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# Usefulness of baseline immature reticulocyte fraction to mature reticulocyte fraction ratio (IMR) as A prognostic predictor for patients with small cell lung cancer

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ABSTRACT

*Background:* Small cell lung cancer (SCLC) has a strong invasive ability and a high degree of malignancy, so accurate prognosis prediction is crucial for making the most favorable treatment decision.Unfortunately, there is a scarcity of prognostic indicators specific to SCLC. Reticulocyte levels in blood parameters have been linked to the prognosis of various malignancies. Given SCLC's aggressive characteristics, identifying reliable prognostic markers, such as reticulocyte counts, becomes pivotal in enhancing prognostic accuracy and guiding effective therapeutic strategies.

*Objective:* This study aimed to evaluate the predictive power of the immature reticulocyte fraction (IRF) to mature reticulocyte fraction (MRF) ratio (IMR) for survival outcomes in patients with SCLC.

*Materials and methods:* A retrospective analysis was conducted on 192 patients with small cell lung cancer (SCLC). The median values of various prognostic indicators, such as IMR, IRF, MRF, reticulocyte count (RET), SII (systemic immune-inflammatory index), were utilized as cutoff points, categorizing patients into high and low groups. The Kaplan–Meier method, univariate, multivariate analyses Cox regression, and C-index were used to analyze the prognostic factors for overall survival (OS).

*Results:* In our cohort, 138 (71.9 %) were male, 119 (62 %) were smokers, and 82 (57.3 %) were older than 60 years old. The median survival time was 18.15 months.Higher mortality was observed in the high IMR and high IRF groups, while the high MRF group exhibited lower mortality. At the same time, mortality was lower in the high MRF group. Univariate analysis showed that smoking history ($P = 0.006$), tumor stage ($P = 0.002$), chemotherapy cycle ($P = 0.014$), IMR ($P = 0.01$), and many other factors significantly affected the prognosis of SCLC.

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Multivariate analysis demonstrated that elevated IMR was an independent adverse predictor of OS ($P = 0.039$, HR = 0.330). Spearman test confirmed that the prognostic indicators IRF, IMR, and SII were positively correlated with the overall survival rate of patients with SCLC. Kaplan-Meier analysis showed that the OS rate of patients with high IMR was significantly worse ($P = 0.0096$). In addition, we found that IMR was superior to IRF in distinguishing patients with different outcomes in the low and high groups ($P < 0.05$). Our novel integration index, combining IMR with the TNM stage system and SII index, exhibited superior prognostic value compared to the original index. Additionally, the combination of prognostic indicators IMR and SII significantly stratified stage I-II SCLC patients ($P < 0.05$).

*Conclusions:* The prognostic index based on peripheral blood IMR stands out as an independent predictor for SCLC patients pre-treatment. Its accessibility through routine blood analysis facilitates immediate clinical application without requiring prolonged scientific research validation. The integration of IMR with the TNM score enhances survival prediction and risk stratification. Notably, when combined with the SII score, the new IMR index demonstrates significant improvements in prognostication for stage I-II small cell lung cancer.

## 1. Introduction

Lung cancer is one of the most common cancers with leading mortality over the world [1]. Lung cancer is divided into two categories, non-small-cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Small cell lung cancer (SCLC), which accounts for about 15%–20 % of lung cancer patients, is a highly aggressive neuroendocrine tumor [2]. SCLC has a strong invasion ability, a high degree of malignancy, and distant metastasis in the early stage of the disease [3]. Although sensitive to chemoradiotherapy, most patients have a poor prognosis due to the high probability of recurrence within 6 months after first-line chemotherapy and the rapid emergence of drug resistance in subsequent chemotherapy [3]. In lung cancer patients approximately 30 % of SCLC is categorized as limited-stage small cell lung cancer (LS-SCLC), with a median survival of 16–24 months [4]; The remaining 70 % of patients are categorized as extensive-stage small cell lung cancer (ED-SCLC), with a median survival of 8–13 months [5]. The 5-year survival rate for SCLC is only 7 % [1], even most patients survive for only < 1 year after diagnosis [6]. So early accurate prediction of patients' prognoses is crucial for making the most favorable treatment decisions.

