Peri-treatment peripheral blood cell score predicts long-term locoregional progression hazard in oesophageal squamous cell carcinoma after definitive chemoradiotherapy

Jianzhou Chen
 Shantou University Medical College Cancer Hospital

Liangyu Xu
 Shantou University Medical College Cancer Hospital

Hong Guo
 Shantou University Medical College Cancer Hospital

Ruihong Huang
 Shantou University Medical College Cancer Hospital

Longjia Guo
 Shantou University Medical College Cancer Hospital

Yuanxiang Yu
 Shantou University Medical College Cancer Hospital

Tiantian Zhai
 Shantou University Medical College Cancer Hospital

Fangcai Wu
 Shantou University Medical College Cancer Hospital

Zhijian Chen
 Shantou University Medical College Cancer Hospital

Derui Li
 Shantou University Medical College Cancer Hospital

ChuangZhen Chen (✉ stccz@139.com)
 Shantou University Medical College Cancer Hospital   https://orcid.org/0000-0002-2342-8099

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Abstract

Background Prediction of response to chemoradiotherapy is critical for the optimal management of oesophageal cancer, yet it is still an unmet clinical need. This study aims to evaluate the predictive potential of peri-treatment peripheral blood cells (PBC) in disease progression hazard in oesophageal cancer following chemoradiotherapy.

Methods 87 patients with primary oesophageal squamous cell carcinoma were subjected to definitive concurrent chemoradiotherapy in a phase II trial. PBC parameters (haemoglobin, neutrophils, platelets, lymphocytes and monocytes) were collected at 7 time points through the course of radiotherapy. The values of peri-treatment PBC parameters in predicting 3-year cumulative hazard of tumour progression were evaluated.

Results Patients with disease progression displayed distinct distribution patterns of peri-treatment PBC compared to patients without. Greater prediction capabilities for risk of locoregional disease progression were found in PBC collected after the start of radiotherapy compared to their pretreatment counterparts, and in individual parameters rather than cell-to-cell ratios. The most predictive PBC parameters were integrated by summation and designated as a PBC score (PBCS), which further augmented their predictive power. Patients divided according to their PBCS (high vs medium vs low) had significantly different 3-year cumulative hazards of locoregional progression (58% vs 29% vs 7%, P = 0.0017). Multivariate analysis confirmed that PBCS high (HR 12.2, 95%CI 2.0-76.3, P = 0.007) and medium (HR 5.8, 95%CI 1.2-27.7, P = 0.028) are independent indicators of locoregional progression.

Conclusion Peri-treatment PBCS can predict the long-term hazard of locoregional progression after definitive chemoradiotherapy in patients with oesophageal squamous cell carcinoma.

Background

Oesophageal cancer (EC) is one of the deadliest malignant diseases. It was estimated that about 572,000 new cases of EC occurred and more than 500, 000 patients died of this disease in 2018 worldwide [1]. Concurrent chemoradiotherapy (CCRT) has gradually become the standard for nonsurgical treatment of locoregional EC. Locoregional tumour control was achieved in approximately half of patients, whereas the other half eventually experienced recurrences [2–4]. The heterogeneity in response to CCRT in patients reflects the complexity of EC and poses a great challenge to clinicians. To address this issue, it is critical to be able to predict the hazard of disease progression in patients after CCRT. This prediction will enable timely and more specific intervention in patients with high risk of tumour control failure and allow sparing of low-risk patients from unnecessary treatment.

Prediction of response to CCRT in EC patients has been an area of great interest. Studies have examined a variety of means, including functional imaging (e.g. Fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography (18^F FDG PET/CT)), clinical parameters (e.g. tumour stage) and biomarkers in tumour samples [5–9]. These all have been shown to correlate with treatment outcomes of

EC patients to some extent. However, none of these means have become the standard or been widely used for clinical patient stratification or decision-making, as each of them still has weaknesses and limitations. Thus, prediction of patient response to CCRT in EC remains an unmet clinical need. Novel means with both accuracy and cost-effectiveness are needed.

Complete blood count is a routine clinical examination for patients undergoing CCRT. This test quantifies various peripheral blood cell (PBC) parameters, including haemoglobin as a surrogate for red blood cells, neutrophils, platelets, lymphocytes and monocytes. There is a growing body of evidence suggesting that these PBC parameters either singly or as a ratio correlate with treatment outcomes of cancer patients [10–13]. Consistently, it has been shown that EC patients with high neutrophil-to-lymphocyte ratio (NLR) or platelet-to-lymphocyte ratio (PLR) are associated with unfavourable outcomes after various treatments, including CCRT [14–19]. The prognostic implication of PBC parameters in EC patients and their clinical availability render them attractive candidates for prediction of tumour control. However, it remains to be determined whether PBC parameters independently correlate with long-term hazard of disease progression in EC patients following CCRT. Moreover, efforts are warranted to maximize their predictive potential, as the reported correlations between PBC parameters and patient survival are relatively modest [10–15].

