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

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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 [4]. 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 [10,19,30]. 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 (18F FDG PET/CT)), clinical parameters (e.g. tumour stage) and biomarkers in tumour samples [6,12,17,20,23]. 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 [22,28,34,35]. 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 [8,11,29,36-38]. 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 [22,28,34-37].

The majority of previous studies relied solely on pretreatment PBC parameters [22,28,34,35], 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 [2,5,24,31,33]. Studies also indicate that the local immune response interplays with systemic inflammation, which consists of a variety of circulating inflammation factors, including PBC parameters [14]. 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 ≥ 18 but ≤ 75 years; (5) Zubrod performance status 0-2; (6) Haemoglobin ≥ 10 gram per litre (g/L), neutrophils ≥ 1.5 x 10^9/L, Platelets ≥ 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 [39]. 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 [15]. 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 [7]. 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 1\textsuperscript{st} 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 2\textsuperscript{nd} - 7\textsuperscript{th} time points). Only neutrophils (3\textsuperscript{rd}) and NLR (3\textsuperscript{rd}) 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 (7\textsuperscript{th}) for local progression, haemoglobin (2\textsuperscript{nd} and 7\textsuperscript{th}) and neutrophils (3\textsuperscript{rd}) 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 [10,30]. 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 [3, 9, 21, 25, 27, 32, 40]. Barbetta A et al show that changes of NLR after CRT are associated with risk of recurrence in patients with oesophageal squamous cell carcinoma [3]. 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 [13, 16]. 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 NLR (<5) [40]. 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 [18]. 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 [1,26]. 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 for publication**

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.

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**Authors' Contributions**

Liangyu Xu and Jianzhou Chen designed the study, performed the statistical analysis, and drafted the manuscript together. Hong Guo, Ruihong H, Longjia Guo, Yuan-xiang Yu, Tiantian Zhai, Fangcai Wu, Zhijian Chen and Derui Li participated in the collection of the
clinical data. Chuangzhen Chen conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Figures

A

Week

0 1 2 3 4 5 6

RT: 2.2Gy x 30F

PF chemotherapy

PBC assessment 1 2 3 4 5 6 7

B

87 patients enrolled in this trial

87 patients received CCRT with peri-treatment PBC assessment

87 patients were followed up for a minimum of 3 years
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.

83 patients were analysed
49 Progression-free
34 Disease progression
  10 Local
  3 Local and regional
  5 Regional
  2 Regional and distant
  14 Distant

4 patients excluded due to lack of information of failure pattern
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).

A

|                  | Area under the ROC curve |
|------------------|--------------------------|
| Hemoglobin       | 0.52 0.56 0.58           |
| Neutrophils      | 0.58 0.67* 0.54 0.57     |
| Platelets        | 0.54 0.56 0.53           |
| Lymphocytes      | 0.55 0.54 0.52 0.55      |
| Monocytes        | 0.59 0.6 0.54 0.56       |
| NLR              | 0.51 0.67* 0.56 0.53     |
| PLR              | 0.57 0.52 0.55 0.58      |
| MLR              | 0.58 0.55 0.54 0.55 0.51 |

Disease progression

B

|                  | Area under the ROC curve |
|------------------|--------------------------|
| Hemoglobin       | 0.58 0.63 0.65 0.53 0.59 0.58 0.55 |
| Neutrophils      | 0.63 0.65 0.56 0.63 0.52 |
| Platelets        |                          |
| Lymphocytes      | 0.58 0.52 0.52 0.54 0.68* |
| Monocytes        | 0.64 0.63 0.52 0.67 0.51 0.55 |
| NLR              | 0.52 0.65 0.55 0.51      |
| PLR              | 0.54                        |
| MLR              | 0.53 0.56 0.55 0.53 0.53   |

Local progression

C

|                  | Area under the ROC curve |
|------------------|--------------------------|
| Hemoglobin       | 0.63 0.73* 0.63 0.64 0.64 0.64 0.71* |
| Neutrophils      | 0.56 0.56 0.76** 0.56 0.54 0.63 |
| Platelets        | 0.57 0.61 0.58 0.64 0.6 |
| Lymphocytes      | 0.52 0.6               |
| Monocytes        | 0.59 0.58 0.55               |
| NLR              | 0.63 0.55 0.68 0.56 0.53 0.6 0.56 |
| PLR              | 0.64 0.57 0.55 0.51 0.68 0.54 |
| MLR              | 0.56 0.57 0.52 0.6 0.56    |

Regional progression

D
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.
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 score 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

A

Locoregional progression

PBCS

\[ P = 0.007 \]

- High: 58%
- Medium: 29%
- Low: 7%

B

Locoregional progression

Clinical stage

\[ P = 0.656 \]

- IV: 34%
- III: 24%
- II: 24%
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 score for regional progression.

Supplementary Files

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