Previous studies have shown that there are many factors affecting the prognosis of SCLC, such as age, gender, smoking, and tumor TNM stage system (such as tumor size, node metastasis, and distant metastasis) [7,8]. Despite many advances in the study of SCLC prognostic indicators such as CTC [9]、PD-L1 [10]、cell-free DNA [11]、pleiotrophin [12]、circulating endothelial cells、microparticles [13]and SII [14] in the past decades. However, the poor prognosis of SCLC has not been significantly improved [15]. Moreover, the method of obtaining the above indexes is not only expensive but also complicated, which makes it difficult to be applied in the clinic quickly. Therefore, practical and rapid means to solve clinical problems are needed.

Many off-the-shelf parameters on automatic hematology analyzers have the advantages of non-invasiveness and no additional cost. For example, neutrophil-lymphocyte ratio (NLR) [1], absolute eosinophil count (AEC) [2], systemic immune-inflammatory index (SII) [3,4], neutrophil-lymphocyte ratio (NLR) [5,6], platelet-to-lymphocyte ratio (PLR) [5], Lymphocyte-monocyte ratio (LMR) [7], absolute platelet count (APC) and other laboratory parameters have attracted increasing research attention as cancer prognostic biomarkers [8]. Reticulocyte in peripheral blood is a useful clinical indicator, in which the reticulum network or granules represent precipitated rough endoplasmic reticulum with associated polyribosomes. During erythropoiesis, reticulocytes are released into the circulation where they gradually lose their RNA, and evolve into mature RBCs [16]. Assessment of reticulocyte maturity is based on the intensity of either fluorescence or light scattering/absorbance, which depends on RNA content. Reticulocytes have now been grouped into the low fluorescent region (LFR), middle fluorescent region (MFR), or high fluorescent region (HFR) corresponding to the lower, middle, and higher RNA content, respectively [17]. The immature reticulocyte fraction (IRF) is a relatively new reticulocyte parameter that includes MFR and HFR and is more reproducible than the HFR [18]. Studies have shown that an increase in IRF is superior to other hematological parameters such as absolute neutrophil count (ANC), immature platelet fraction (IPF), reticulocyte counts as an early indicator of bone marrow recovery or hematopoietic stem cell transplantation [19–21]. The clinical utility of IRF has been reported in a variety of conditions such as assessing bone marrow recovery after chemotherapy [22] , monitoring of diagnosis of anemia and its treatment [23], verifying aplastic anemia [24], and assessing the need for RBC transfusion in an anemic patient, etc. [22].

Cancer-related anemia is either a tumor-driven blood disorder or a result of the patient's chemotherapy or progressive disease. Anemia can increase hypoxia in the tumor microenvironment, leading to tumor growth, tissue invasion, metastasis, and resistance to radiation and chemotherapy. The presence and severity of anemia were significantly associated with cancer stage [25]. Therefore, cancer-related anemia seriously affects the quality of life and overall prognosis of cancer patients. Cancer-related anemia activates the stress erythropoietic machinery, which may lead to ineffective erythropoiesis [26]. It is manifested by an enlarged pool of erythroid progenitor cells, low reticulocyte count, and largely unable to differentiate and produce mature red blood cells, which further aggravates cancer-related anemia. The reticulocyte count serves as a key tool to assess the bone marrow's ability to increase erythrocyte production in response to various types of anemias [27]. Hence, we posit the proposal that IMR can serve as a rapid and efficient indicator for stratifying the prognosis of SCLC, addressing the challenge of prognostic stratification.

In this retrospective analysis, clinical data from 192 pre-treatment small cell lung cancer (SCLC) patients were examined to assess the prognostic utility of IMR and its correlation with overall survival (OS). The study aims to offer a valuable reference for clinical

decision-making, providing insights into the predictive potential of IMR in small cell lung cancer prognosis. The findings aspire to contribute to informed clinical practices and stimulate further research endeavors in the context of SCLC.

## 2. Materials and Methods

### 2.1. Patient selection

The study was approved by the Medical Committee of Sichuan Cancer Hospital (No. KY-2021-076). This study was a retrospective study, and the informed consent exemption statement was completed. The inclusion criteria were as follows: (1) The patient was pathologically diagnosed as SCLC according to the NCCN (National Comprehensive Cancer Network) Clinical Practice Guidelines in Oncology (NCCN guidelines) [28]; (2) blood analysis before surgery or treatment; and (3) available follow-up data and clinical data. Exclusion criteria：(1) the patient had a severe cardio-cerebrovascular disease or other diseases that may have had a significant impact on prognosis, and; (2) the patient was lost to follow-up or patient medical records important information was incomplete or missing.