The majority of previous studies relied solely on pretreatment PBC parameters [10–13], whilst emerging data suggest that radiation therapy (RT) can trigger an anti-tumour immune response, which has an important role in determining tumour response to RT in animal models [20–24]. Studies also indicate that the local immune response interplays with systemic inflammation, which consists of a variety of circulating inflammation factors, including PBC parameters [25]. Thus, changes of PBC parameters after RT may provide substantial inputs to their predictive capacity for patient outcomes. Furthermore, RT-induced immune responses appear to be transient in animal models, indicating that prompt assessment of PBC parameters after RT may better reflect the ongoing immune response. However, it is difficult to determine a specific time point for PBC assessment in patients. Definitive RT generally takes 5–7 weeks and the crosstalk between the local immune response and systemic reactions during this period of time is still not clear. Therefore, a more comprehensive analysis of PBC parameters with multiple assessments during radiotherapy (peri-treatment) may help better evaluate their predictive value.

We launched a phase II clinical trial in 2012, in which patients with oesophageal squamous cell carcinoma were subjected to definitive CCRT with radiation dose escalation. One of the objectives of this trial is to explore the predictive potential of peri-treatment PBC parameters for tumour control. PBC assessment was performed at 7 time points throughout the course of RT. We hypothesized that systematic assessment of peri-treatment PBC parameters may provide a tool of predictive value for evaluation of disease progression hazard in patients following treatment. Here, we report the results of this prospective cohort study. We found that peri-treatment PBC had moderate predictive potential for hazard of locoregional disease progression in this patient cohort. Interestingly, greater predictive capability was found in parameters collected after the start of RT compared to their pretreatment counterparts, and in individual parameters rather than cell-to-cell ratios. With regard to these emerging

features, we developed an approach to integrate multiple peri-treatment PBC parameters as a PBC score (PBCS), which led to greater predictive power. Further analysis shows that PBCS is an independent predictor of tumour control failure at locoregional sites and is able to separate patients into subgroups with significantly different long-term hazards of locoregional disease progression.

Methods

Patient inclusion criteria

Patients who met the following criteria were enrolled in this trial: (1) Pathologically confirmed primary oesophageal squamous cell carcinoma; (2) Disease located in cervical, upper or middle thoracic oesophagus; (3) Stage I-IVA, including cervical nodes, according to the 6th edition of American Joint Committee on Cancer (AJCC) TNM staging system; (4) Age $\geq$ 18 but $\leq$ 75 years; (5) Zubrod performance status 0-2; (6) Haemoglobin $\geq$ 10 gram per litre (g/L), neutrophils $\geq$ 1.5 x 10^9/L, Platelets $\geq$ 150 x 10^9/L; (7) Adequate liver and renal function; (8) No history of prior chemotherapy, radiotherapy or major oesophageal surgery. This clinical trial was approved by the clinical research ethical review committee of the centre and registered at clinicaltrial.gov (NCTxxxxxxxx). All enrolled patients had written informed consent.

Treatment

Patients recruited to this trial were treated with definite CCRT (treatment scheme in **Figure 1A**). RT was delivered using the simultaneous modulated accelerated radiotherapy approach, which has been previously described [26]. Briefly, the gross tumour (primary and regional metastatic lymph nodes) was determined by CT imaging, endoscopic reports and barium swallow fluoroscopy. The region of subclinical disease was derived from the gross tumour, including high-risk lymph node regions. The prescribed dose was 66 Gray (Gy) to the gross tumour and at the same time 54Gy to the subclinical disease in 30 fractions (F) given over 6 weeks. RT was delivered using intensity modulated radiotherapy with cone-beam CT guidance with a TrueBeam linear accelerator (Varian Medical system, Palo Alto, CA). Patients were also treated with 2 cycles of concurrent chemotherapy on weeks 1 and week 5 during radiotherapy, and another 2 cycles of adjuvant chemotherapy after radiotherapy at week 8 and 11. The chemotherapy regimen consisted of cisplatin, 75 mg/m^2, intravenous on day 1 and fluorouracil, 0.5 g/m^2, intravenous on day 1 to 4.

Assessment of PBC parameters

Patients had assessment of PBC parameters at baseline (within 1 week before RT), and after every 5 fractions of RT (7 time points in total). PBC assessments at other than these time points were allowed,

but were not included in the current analysis. All PBC assessments were performed by a Coulter LH 750 Haematology Analyzer (Beckman Coulter, Brea, CA). The following PBC parameters were analysed: haemoglobin, neutrophils, platelets, lymphocytes, monocytes, NLR, PLR and monocyte-to-lymphocyte ratio (MLR).

Follow-up and evaluation of therapeutic response

Follow-up for patients was scheduled for every 3 months for 2 years and then every 6 months for 3 years. Assessment included history, physical examination, hematologic and biochemical profiles, chest X-ray plus oesophageal barium swallow test or contrast-enhanced CT scan, and abdominal ultrasound. Endoscopic ultrasound of the oesophagus with biopsy or PET/CT was performed if clinically indicated. Tumour response to CCRT was evaluated according to the Response Evaluation Criteria in Solid Tumours guideline 1.1 [27]. Disease progression was confirmed with pathological proof or longitudinal imaging when biopsy was not suitable.

