of patients with EC[10]. The minimum ALC may be a prognostic factor indicating a worse outcome for nasopharyngeal cancer and hepatocellular carcinoma[11,12]. Pretreatment lymphopenia is a predictor of a good outcome as well as a predictive factor for tumor response and chemotherapy-related hematological toxicity in metastatic ESCC[13]. Likewise, in patients with ESCC treated with CRT, we found that ALC2 can predict the patients' outcomes. Furthermore, middle and lower thoracic EC were more likely to cause a decrease in peripheral blood lymphocytes than cervical EC. This result is consistent with previous studies[10] and is probably because the heart and spleen are greatly affected by RT during the treatment of middle and lower thoracic EC. As the distal esophageal location passes across the heart, a significant pool of lymphocytes will be exposed to radiation treatment for distal esophageal tumors[14]. Yue et al. showed that higher spleen irradiation doses were significantly correlated with a lower Min ALC during RT for hepatocellular carcinoma[15].

We found that the relative volume of sternum BM irradiated by more than 20 Gy could obviously affect the peripheral blood lymphocytes in patients with ESCC. Miyoshi N et al. have shown that BM chemical toxicity is an important prognostic factor in patients with T4 EC who underwent radical resection after CRT[16]. The sternum volume exposure to 50 Gy contributed to the BM suppression in breast cancer[17]. Low-dose radiation to the pelvis was also significantly associated with hematotoxicity in patients with pelvic tumors, such as cervical cancer and rectal squamous cell carcinoma[8,18]. However, WU et al. reported that radiation doses to the thoracic vertebrae and ribs in esophageal cancer patients treated with neoadjuvant CRT, including the average dose and the V5–30 of the thoracic vertebrae, and the average dose and V5–20 of the ribs, were related to lymphopenia of grade 3 or above. However, the exposure to the sternum, scapular and clavicular BM did not affect the occurrence of hematotoxicity[19]. The reasons for these different results may be due to differences in patient inclusion criteria, treatment mode, statistical methods and so on.

According to the existing studies, the effect of ionizing radiation on BM is related to the BM microvascular system. Weintraub NL et al. definitively showed a causal relationship between radiation and vascular diseases[20]. After the BM microvessels are exposed to ionizing radiation, not only is the blood supply of the BM interrupted but also other normal physiological functions may be negatively affected directly or indirectly. In a study by Slayton et al., mice were lethally irradiated with 9.5 Gy and a week after irradiation they lost

homeostasis between the extravascular and intravascular space within the BM. BM sinusoidal dilations with unusual shapes and hemorrhages within the BM of irradiated mice were also detected[21]. In our study, we found that the level of ALC2 could predict the outcomes of the patients, which may be due to the damage of the BM microvascular system caused by the cumulative dose of radiation after 6–10 sessions. At this time point, the level of lymphocytes can better reflect the immune capacity of each patient, which could affect the prognosis of the patients. However, the abovementioned studies are all preclinical studies, and further clinical studies are needed to support this conclusion.

There are several limitations of this study: 1. All data were derived from a retrospective cohort and we could not standardize the times of blood testing during patient treatment; 2. Limited by its sample size, the current data are not generally representative; 3. There are no standard criteria for delineating BM ranges and different delineation methods may affect the final results. However, this is the first comprehensive retrospective study on the relationships among lymphocyte levels, survival rate and the sternum and vertebral body BM in patients with unresectable ESCC. Therefore, these results still need to be confirmed in large-scale prospective studies in the future.

Conclusions

In patients with ESCC, the relative volume of sternum BM irradiated by more than 20 Gy was associated with lymphocyte. The V20 of the sternum BM and the V50 of the vertebra body BM were related to the OS of the patients. The level of ALC2 is a significant prognostic factor in esophageal carcinoma patients.