To ensure comprehensive inclusion, we accessed all lung cancer cases documented in the Sichuan Cancer Hospital follow-up system from 2013 to September 2022. Of the 2830 pathologically confirmed lung cancer cases with complete follow-up data, 226 were identified as small cell lung cancer (SCLC) through histopathological analysis. Fourteen patients lacking reticulocyte fluorescence intensity data in peripheral blood analysis were excluded. In cases where multiple measurements existed in the clinical laboratory information system, the initial measurement was chosen, resulting in the exclusion of twenty cases with repeated measurements. The study flow chart is shown in Fig. 1. At last, 192 SCLC patients were ultimately enrolled in this research.

### 2.2. Follow-up and clinical data collection

Patients were followed every 3 months during the first 2 years after treatment, every 6 months for 2–5 years, and every 1 year after 5 years. The survival information was assembled by interviewing medical records or telephoning. The primary endpoint of this study was overall survival (OS), which was defined as the time from the date of diagnosis to death or last follow-up. Clinical data including patients' age, gender, smoking history, the tumor, node, metastasis (TNM) staging system, treatment, and differentiation were collected from retrospective electronic medical records. TNM staging was based on the 8th edition of the TNM classification [29]. The laboratory data of high fluorescence intensity reticulocytes, medium fluorescence intensity reticulocytes, low fluorescence intensity reticulocytes, platelet count (PLT)， neutrophil count (NEUT)， lymphocyte count (LY) and reticulocyte count (RET) were extracted from medical records. All experimental results were analyzed by an automatic hematology analyzer (Shenzhen Mindray, BC-5390). The systemic immune-inflammation index (SII) was calculated according to the formula: platelet count × neutrophil count/lymphocyte count [14]. TNM stage system, SII, and IMR were also used to perform survival analysis.

### 2.3. Treatment

According to the NCCN guidelines patients with stage T1-2N0M0 LS-SCLC can be treated with radical surgery and adjuvant

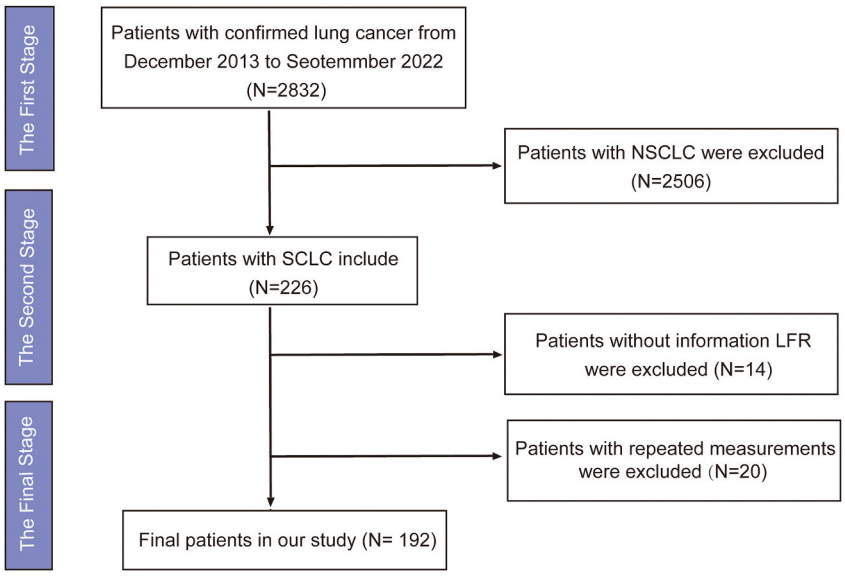


**Fig. 1. Flow chart of the patient selection process.** Abbreviations: NSCLC, non-small-cell lung cancer; SCLC, Small cell lung cancer; low fluorescent region (LFR), middle fluorescent region (MFR), or high fluorescent region (HFR).

platinum-based chemotherapy. For limited-stage SCLC exceeding T1-2N0, concurrent radiation, and platinum-based chemotherapy are recommended. Chemotherapy or combined immunotherapy based on chemotherapy is recommended for extensive-stage SCLC [30]. The chemotherapy regimens are EP, EC, irinotecan combined with cisplatin (IP), irinotecan combined with carboplatin (IC), or etoposide combined with lobaplatin (EL). Second-line treatment options (irinotecan, gemcitabine, vinorelbine, or paclitaxel, etc.) are available for patients with recurrence or progression within 6 months after first-line chemotherapy.