Endpoints and statistical analysis

The endpoints of this study included peri-treatment PBC parameters, first failure patterns (local, regional or distant) within 3 years after RT and survival. Multiple sites of tumour progression were considered simultaneous, if they occurred within 3 months. Time-to-event (cumulative hazard or survival) was measured from the date when radiotherapy was completed to the date of event (tumour progression or death) or the last clinic visit. The analysis was performed using the Statistical Package for Social Sciences (SPSS 25.0, Chicago, IL). Mean comparisons of two groups were performed using two-tailed unpaired Student's t test or the Wilcoxon rank-sum test when appropriate. Time-to-event curves were generated using the Kaplan-Meier method and compared using the log-rank's test. Receiver operating characteristic (ROC) curves were used to assess the prediction capability of PBC parameters. The Area Under a Curve (AUC) for ROC curves was reported. The cut-off of ROC curves was determined by the Youden index (sensitivity + specificity - 1). Univariate analysis was performed using the log-rank's test. Multivariate cox regression with the enter selection method was used for hazard evaluation. $P < 0.05$ was considered statistically significant.

Results

A total of 87 patients were enrolled in this study between August 2012 and August 2015. The interim analysis of this trial has been previously reported [28]. The current analysis includes all enrolled patients (trial diagram in **Figure 1B**). There were 67 males and 20 females with a median age of 61 years (range 37-73 years). Their clinical stages were as follows: stage II, 30 cases (34.5%), stage III 44 cases (50.6%) and stage IV 13 cases (14.9%). All patients completed the whole course of CCRT and the majority of them

(85.1%) also received 1-2 cycles of adjuvant chemotherapy. The data provided in this report were current as of September 15, 2018 when all patients had a minimum 3-year follow-up after RT. Four patients (4.6%) were excluded from the analysis due to lack of information of disease progression. Of the 83 patients analysed, there were 34 (41%) cases with disease progression.

The distribution of peri-treatment PBC parameters in patients with or without disease progression is summarized in **Figure 2**. These parameters in both groups exhibited a generally similar trend over time, including periodic oscillation of neutrophils, platelets and monocytes, gradual decline of haemoglobin and lymphocytes, and steady increase of NLR, PLR and MLR. However, significant differences were found in some parameters. Patients with disease progression had elevated levels of neutrophils at the 3rd time point (after 10F of RT) and platelets at the 6th time point (after 25F of RT) compared to patients without. They also showed a trend of increasing level of haemoglobin through the course of RT. These distinct distribution patterns of PBC parameters in patients who failed to respond to CCRT suggest that they have potential predictive value.

The discrimination capacity of 56 peri-treatment PBC parameters in predicting hazard of disease progression was analysed using ROC curves (**Figure 3A**). Pretreatment (the 1st time point) PBC parameters did not appear to have predictive potential (AUC range 0.52-0.58), whereas modest capacity (AUC up to 0.67) was found in PBC parameters collected after the start of RT (the 2nd - 7th time points). Only neutrophils (3rd) and NLR (3rd) exhibited statistical significance which is consistent with their mean differences in **Figure 2**. Further analysis reveals that the prediction capability of PBC parameters varies markedly for different progression patterns (**Figure 3B-D**). Greater discrimination capacity was found for prediction of local (**Figure 3B**, AUC up to 0.68) or regional progression hazard (**Figure 3C**, AUC up to 0.76) in a number of PBC parameters, whereas their potential was much less for risk of distant progression (**Figure 3D**, AUC up to 0.63). Therefore, subsequent analyses concentrated only on local and regional progression. Consistently, higher AUC values were found with PBC parameters during the course of treatment compared to pretreatment PBC parameters for these two failure patterns (**Figure 3B,C**). Individual PBC parameters also exhibited greater potential than cell ratios (NLR, PLR and MLR) in most cases. Moreover, the panel of parameters with higher AUC (> 0.6) differed between local and regional progression. Parameters with statistical significance include lymphocytes (7th) for local progression, haemoglobin (2nd and 7th) and neutrophils (3rd) for regional progression.

To further augment the predictive power of PBC parameters, integration of multiple parameters was examined. Cell-to-cell ratios (NLR, PLR and MLR) from a single time point only led to inferior predictive potential, removing them from consideration (**Figure 3B,C**). The distinctive distribution of predictive PBC parameters that emerged from the screen suggested that a novel integration approach based on peri-treatment PBC might stratify patient prognosis. Since the panel of predictive PBC parameters varied between local and regional progression, these two patterns were analysed separately. All 35 PBC parameters were ranked according to their AUC values from high to low (**Figure 4A,B**). The top 10 parameters were selected for an initial test of integration. These parameters showed a consistent trend of

elevated levels in patients with progression versus patients without, suggesting that their summation might integrate their predictive potential (**Figure S1,S2**). Because PBC parameters have different ranges of normal values, they were first normalized to the mean of each parameter of the whole group. Normalization did not alter their AUC values and rankings (data not shown). Means of the top parameters (1~10) were then calculated and designated as a PBC score (PBCS) to test for hazard prediction of local (PBCS-L) or regional progression (PBCS-R). As shown in **Figure 4C**, combinations of multiple PBS parameters resulted in greater AUC values. PBCS-L with the highest AUC (0.73) was derived from the top 2 parameters, comprising lymphocyte 7th and monocytes 5th (**Figure 4A**). PBSC-R with the highest AUC (0.83) was based on the top 4 parameters, including neutrophils (3rd), haemoglobin (2nd and 7th) and platelets (6th) (**Figure 4B**). They were used in the subsequent analysis of this cohort of patients.