Abbreviations

BM: bone marrow; **RT:** radiotherapy; **EC:** esophageal cancer; **OS:** overall survival; **CRT:** chemoradiotherapy; **ESCC:** esophageal squamous cell carcinoma; **ALC:** absolute value of lymphocytes; **DVH:** dose-volume histograms; **LIR:** lymphopenia induced by radiation; **GTV:** gross tumor volume; **CTV:** clinical tumor volume; **PTV:** planning target volume; **KPS:** Karnofsky Performance Status; **IMRT:** Intensity-modulated radiation therapy; **S1**: sternum of group1; **S2**: sternum of group2; **V1**: vertebra body of group1; **V2**: vertebra body of group2

Declarations

Ethics approval and consent to participate: The study was conducted in agreement with the Declaration of Helsinki. All the patients signed a consent to share their clinical data and information for clinical studies.

Consent for publication Not applicable

Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests

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Tables

Table 1. Relationship between basic clinicopathological characteristics of patients and OS

Characteristics	Number (%) (Total patients =476)	P value
Age(median (range))	63(37-85)	
Age（<60 vs. ≥60）	169 (35.5) vs. 307(64.5)	0.186
Gender (male vs. female)	367 (77.1) vs. 109(22.9)	0.141
KPS（70 vs. 80 vs. 90）	15(3.2) vs. 235(49.4) vs. 226(47.5)	0.011
Tumor location (upper vs. middle vs. lower）	196(41.2) vs.205(43.1) vs.75(15.8)	0.245
TNM（I、II vs. III vs. IV）	54(11.3) vs. 293(61.6) vs.129(27.1)	<0.0001
RT-technology (IMRT vs. CRT)	313(65.8) vs. 163(34.2)	0.232
Dose(50group vs. 60 group)	74 (15.5) vs. 402(84.5)	0.828
RT-pattern (Concurrent vs. Non-concurrent)	58(12.2) vs. 418(87.8)	0.173
Chemotherapy（Paclitaxel group vs. 5-FU group）	276 vs. 200	0.734
Chemotherapy cycle（1~2 vs. 3~4 vs. 5~6 vs.>6）	104(24.2) vs.183(38.9) vs.143(37.0) vs.46(9.7)	0.154
ALC0		0.636
ALC1		0.261
ALC2		0.025
ALC3		0.961
ALC4		0.325
ALC5		0.112
ALC6		0.978
CEA before treatment		0.204
Cyfra before treatment		0.002

Abbreviations: KPS Karnofsky Performance Status,RTradiotherapy,IMRT Intensity-modulated radiation therapy,CRT chemoradiotherapy, ALC absolute value of lymphocytes

Table 2. Relationship between dose-relative volume of BM and OS

Parameters		HR(CI 95%)	P value
RV5Gy	sternum	1.05(0.90-1.21)	0.515
	vertebra body	0.92(0.68-1.25)	0.594
RV10Gy	sternum	0.96(0.85-1.09)	0.581
	vertebra body	1.10(0.87-1.40)	0.414
RV15Gy	sternum	1.12(0.99-1.26)	0.051
	vertebra body	1.02(0.81-1.29)	0.878
RV20Gy	sternum	0.92(0.86-0.99)	0.044
	vertebra body	0.99(0.79-1.25)	0.950
RV25Gy	sternum	1.03(0.97-1.09)	0.414
	vertebra body	0.97(0.83-1.13)	0.680
RV30Gy	sternum	1.01(0.96-1.06)	0.652
	vertebra body	1.05(0.92-1.20)	0.482
RV35Gy	sternum	0.97(0.93-1.01)	0.122
	vertebra body	0.99(0.92-1.06)	0.731
RV40Gy	sternum	1.03(0.99-1.07)	0.178
	vertebra body	0.99(0.94-1.04)	0.675
RV45Gy	sternum	0.99(0.95-1.02)	0.463
	vertebra body	0.97(0.91-1.02)	0.233
RV50Gy	sternum	1.01(0.99-1.04)	0.428
	vertebra body	1.04(1.00-1.08)	0.027