### *2.4. Statistical analysis*

The median of immature reticulocyte fraction (IRF) 、 mature reticulocyte fraction (MRF) 、immature reticulocyte fraction to mature reticulocyte fraction ratio (IMR)、reticulocyte count (RET) and systemic immune-inflammation index (SII) were used as the cutoff of survival analysis. The clinicopathologic characteristics were evaluated by descriptive analysis. The clinicopathological characteristics grouped by IMR were compared by the Chi-squared tests or Fisher's exact tests. The Kaplan–Meier method was utilized to estimate survival time with Log-rank tests. The prognostic factors of survival were identified with univariate and multivariate analyses of Cox proportional hazards regression models. The multivariate Cox analysis was based on the factors with significant prognostic values in the univariate Cox analysis. The calibration index (C-index) was evaluated to assess the consistency between the predicted and observed probabilities [31]. The C-index can evaluate the model's ability to classify individual patients into risk groups with different prognoses by estimating the probability of concordance between predicted and observed outcomes. C-index was calculated using the Hmisc R package in R software version 4.2.3 [31,32]. All statistical analyses were conducted using SPSS 26.0 and. Plotting for survival and prognostic analysis was done by Hiplot Pro [33]. A two-tailed $P$ value $< 0.05$ was considered statistically significant.

**Table 1**
The clinical characteristics of 192 patients with SCLC.

| Clinical Characteristics | Number (%)/median (range) | |
|---|---|---|
| **Age** | | |
| ≤60 | 110 | (42.7) |
| >60 | 82 | (57.3) |
| **Smoking history** | | |
| Nonsmoking | 73 | (38) |
| moking | 119 | (62) |
| **Gender** | | |
| Female | 54 | (28.1) |
| Male | 138 | (71.9) |
| **Event** | | |
| Alive | 66 | (34.4) |
| Dead | 126 | (65.6) |
| **Time(month)** | 18.15 | (0.3–80.8) |
| Immature reticulocyte fraction (IRF) | 4.40 | (0–29.0) |
| Mature reticulocyte fraction (MRF) | 95.55 | (71–100.0) |
| Immature reticulocyte fraction to mature reticulocyte fraction ratio (IMR) | 0.046 | (0–0.41) |
| Platelet count (PLT) | 198 | (73–472) |
| Neutrophil count (NEUT) | 4.24 | (1.75–13.02) |
| Lymphocyte count (LY) | 1.425 | (0.28–6.72) |
| Reticulocyte count (RET) | 0.045 | (0.01–0.14 ) |
| **T stage** | | |
| T1-2 | 72 | (37.5) |
| T3-4 | 109 | (56.8) |
| Tx | 11 | (5.7) |
| **Node metastasis** | | |
| No | 56 | (29.2) |
| Yes | 125 | (65.1) |
| Uncertain | 11 | (5.7) |
| **Distant metastasis** | | |
| M0 | 129 | (67.2) |
| M1 | 54 | (28.1) |
| Mx | 9 | (4.7) |
| **Tumor stage** | | |
| I-II | 44 | (22.9) |
| III | 82 | (42.7) |
| IV | 53 | (27.6) |
| X | 13 | (6.8) |
| **Treatment** | | |
| Surgery/chemotherapy/radiotherapy | 43 | (22.4) |
| Chemotherapy/radiotherapy/concurrent chemoradiotherapy/immunotherapy | 99 | (51.6) |
| Uncertain | 50 | (26) |
| **Chemo-cycle** | 4 | (1–16) |

## 3. Results

### 3.1. Baseline characteristics of patients and their relationship to IMR levels

The distribution of clinical characteristics of 192 SCLC patients is summarized in Table 1. All patients consisted of 138 males (71.9 %) and 54 females (28.1 %). The median age of patients was 60 years (range: 28–79 years) and there were 82 patients (57.3 %) older than 60 years. In all patients, the median follow-up length was 18.15 months, and 126 patients (65.6 %) died during follow-up. The staging was also carried out based on the TNM staging criteria. 72 of the tumors (37.5 %) wereT1-2, 109 (56.8 %) T3-4. 56 (29.2 %)