The optimal cut-off of PBCS-L was set at 86 as determined by the Youden index (**Figure 5A**). It had a sensitivity of 92.3% and a specificity of 57.1% for prediction of risk of local progression. Patients with high PBCS-L had greater 3-year cumulative hazard of local progression compared to patients with low PBCS-L (30% vs 3%, P = 0.002) (**Figure 5B**). Twelve out of 13 (92.3%) patients confirmed with local progression were PBCS-L high. The cut-off of PBSC-R was set at 107 with a sensitivity of 90.0% and a specificity of 79.5% (**Figure 5C**). Patients with high PBCS-R had significantly higher 3-year cumulative hazard of regional progression than patients with low PBCS-R (41% vs 2%, P < 0.001) (**Figure 5D**). Nine out of 10 (90%) patients confirmed with regional progression were PBCS-R high. Multivariate cox regression confirmed that high PBCS-L (HR 16.0, 95%CI 1.9-132.5, P = 0.01) and high PBCS-R (HR 28.6, 95%CI 3.2-254.8, P = 0.003) were independent indicators of local and regional progression, respectively (**Table S1,2**).

Overall, there were 20 cases (24%) of patients with local or regional disease progression. To evaluate the predictive value of PBCS in hazard of local and regional progression as a whole, patients were divided into 3 subgroups according to their PBCS-L and PBCS-R: PBCS high: both PBCS-L and PBCS-R high; PBCS medium: either PBCS-L or PBCS-R high; PBCS low: both PBCS-L and PBCS-R low. These subgroups of patients displayed significantly different 3-year cumulative hazards of locoregional failure (58 vs 29% vs 7%, P = 0.0017, **Figure 6A**). In contrast, no significant difference was found in patients with different clinical stages (**Figure 6B**). Multivariate cox regression analysis confirmed that PBCS was the only independent variable associated with the hazard of locoregional progression (**Table 1**). Higher risk of locoregional failure was found in patients with PBCS high (HR 12.2, 95%CI 2.0-76.3, P = 0.007) or medium (HR 5.8, 95%CI 1.2-27.7, P = 0.028) versus patients with PBCS low.

Discussion

The heterogeneous response among EC patients after definite CCRT is a major challenge for the optimal management of this disease [3, 4]. The ability to predict patient response to CCRT could be a critical advance leading to stratified treatment and better outcomes; yet it is still an unmet clinical need. The prognostic implication of pretreatment PBC parameters in EC patients suggests that they may help address this issue, though their predictive capacity was often modest. In this study, we show that PBC

parameters collected after the start of RT exhibited greater discrimination capacity in predicting disease progression hazard compared to their pretreatment counterparts. Moreover, superior potentials were found in individual parameters instead of cell-to-cell ratios. In the face of these emerging features from this peri-treatment PBC screen, an approach was developed to integrate the most predictive PBC parameters as a PBCS which further augmented their predictive potential. Patients with high PBCS had significantly higher hazard of locoregional progression compared to patients with low PBCS. Multivariate analysis confirms that high peri-treatment PBCS is an independent indicator of tumour control failure at locoregional sites.

The sample size of this study is medium, which represents a common challenge in the clinic that researchers very often have to rely on small to medium clinical datasets to develop response predictors. This limitation may impede the discovery of potential predictors or undermine their predictive values. Indeed, the majority of patient clinical characteristics and pretreatment PBC fail to discriminate patients with hazard of disease progression from patients without in this study. Nonetheless, our results show that systematic assessment and appropriate integration could help reveal the predictive value of peri-treatment PBC for tumour response to therapy. PBCS is able to separate patients into subgroups with markedly distinct hazards of disease progression, suggesting that it is an effective methodology to tackle the conflict between the clinical need for cost-effective predictors and limited resources.

Our results suggest that the timing of PBC assessment is important for a thorough evaluation of their predictive value. In our hands, pretreatment PBC parameters did not correlate with local progression hazard and only displayed modest discrimination capacity for risk of regional progression. Peri-treatment PBC parameters proved to be better indicators. Indeed, all parameters with significant discrimination capacity were collected after the start of RT, rather than at baseline. In line with our results, a number of recently published studies suggest that post-treatment PBC parameters are associated with outcomes of cancer patients after RT or immunotherapy [29−35]. Barbetta A et al show that changes of NLR after CRT are associated with risk of recurrence in patients with oesophageal squamous cell carcinoma [35]. Lin SH and colleagues found that lymphocyte nadir during CRT for EC was associated with poor outcomes and a higher level of lymphocyte count during neoadjuvant CRT was associated with a higher rate of pathologic complete response in EC [36, 37]. The majority of these studies, however, have only a single assessment of PBC after the start of treatment, which may have limited their predictive ability. For instance, Zhou and colleagues examined the predictive role of NLR in EC patients after definite CRT. They found that post-treatment NLR only had prognostic value in patients with high NLR (> 5) at baseline, but not in patients with low NRL (< 5) [33]. The majority (98%) of patients enrolled in this study have low NLR (< 5) at baseline (1st). Their post-treatment NLR (7th) does not show apparent association with disease progression hazard. Nevertheless, a number of PBC parameters with statistical significance emerged in our screen and most of them were collected during RT. This suggests that the correlations between PBC parameters and patient response to RT follow a temporal pattern with considerable variation, which requires longitudinal and more comprehensive assessments.