Relationship between dose-relative volume of BM and OS

Parameters		HR(CI 95%)	P value
AV5Gy	sternum	1.00(0.78-1.29)	0.991
	vertebra body	0.92(0.70-1.21)	0.554
AV10Gy	sternum	0.84(0.47-1.53)	0.575
	vertebra body	1.09(0.70-1.69)	0.716
AV15Gy	sternum	1.66(0.90-3.04)	0.102
	vertebra body	0.99(0.66-1.49)	0.966
AV20Gy	sternum	0.59(0.37-1.94)	0.027
	vertebra body	1.06(0.72-1.54)	0.781
AV25Gy	sternum	1.34(0.95-1.91)	0.100
	vertebra body	0.91(0.72-1.15)	0.430
AV30Gy	sternum	0.97(0.77-1.24)	0.823
	vertebra body	1.10(0.91-1.33)	0.329
AV35Gy	sternum	0.86(0.72-1.03)	0.102
	vertebra body	0.99(0.90-1.10)	0.826
AV40Gy	sternum	1.18(0.99-1.38)	0.053
	vertebra body	0.97(0.90-1.04)	0.381
AV45Gy	sternum	0.90(0.76-1.06)	0.192
	vertebra body	0.96(0.89-1.04)	0.284
AV50Gy	sternum	1.04(0.94-1.14)	0.440
	vertebra body	1.06(1.01-1.11)	0.024

Abbreviations:BM bone marrow,OS overall survival,HR hazard ratio,RVxGy relative volume irradiated by xGy, AVxGy absolute volume irradiated by xGy

Table 3. Comparative analysis of patient clinicopathological characteristics from unmatched and propensity-matched groups

Characteristic	Before matching			After matching		
	LC<0.8(n=258) (%)	LC≥0.8(n=218) (%)	P value	LC<0.8(n=55) (%)	LC≥0.8(n=55) (%)	P value
Age(average)	61.8	62.5	0.0971	63.8	63.0	1
Age<60	97(20.4)	72(15.1)		16(14.5)	16(14.5)	
Age≥60	161(33.8)	146(30.7)		39(35.5)	39(35.5)	
Gender			0.500			0.654
Male	202(42.4)	165(34.7)		41(37.3)	43(39.1)	
Female	56(11.8)	53(11.1)		14(12.7)	12(10.9)	
KPS			0.124			0.615
70	10(2.1)	5(1.1)		2(1.8)	3(2.7)	
80	136(28.6)	99(20.8)		31(28.2)	26(23.6)	
90	112(23.5)	114(23.9)		22(20.0)	26(23.6)	
Tumor location location			<0.0001			0.496
upper(cervical and upper thoracic)	92(19.3)	104(21.8)		21(19.1)	20(18.2)	
Middle thoracic	119(25.0)	86(18.1)		23(20.9)	28(25.5)	
Lower thoracic	47(9.9)	28(5.9)		11(10.0)	7(6.4)	
Tumor length Length(cm)			0.634			0.603
1-5	167(35.1)	138(29.0)		37(33.6)	38(34.5)	
5.1-10	84(17.6)	77(16.2)		17(15.5)	17(15.5)	
10.1-15	6(1.3)	2(0.4)		1(0.9)	0	
15.1-20	1(0.2)	1(0.2)		0	0	
TNM			0.224			0.699
I□II	29(6.1)	25(5.3)		6(5.5)	7(6.4)	
III	149(31.3)	144(30.3)		40(36.4)	36(32.7)	

IV	80(16.8)	49(10.3)		9(8.2)	12(10.9)	
cTstage			0.105			0.823
T1T2	29(6.1)	22(4.6)		5(4.5)	6(5.5)	
T3	175(36.8)	150(31.5)		40(36.4)	37(33.6)	
T4	54(11.3)	46(9.7)		10(9.1)	12(10.9)	
cNstage			0.951			0.792
N0	35(7.4)	30(6.3)		9(8.2)	8(7.3)	
N+	223(46.8)	188(39.5)		46(41.8)	47(42.7)	
RT-technology			0.946			1
IMRT	170(35.7)	143(30.0)		34(30.9)	34(30.9)	
CRT	88(18.5)	75(15.8)		21(19.1)	21(19.1)	
Dose(Gy)			0.463			0.376
50 group	43(9.0)	31(6.5)		8(7.3)	5(4.5)	
60 group	215(45.2)	187(39.3)		47(42.7)	50(45.5)	
RT-pattern			0.036			0.808
Concurrent	24(5.0)	34(7.1)		45(40.9)	44(40.0)	
Non-concurrent	234(49.2)	184(38.7)		10(9.1)	11(10.0)	
Chemotherapy			0.024			0.251
Paclitaxel+	166(34.9)	118(24.8)		22(20.0)	28(25.5)	
5-FU+	92(19.3)	100(21.0)		33(30.0)	27(24.5)	
Chemotherapy cycle			0.658			0.763
1~2	55(11.6)	49(10.3)		16(14.5)	13(11.8)	
3~4	94(19.7)	89(18.7)		21(19.1)	21(19.1)	
5~6	82(17.2)	61(12.8)		18(16.4)	21(19.1)	