**Table 2**
Relationship between the High IMR group and Low IMR group of 192 Patients with SCLC (Immature Reticulocyte Fraction to Mature Reticulocyte Fraction Ratio, IMR).

| Clinical Characteristics | IMR, Number (%) | | |
|---|---|---|---|
| | ≤0.046 | > 0.046 | *P* |
| Age | | | 0.663 |
| < 60 | 38 ( 40.9 ) | 44 ( 44.4 ) | |
| ≥60 | 55 ( 59.1 ) | 55 ( 55.6 ) | |
| Gender | | | 0.873 |
| Female | 27 ( 29.0 ) | 27 ( 27.3 ) | |
| Male | 66 ( 71.0 ) | 72 ( 72.7 ) | |
| Smoking history | | | 0.882 |
| Nonsmoker | 37 ( 39.8 ) | 36 ( 36.4 ) | |
| smoking | 56 ( 60.2 ) | 63 ( 63.6 ) | |
| Event | | | **0.007** |
| Alive | 41 ( 44.1 ) | 25 ( 25.3 ) | |
| Dead | 52 ( 55.9 ) | 74 ( 74.7 ) | |
| T stage | | | **0.043** |
| T1-2 | 40 ( 43.0 ) | 32 ( 32.3 ) | |
| T3-4 | 45 ( 48.4 ) | 64 ( 64.7 ) | |
| Tx | 8 ( 8.6 ) | 3 ( 3.0 ) | |
| Node metastasis | | | 0.732 |
| No | 29 ( 31.2 ) | 27 ( 27.3 ) | |
| Yes | 58 ( 62.4 ) | 67 ( 67.7 ) | |
| Uncertain | 6 ( 6.4 ) | 5 ( 5.0 ) | |
| Distant metastasis | | | 0.741 |
| No | 65 ( 69.9 ) | 64 ( 64.6 ) | |
| Yes | 24 ( 25.8 ) | 30 ( 30.3 ) | |
| Uncertain | 4 ( 4.3 ) | 5 ( 5.1 ) | |
| Tumor stage | | | 0.679 |
| I-II | 24 ( 24.8 ) | 20 ( 20.2 ) | |
| III | 38 ( 40.9 ) | 44 ( 44.4 ) | |
| IV | 23 ( 24.7 ) | 30 ( 30.3 ) | |
| X | 8 ( 8.6 ) | 5 ( 5.1 ) | |
| Chemo-cycle | | | 1.000 |
| ≤4 | 35 ( 53.8 ) | 37 ( 54.4 ) | |
| > 4 | 30 ( 46.2 ) | 31 ( 45.6 ) | |
| Systemic immune-inflammation index | | | 0.386 |
| ≤612.35 | 50 ( 53.8 ) | 46 ( 46.5 ) | |
| > 612.35 | 43 ( 46.2 ) | 53 ( 53.5 ) | |
| Immature reticulocyte fraction | | | **< 0.001** |
| ≤4.40 | 93 ( 100 ) | 4 ( 4.0 ) | |
| > 4.40 | 0 | 95 ( 96.0 ) | |
| Mature reticulocyte fraction | | | **< 0.001** |
| ≤95.5 | 25 ( 26.9 ) | 71 ( 71.7 ) | |
| > 95.5 | 68 ( 73.1 ) | 28 ( 28.3 ) | |
| Platelet count | | | 0.194 |
| ≤198 | 42 ( 45.2 ) | 55 ( 55.6 ) | |
| > 198 | 51 ( 54.8 ) | 44 ( 44.4 ) | |
| Neutrophil count | | | **0.043** |
| ≤4.24 | 54 ( 58.1 ) | 42 ( 42.4 ) | |
| > 4.24 | 39 ( 41.9 ) | 57 ( 57.6 ) | |
| Lymphocyte count | | | 0.773 |
| ≤1.424 | 48 ( 51.6 ) | 48 ( 48.5 ) | |
| > 1.424 | 45 ( 48.4 ) | 51 ( 51.5 ) | |
| Reticulocyte count | | | **< 0.001** |
| ≤0.045 | 65 ( 69.9 ) | 33 ( 33.3 ) | |
| > 0.045、 | 28 ( 30.1 ) | 66 ( 66.7 ) | |