Our results also indicate that integration of multiple parameters into a score could provide superior discrimination capacity over any single parameter or over cell-to-cell ratios. The specific distribution of predictive PBC in patients with locoregional progression led us to develop an integrated score for hazard prediction. This score is composed of a variety of PBC parameters at multiple time points during RT. Consistent with these results, a recently published report by Xiaoyan Feng and colleagues shows that integrating multiple PBC parameters could predict the survival of patients with adult T-lymphoblastic lymphoma [38]. Thus, combining multiple PBC parameters through appropriate integration may reveal their predictive power. This is practically feasible, as PBC assessment at multiple time points are readily available in patients undergoing CCRT. Nonetheless, how these parameters collectively influence locoregional tumour control requires further investigation.

One interesting observation from this study is that the best predictive PBC parameters for local and regional progression differ. PBCS-L is based on lymphocytes (7th) and monocytes (5th), while neutrophils (3rd) haemoglobin (2nd, and 7th), and platelets (6th) make up PBCS-R. This discrepancy provides a way to distinguish regional progression from local progression, which could be helpful for clinical stratification. Further studies will be needed to address how increases of circulating lymphocytes and monocytes lead to greater risk of local recurrence, while regional progression is more often found in patients with augmentation of neutrophils, haemoglobin and platelets.

In addition to its predictive role, peri-treatment PBCS could also provide insights for investigation of novel targets. A variety of PBC parameters have been shown to increase at differing time points in patients with disease progression and these cells may contribute to the treatment failure. For instance, the augmented number of neutrophils and monocytes in patients with disease progression is in accord with findings from animal models that IR leads to recruitment of myeloid-derived suppressor cells (derived from granulocytes and monocytes) in the tumours, which then mediates their resistance to IR [39, 40]. Targeting these cells may reverse resistance to therapy. In contrast to neutrophils and monocytes, there are fewer studies in regard to the potential detrimental effect of haemoglobin variation on tumour control. Yet our results suggest that patients with hazard of treatment failure are associated with elevated haemoglobin levels, which may provide clues for exploring potential targets to improve response to CCRT.

There are a number of limitations in this study that could be addressed in further research. First, the sample size of this study is relatively small and all patients were enrolled in a single centre. Further validation in multicentre studies with larger cohort of patients is warranted. Second, we only used a summation of PBC parameters as a score for discrimination of disease progression. There may be other integration methods for example those that weight different components that could provide greater predictive power. Additionally, there is no information regarding local and systemic immune responses. These data could help further improve the peri-treatment PBC-based prediction system and may provide important insights into the underlying mechanisms.

Conclusions

In this prospective cohort study, we show that peri-treatment PBCS can predict long-term hazard of locoregional progression after definitive CCRT in patients with oesophageal squamous cell carcinoma. This could provide useful insights for considerations of personalized adjuvant therapy. Further validation in multicentre studies with larger cohort of patients is warranted.

Abbreviations

PBC:peripheral blood cells; PBCS:PBC score; PBCS-L:PBC score (PBCS) to test for hazard prediction of local progression; PBCS-R:PBC score (PBCS) to test for hazard prediction of regional progression; EC:Oesophageal cancer; CCRT:Concurrent chemoradiotherapy; NLR:neutrophil-to-lymphocyte ratio; PLR:platelet-to-lymphocyte ratio; MLR:monocyte-to-lymphocyte ratio; AJCC:American Joint Committee on Cancer; ROC:Receiver operating characteristic; AUC:Area Under a Curve

Declarations

- Ethics approval and consent to participate

This study was in accordance with the Helsinki Declaration (2000) and was approved by the clinical research Ethics Review Committee of Cancer Hospital of Shantou University Medical College. Each patient had signed informed consent.

- Consent to publish

Not applicable.

- Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests.

- Funding

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018, 68(6):394-424.

2. Herskovic A, Martz K, al-Sarraf M, Leichman L, Brindle J, Vaitkevicius V, Cooper J, Byhardt R, Davis L, Emami B: Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with cancer of the esophagus. N Engl J Med 1992, 326(24):1593-1598.

3. Cooper JS, Guo MD, Herskovic A, Macdonald JS, Martenson JA, Jr., Al-Sarraf M, Byhardt R, Russell AH, Beitler JJ, Spencer S et al: Chemoradiotherapy of locally advanced esophageal cancer: long-term follow-up of a prospective randomized trial (RTOG 85-01). Radiation Therapy Oncology Group. JAMA 1999, 281(17):1623-1627.

4. Minsky BD, Pajak TF, Ginsberg RJ, Pisansky TM, Martenson J, Komaki R, Okawara G, Rosenthal SA, Kelsen DP: INT 0123 (Radiation Therapy Oncology Group 94-05) phase III trial of combined-modality therapy for esophageal cancer: high-dose versus standard-dose radiation therapy. J Clin Oncol 2002, 20(5):1167-1174.