>6	27(5.7)	19(4.0)		0	0	
CEA			0.674			0.644
<1.6	61(12.8)	48(10.1)		13(11.8)	11(10.0)	
≥1.6	197(41.4)	170(35.7)		42(38.2)	44(40.0)	
Cyfra			0.018			0.297
<6.4	223(46.8)	203(42.6)		49(44.6)	52(47.3)	
≥6.4	35(7.4)	15(3.2)		6(5.4)	3(2.7)	
Differentiation			0.317			0.915
hyper	18(3,8)	23(4.8)		4(3.6)	5(4.5)	
Middle	25(5.3)	24(5.0)		8(7.2)	7(6.3)	
lower	215(45.2)	171(35.9)		43(39.1)	43(39.1)	

Abbreviations: KPS Karnofsky Performance Status,RT radiotherapy,IMRT Intensity-modulated radiation therapy,CRT chemoradiotherapy, LC lymphocytes,ALC absolute value of lymphocytes

Table.4. Relative volume of sternum, vertebra body BM between the two groups

Parameters		Relative volume	P value
RV5Gy	S1 vs. S2	96.5vs. 95.0	0.221
	V1 vs.V2	99.9 vs. 99.6	0.488
RV10Gy	S1 vs. S2	94.0 vs. 93.7	0.134
	V1 vs.V2	99.0 vs. 99.2	0.859
RV15Gy	S1 vs. S2	86.5 vs. 90.0	0.094
	V1 vs.V2	95.6 vs. 97.5	0.699
RV20Gy	S1 vs. S2	83.5 vs. 85.0	0.033
	V1 vs.V2	94.0 vs. 95.5	0.734
RV25Gy	S1 vs. S2	75.0 vs. 75.0	0.002
	V1 vs.V2	91.0 vs. 92.0	0.802
RV30Gy	S1 vs. S2	64.0 vs. 63.0	0.0004
	V1 vs.V2	87.5 vs. 89.1	0.782
RV35Gy	S1 vs. S2	50.8 vs. 46.4	<0.0001
	V1 vs.V2	83.5 vs. 83.5	0.922
RV40Gy	S1 vs. S2	30.0 vs. 36.6	<0.0001
	V1 vs.V2	73.5 vs. 73.3	0.888
RV45Gy	S1 vs. S2	23.5 vs. 13.5	<0.0001
	V1 vs.V2	60.0 vs. 57.5	0.789
RV50Gy	S1 vs. S2	6.0 vs. 12.5	<0.0001
	V1 vs.V2	45.0 vs. 42.0	0.584
RV55Gy	S1 vs. S2	2.5 vs. 5.5	<0.0001
	V1 vs.V2	30.5 vs. 37.5	0.669
RV60Gy	S1 vs. S2	1.0 vs. 3.0	<0.0001
	V1 vs.V2	18.0 vs. 15.0	0.351

Abbreviations:BM bone marrow,RVxGy relative volume irradiated by xGy,S1 sternum of group1,S2 sternum of group2,V1 vertebra body of group1,V2 vertebra body of group2