Abbreviations: SII, systemic immune-inflammation index. X Characteristics with *P* values < 0.05 are marked in bold. X represents that information such as tumor size and metastasis cannot be determined.

had no node metastasis, 125 ( 65.1 % ) had no node metastasis.129 (67.2 %) had no distant metastasis, and 54 (28.1 %) had distant metastasis. 44 (22.9 %) of the tumors were stage I-II, 82 (42.7 %) stage III, and 53 (27.6 %) stage IV. The median chemo-cycle of patients was 4 cycles (range: 1–16 cycles).

The relationship between the high IMR group and the low IMR group of 192 patients with SCLC is shown in Table 2. The mortality rate was 74.7 % (74/99) in the high IMR group( > 0.046), and 55.9 % (52/93) in the low IMR group (≤0.046). The number of deaths in the high IRF group was higher than that in the low IRF group, 55.6 % (70/95) and 44.4 % (56/97), respectively. In addition, the high MRF group had fewer deaths than the low MRF group: 48.4 % (61/96) vs 51.6 % (65/96), as shown in the table below (Supplementary Material Table S1). The IMR prognostic factors were significantly correlated with IRF, MRF, and reticulocyte count ($P < 0.001$). In addition ， IMR prognostic factors are related to tumor size, and the high group has significantly more T3-4 cases than the low group 64.7 % (64/99) and 48.4 % (45/93), respectively). Other characteristics (gender, smoking, tumor stage, chemo-cycle) were not significantly different between the high or low-risk groups with different IMR levels ($P > 0.05$).

### 3.2. Univariate and multivariate Cox analyses

Table 3 shows the association between Clinical characteristics variables and OS. Univariate analyses showed significant prognostic factors of poor survival containing smoking history ($P = 0.006$), node metastasis ($P = 0.004$), distant metastasis ($P = 0.005$), tumor stage ($P = 0.002$), treatment ($P = 0.015$), chemo-cycle ($P = 0.014$), IRF ($P = 0.029$), IMR ($P = 0.01$). The factors included in the final multivariate Cox regression analysis were unadjusted closely related to survival and progress in univariate analysis ($P < 0.05$). In multivariate analysis, independent risk factors of poor patient survival consisted of smoking history (HR: 0.553, 95 %, CI: 0.371–0.826, $P = 0.004$) and IMR (HR: 0.330, 95 %, CI: 0.115–0.945, $P = 0.039$). Therefore, the results of our study suggest that IMR is a better independent prognostic predictor for SCLC.

### 3.3. Prognostic indicators (IRF, MRF, IMR) overall survival analysis

Spearman test confirmed that the prognostic indicators IRF, IMR, and SII were positively correlated with the overall survival rate of patients with SCLC. small cell lung cancer. In addition, RET was positively correlated with survival time, while it was negatively correlated with SII (Supplementary Material, Table S2).

The median (4.40, 95.5, 0.046) of IRF, MRF, and IMR were selected as the risk cut-off value to classify patients into the low or high group. We examined the association between IRF, MRF, and IMR with OS of patients with SCLC by performing the Kaplan-Meier survival analysis. We found that patients with the high IRF group and the high IMR group had significantly worse OS rates ($P = 0.032$, Fig. 2A; $P = 0.0096$, Fig. 2C, respectively). We also found that there was no statistically significant difference in OS between the two groups for the prognostic indicator MRF ($P = 0.5$, Fig. 2B). ROC curves were drawn to determine the diagnostic efficiency of prognostic indicators IRF, MRF, and IMR for SCLC (Supplementary Material, Fig. S1 A).