5. Kwee RM: Prediction of Tumor Response to Neoadjuvant Therapy in Patients with Esophageal Cancer with Use of F-18 FDG PET: A Systematic Review. Radiology 2010, 254(3):707-717.

6. Cremonesi M, Garibaldi C, Timmerman R, Ferrari M, Ronchi S, Grana CM, Travaini L, Gilardi L, Starzynska A, Ciardo D et al: Interim (18)F-FDG-PET/CT during chemo-radiotherapy in the management of oesophageal cancer patients. A systematic review. Radiother Oncol 2017, 125(2):200-212.

7. Ishihara R, Yamamoto S, Iishi H, Takeuchi Y, Sugimoto N, Higashino K, Uedo N, Tatsuta M, Yano M, Imai A et al: Factors predictive of tumor recurrence and survival after initial complete response of esophageal squamous cell carcinoma to definitive chemoradiotherapy. Int J Radiat Oncol Biol Phys 2010, 76(1):123-129.

8. Chao YK, Tseng CK, Wen YW, Liu YH, Wan YL, Chiu CT, Chang WC, Chang HK: Using Pretreatment Tumor Depth and Length to Select Esophageal Squamous Cell Carcinoma Patients for Nonoperative Treatment After Neoadjuvant Chemoradiotherapy. Annals of Surgical Oncology 2013, 20(9):3000-3008.

9. Fareed KR, Kaye P, Soomro IN, Ilyas M, Martin S, Parsons SL, Madhusudan S: Biomarkers of response to therapy in oesophago-gastric cancer. Gut 2009, 58(1):127-143.

10. Knight K, Wade S, Balducci L: Prevalence and outcomes of anemia in cancer: a systematic review of the literature. Am J Med 2004, 116 Suppl 7A:11S-26S.

11. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran 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(6):dju124.

12. Templeton AJ, Ace O, McNamara MG, Al-Mubarak M, Vera-Badillo FE, Hermanns T, Seruga B, Ocana A, Tannock IF, Amir E: Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2014, 23(7):1204-1212.

13. Mao Y, Chen D, Duan S, Zhao Y, Wu C, Zhu F, Chen C, Chen Y: Prognostic impact of pretreatment lymphocyte-to-monocyte ratio in advanced epithelial cancers: a meta-analysis. Cancer Cell Int 2018, 18:201.

14. Yodying H, Matsuda A, Miyashita M, Matsumoto S, Sakurazawa N, Yamada M, Uchida E: Prognostic Significance of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Oncologic Outcomes of Esophageal Cancer: A Systematic Review and Meta-analysis. Ann Surg Oncol 2016, 23(2):646-654.

15. Yoo EJ, Park JC, Kim EH, Park CH, Shim CN, Lee HJ, Chung HS, Lee H, Shin SK, Lee SK et al: Prognostic value of neutrophil-to-lymphocyte ratio in patients treated with concurrent chemoradiotherapy for locally advanced oesophageal cancer. Dig Liver Dis 2014, 46(9):846-853.

16. Cox S, Hurt C, Grenader T, Mukherjee S, Bridgewater J, Crosby T: The prognostic value of derived neutrophil to lymphocyte ratio in oesophageal cancer treated with definitive chemoradiotherapy. Radiother Oncol 2017, 125(1):154-159.

17. Miao C, Zhu S, Pan H, Cao X, Yuan S, Hu X: Combined neutrophil-platelet score and hemoglobin level predict survival in esophageal squamous cell carcinoma patients treated with chemoradiotherapy. Oncotarget 2017, 8(50):87971-87979.

18. Chen MF, Chen PT, Kuan FC, Chen WC: The Predictive Value of Pretreatment Neutrophil-To-Lymphocyte Ratio in Esophageal Squamous Cell Carcinoma. Ann Surg Oncol 2019, 26(1):190-199.

19. Zenda S, Hironaka S, Boku N, Yamazaki K, Yasui H, Fukutomi A, Yoshino T, Onozawa Y, Nishimura T: Impact of hemoglobin level on survival in definitive chemoradiotherapy for T4/M1 lymph node esophageal cancer. Dis Esophagus 2008, 21(3):195-200.

20. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P et al: Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007, 13(9):1050-1059.

21. Lee Y, Auh SL, Wang Y, Burnette B, Wang Y, Meng Y, Beckett M, Sharma R, Chin R, Tu T et al: Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood 2009, 114(3):589-595.

22. Swann JB, Smyth MJ: Immune surveillance of tumors. J Clin Invest 2007, 117(5):1137-1146.

23. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J et al: Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 2006, 203(5):1259-1271.

24. Burnette BC, Liang H, Lee Y, Chlewicki L, Khodarev NN, Weichselbaum RR, Fu YX, Auh SL: The Efficacy of Radiotherapy Relies upon Induction of Type I Interferon-Dependent Innate and Adaptive Immunity. Cancer Res 2011, 71(7):2488-2496.

25. Diakos CI, Charles KA, McMillan DC, Clarke SJ: Cancer-related inflammation and treatment effectiveness. Lancet Oncol 2014, 15(11):E493-E503.

26. Zhang WZ, Chen JZ, Li DR, Chen ZJ, Guo H, Zhuang TT, Li DS, Zhou MZ, Chen CZ: Simultaneous modulated accelerated radiation therapy for esophageal cancer: a feasibility study. World J Gastroenterol 2014, 20(38):13973-13980.

27. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer 2009, 45(2):228-247.

28. Chen JZ, Guo H, Zhai TT, Chang D, Chen ZJ, Huang RH, Zhang WZ, Lin K, Guo LJ, Zhou MZ et al: Radiation dose escalation by simultaneous modulated accelerated radiotherapy combined with chemotherapy for esophageal cancer: a phase II study. Oncotarget 2016, 7(16):22711-22719.

29. Chowdhary M, Switchenko JM, Press RH, Jhaveri J, Buchwald ZS, Blumenfeld PA, Marwaha G, Diaz A, Wang D, Abrams RA et al: Post-treatment neutrophil-to-lymphocyte ratio predicts for overall survival in brain metastases treated with stereotactic radiosurgery. J Neuro-Oncol 2018, 139(3):689-697.

30. Lin AJ, Rao YJ, Chin RI, Campian J, Mullen D, Thotala D, Daly M, Gay H, Oppelt P, Hallahan D et al: Post-operative radiation effects on lymphopenia, neutrophil to lymphocyte ratio, and clinical outcomes in palatine tonsil cancers. Oral Oncol 2018, 86:1-7.

31. Suh KJ, Kim SH, Kim YJ, Kim M, Keam B, Kim TM, Kim DW, Heo DS, Lee JS: Post-treatment neutrophil-to-lymphocyte ratio at week 6 is prognostic in patients with advanced non-small cell lung cancers treated with anti-PD-1 antibody. Cancer Immunol Immun 2018, 67(3):459-470.

32. Khunger M, Patil PD, Khunger A, Li M, Hu B, Rakshit S, Basu A, Pennell N, Stevenson JP, Elson P et al: Post-treatment changes in hematological parameters predict response to nivolumab monotherapy in non-small cell lung cancer patients. PLoS One 2018, 13(10):e0197743.

33. Zhou XL, Li YQ, Zhu WG, Yu CH, Song YQ, Wang WW, He DC, Tao GZ, Tong YS: Neutrophil-to-lymphocyte ratio as a prognostic biomarker for patients with locally advanced esophageal squamous cell carcinoma treated with definitive chemoradiotherapy. Sci Rep-Uk 2017, 7.

34. Lee YJ, Lee SB, Beak SK, Han YD, Cho MS, Hur H, Lee KY, Kim NK, Min BS: Temporal changes in immune cell composition and cytokines in response to chemoradiation in rectal cancer. Sci Rep 2018, 8(1):7565.

35. Barbetta A, Nobel TB, Sihag S, Hsu M, Tan KS, Bains MS, Isbell JM, Janjigian YY, Wu AJ, Bott MJ et al: Neutrophil to Lymphocyte Ratio as Predictor of Treatment Response in Esophageal Squamous Cell Cancer. Ann Thorac Surg 2018, 106(3):864-871.

36. Davuluri R, Jiang W, Fang P, Xu C, Komaki R, Gomez DR, Welsh J, Cox JD, Crane CH, Hsu CC et al: Lymphocyte Nadir and Esophageal Cancer Survival Outcomes After Chemoradiation Therapy. Int J Radiat Oncol Biol Phys 2017, 99(1):128-135.

37. Fang P, Jiang W, Davuluri R, Xu C, Krishnan S, Mohan R, Koong AC, Hsu CC, Lin SH: High lymphocyte count during neoadjuvant chemoradiotherapy is associated with improved pathologic complete response in esophageal cancer. Radiother Oncol 2018, 128(3):584-590.

38. Feng X, Li L, Wu J, Zhang L, Sun Z, Li X, Wang X, Yu H, Chang Y, Wu X et al: Complete Blood Count Score Model Integrating Reduced Lymphocyte-Monocyte Ratio, Elevated Neutrophil-Lymphocyte Ratio, and Elevated Platelet-Lymphocyte Ratio Predicts Inferior Clinical Outcomes in Adult T-Lymphoblastic Lymphoma. Oncologist 2019.

39. Ahn GO, Tseng D, Liao CH, Dorie MJ, Czechowicz A, Brown JM: Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. Proc Natl Acad Sci U S A 2010, 107(18):8363-8368.

40. Liang H, Deng L, Hou Y, Meng X, Huang X, Rao E, Zheng W, Mauceri H, Mack M, Xu M et al: Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. Nat Commun 2017, 8(1):1736.

Table

Parameters	Subgroups	No	locoregional progression hazard (%)	Log-rank		Cox regression	
				Chi-Square	P value	HR (95%CI)	P value
Age	≤ 60	41	30	0.683	0.409	1.2 (0.5-3.1)	0.716
	> 60	42	22			1	
Gender	Male	64	33	4.686	0.030	6.7 (0.8-55.3)	0.078
	Female	19	5			1	
T stage *	T1-2	18	23	0.660	0.719	1	
	T3	38	31			1.2 (0.3-4.5)	0.738
	T4	27	20			0.4 (0.1-3.5)	0.436
N stage *	0	31	26	0.000	0.997	1	0.579
	1	52	25			0.7 (0.2-2.8)	
M stage *	0	71	24	0.821	0.365		
	1	12	34				
Clinical stage *	II	30	24	0.845	0.656	1	
	III	41	24			1.1 (0.1-3.9)	0.538
	IV	12	34			2.1 (0.3-13.3)	0.422
PBCS	Low	30	7	9.857	0.007	1	
	Medium	40	29			5.8 (1.2-27.7)	0.028
	High	13	58			12.2 (2.0-76.3)	0.007

Table 1. Univariate and multivariate cox regression analysis for 3-year cumulative hazard of locoregional tumour progression.