Figures

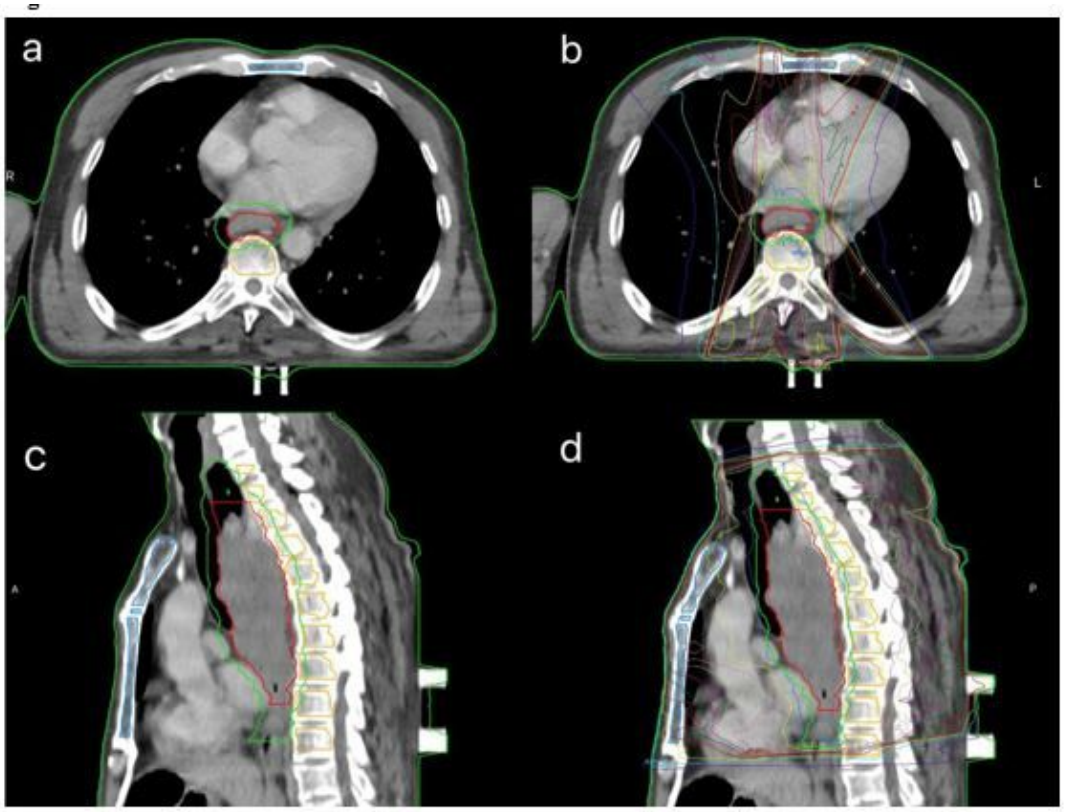

Figure 1

Delineation on cross-sectional(a,b) and sagittal planes(c,d) CT. b and d shows the isodose line of target. Structures included tumor PTV (green), GTV(red), vertebra body BM (yellow) and sternum BM (blue)

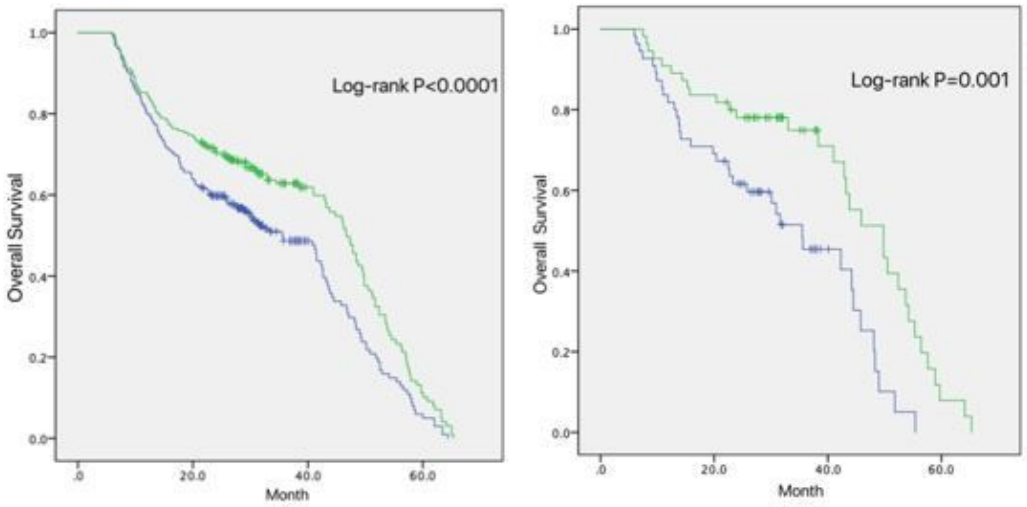

Figure 2

Kaplan-Meier curves showing overall survival between group1(green line) and group2(blue line) before(left) and after(right) Propensity score matching