**Table 3**
Univariate and Multivariate Cox analyses of baseline Characteristics and Risk on Survival in SCLC Patients.

| Characteristics | OS | | | | | |
|---|---|---|---|---|---|---|
| | Univariate analysis | | | Multivariate analysis | | |
| | HR (95%CI) | P | Beta | HR (95%CI) | P | Beta |
| Age | 0.768(0.537–1.100) | 0.15 | −0.263 | | | |
| Gender (male vs. female) | 1.461(0.969–2.203) | 0.07 | 0.379 | | | |
| Smoking history | 1.702(1.165–2.486) | **0.006** | −0.532 | 0.553(0.371–0.826) | **0.004** | −0.592 |
| Immature reticulocyte fraction to mature reticulocyte fraction ratio (IMR) | 0.628(0.440–0.896) | **0.01** | −0.466 | 0.330(0.115–0.945) | **0.039** | −1.108 |
| Immature reticulocyte fraction (IRF) | 1.482(1.042–2.107) | **0.029** | −0.393 | 0.566(0.201–1.590) | 0.280 | −0.570 |
| Mature reticulocyte fraction (MRF) | 1.116(0.787–1.583) | 0.539 | 0.110 | | | |
| Chemo-cycle | 0.583(0.380–0.896) | **0.014** | −0.54 | | | |
| Treatment | 1.373(1.064–1.772) | **0.015** | 0.317 | 1.243 (0.951–1.6250 | 0.112 | 0.217 |
| T stage (T1-2 vs T3-4) | 1.272(0.933–1.734) | 0.128 | 0.241 | | | |
| Node metastasis | 1.584(1.157–2.169) | **0.004** | 0.46 | 1.308(0.896–1.909) | 0.165 | 0.268 |
| Distant metastasis | 1.480(1.129–1.940) | **0.005** | 0.392 | 1.131(0.814–1.571) | 0.465 | 0.123 |
| Tumor stage | 1.312(1.107–1.554) | **0.002** | 0.271 | 1.144 (0.968–1.3520 | 0.114 | 0.135 |
| Reticulocyte count | 0.704 (0.495–1.001) | 0.051 | −0.351 | | | |
| Systemic immune-inflammation index | 1.331(0.937–1.891) | 0.110 | 0.286 | | | |
| Sum the betas | | | −0.089 | | | −1.527 |

Abbreviations: SII, systemic immune-inflammation index; IMR, Immature reticulocyte fraction to mature reticulocyte fraction ratio; RET, Reticulocyte count; Characteristics with $P$ values < 0.05 are marked in bold. When the $P$ value was lower than 0.05, the corresponding factor was added to the multivariate analysis, and only the significant factors were listed for the results of the multivariate analysis.