* According to the 6th edition of American Joint Committee on Cancer (AJCC) TNM staging system.

HR: hazard ratio; CI: confidence interval; HR = 1 for references.

PBCS: peripheral blood cell score.

Figures

Figure 1

The treatment scheme and trial diagram The treatment scheme and the timing of peripheral blood cell (PBC) assessment are shown in (A). RT: radiotherapy; Gy: gray; F: fraction; PF: cisplatin and fluorouracil. The trial diagram was summarized in (B). CCRT: concurrent chemoradiotherapy.

Figure 2

Distribution of peri-treatment peripheral blood cell parameters in patients with or without disease progression following definite chemoradiotherapy for oesophageal squamous cell carcinoma. Eight peripheral blood cell parameters were collected from patients at 7 time points during radiotherapy, including haemoglobin (A), neutrophils (B), platelets (C), lymphocytes (D), monocytes (E), neutrophil-to-lymphocyte ratio (NLR, F), platelet-to-lymphocyte ratio (PLR, G) and monocyte-to-lymphocyte ratio (MLR, H). These parameters in patients with (red, n = 34) or without (black, n = 49) disease progression were plotted in parallel and compared. Values represent mean with 95% confidence interval. Comparisons of means were performed using the two-tailed unpaired Student's t test (* P < 0.05).

Figure 3

The discrimination capacity of peri-treatment peripheral blood cell parameters for hazard of disease progression. The discrimination capacity of peri-treatment peripheral blood cell parameters (PBC) for hazard of disease progression (A), local (B), regional (C) and distant (D) progression were evaluated individually by receiver operating characteristic (ROC) curves. Peri-treatment PBC includes haemoglobin, neutrophils, platelets, lymphocytes, monocytes, neutrophil-to-lymphocyte ratio (NLR), platelet-to-

lymphocyte ratio (PLR) and monocyte-to-lymphocyte ratio (MLR). The area under a curve (AUC) of these curves were summarized as a matrix with parameter type on the column and time point on the row. AUCs with statistical significance are indicated with an asterisk. AUC < 0.5 is not shown (* P < 0.05. ** P < 0.01). The background of each AUC corresponds to its value as indicated.

Figure 4

Development of peripheral blood cell score Peri-treatment peripheral blood cell (PBC) parameters were ranked according to their area under a curve (AUC) for discrimination of local (A) and regional (B) progression using Receiver Operating Characteristic (ROC) curves. The top 10 parameters are indicated. The values of peripheral blood cell (PBC) parameters were normalized to the mean of each parameter of the whole group (mean = 100). The mean of the top PBC (n = 2-10) parameters from (A) and (B) were calculated and designated as PBC score for local and regional progression (PBCS-L and PBCS-R), respectively. The discrimination capacity of PBCS-L and PBCS-R were evaluated by ROC curves and summarized in (C). The background of each AUC corresponds to its value as indicated.

Figure 5

Peri-treatment peripheral blood cell score (PBCS) predict long-term hazard of local or regional progression in oesophageal cancer patients after chemoradiotherapy. The receiver operating characteristic curves of PBCS-L and PBCS-R were shown in (A) and (C). The cut-off of the curve was determined by Youden index. The percentage and 3-year cumulative hazard of local progression in patients with PBCS-L > 86 (red, n = 42) or PBCS-L ≤ 86 (black, n = 41) was plotted in (B). The 3-year cumulative hazard of regional progression in patients with PBCS-R > 108 (red, n = 24) or PBCS-R ≤ 108 (black, n = 59) was plotted in (D). Red in pie charts indicates the percentage of patients with progression. Comparison of 3-year cumulative hazards was performed using the log rank's test. PBCS-L: peripheral blood cell score for local progression. PBCS-R: peripheral blood cell sore for regional progression.

Figure 6

Peri-treatment peripheral blood cell score (PBCS) predicts long-term hazard of locoregional recurrence in patients with oesophageal squamous cell carcinoma after chemoradiotherapy Patients were divided into 3 subgroups according to their PBCS-L and PBCS-R: PBCS high (red solid line): both PBCS-L and PBCS-R high; PBCS medium (red dash line): either PBCS-L or PBCS-R high; PBCS low (black solid line): both PBCS-L and PBCS-R low. The percentage and 3-year cumulative hazard of locoregional progression in patients categorized according to their PBCS is plotted in (A). The 3-year cumulative hazard of locoregional progression in patients categorized according to their clinical stage (II (black solid line) vs III (red dash line) vs IV (red solid line)) is plotted in (B). Red in pie charts indicates the percentage of patients

with progression. Comparison of 3-year cumulative hazard was performed using the log rank's test. PBCS-L: peripheral blood cell score for local progression. PBCS-R: peripheral blood cell sore for regional progression.

Supplementary Files

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