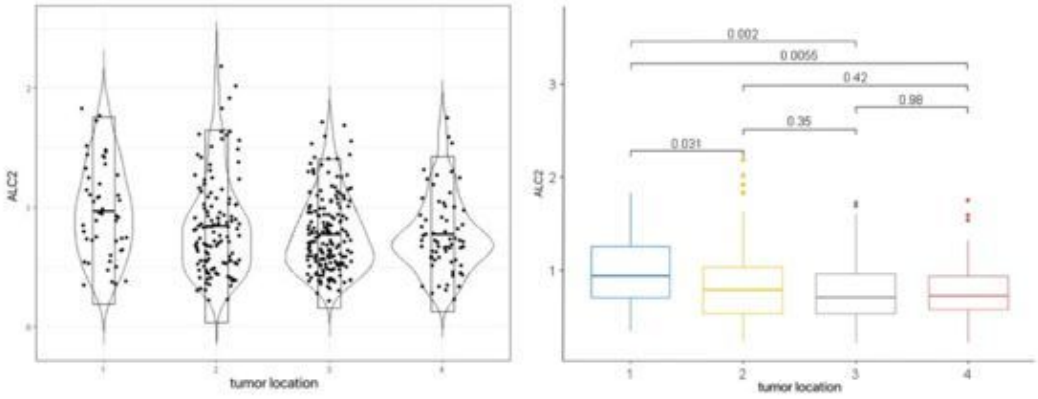

Figure 3

The left shows data distribution of ALC2 in patients with tumor location in each group. The right shows the differences of ALC2 in patients with tumor location in each group by pairwise comparison.1-Cervical EC; 2-Upper thoracic EC; 3-Middle thoracic EC; 4-Lower thoracic EC

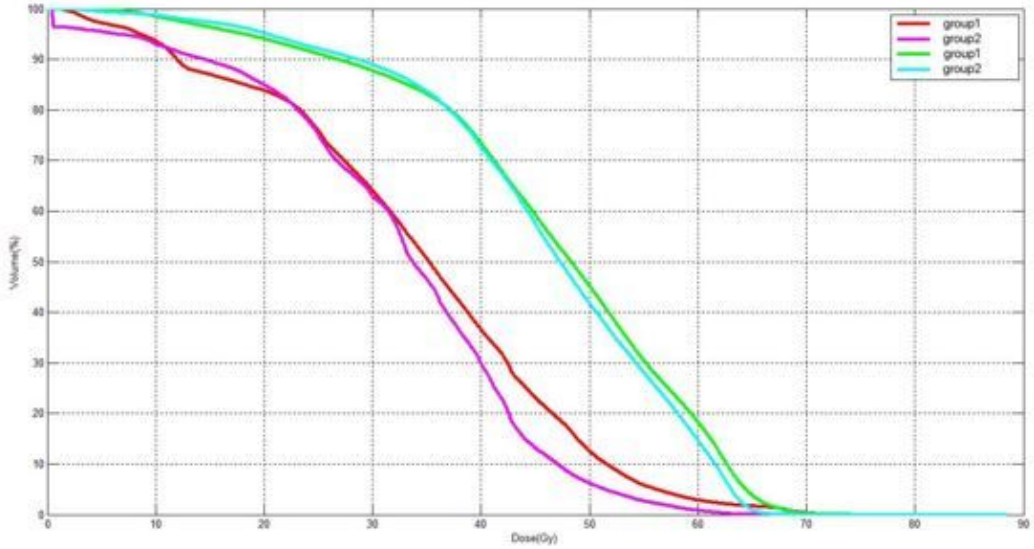

Figure 4

DVH of sternum BM(two red line) and vertebra body BM(two green line) of two groups after Propensity score matching,four solid line is the mean values of each groups.