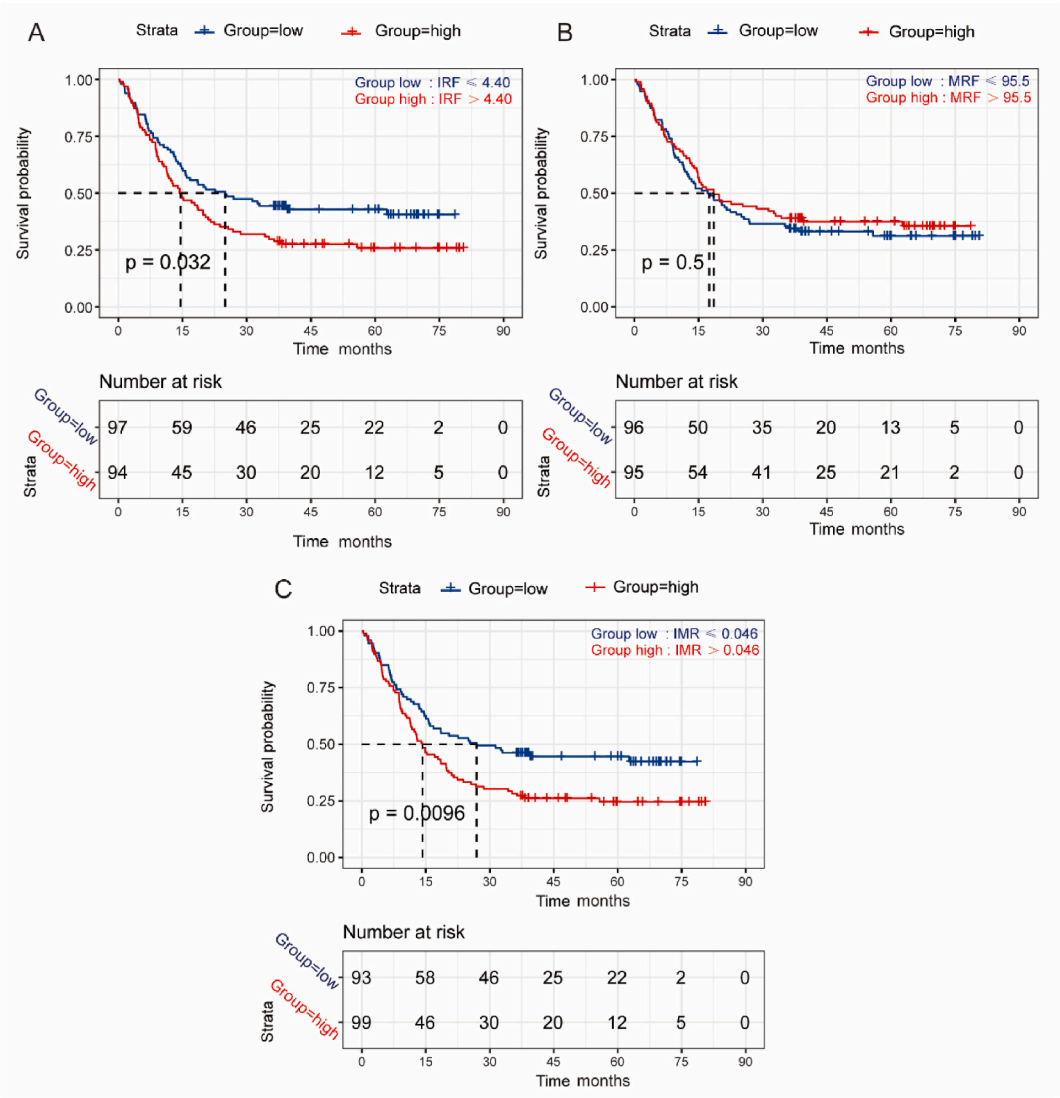


**Fig. 2. Kaplan-Meier curves for overall survival (OS) in patients with SCLC by IRF, MRF, and IMR index** (group-high: **>** median (4.40, 95.55, 0.0460); group-low:≤median (4.40, 95.55, 0.0460). Abbreviations: IMR, Immature reticulocyte fraction to mature reticulocyte fraction ratio; IRF, Immature reticulocyte fraction; MRF, Mature reticulocyte fraction.

### *3.4. Multiple prognostic indexes (IMR、RET、SII) were compared by overall survival analysis*

Further, a comparative analysis was conducted to assess the diagnostic efficiency of the IMR prognostic indicators in comparison with those of previous studies. ROC curves were generated to evaluate the diagnostic performance of prognostic indicators, including RET, SII, LY, and NEUT, in predicting outcomes for SCLC. (Supplementary Material, Fig. S1 B). The median (0.045,612.35) of RET and SII were selected as the risk cut-off value to classify patients into the low and high groups. We examined the association between RET , SII, and TNM staging with OS of patients with SCLC by performing the Kaplan-Meier survival analysis. RET, SII prognostic index there was no significant difference in OS between the two groups ($P > 0.05$ Supplementary Material, Fig. S2 A and B, respectively).

Our results in this study are consistent with previous data that the TNM stage is an important predictor of overall survival in patients with SCLC ($P = 0.00011$, Supplementary Material, Fig. S3 A). This indicates that the staging of the patients we included in this study is accurate. However, we found that the TNM stage was not significantly stratified in patients with stage I-II SCLC ($P = 0.33$, Supplementary Material, Fig. S3 B). We investigated the association between IMR and OS in SCLC patients with stage I-II by Kaplan-Meier survival analysis. It is a pity that IMR failed to show significant prognostic value in patients with stage I-II SCLC (Supplementary Material, Fig. S3 C).

### 3.5. New index combining IMR with RET, SII, and TNM scores

We used the new index generated by combining IMR with RET, SII, and TNM staging scores to analyze the survival and prognosis of SCLC. Patients with IMR, RET, and SII above the median were scored 1 and those below the median were scored 0. One point for I-III stage (limit-stage) and two points for IV (extensive-stage) after excluding patients with indeterminate stage. We performed a C-index analysis to evaluate the discriminatory impact of IMR on OS. TNM stage system scores were found to be significant with C-index (0.579) analysis in OS (Fig. 3A, Table 4). SII scores were found to be no significant with C-index (0.535) analysis in OS (Supplementary Material, Fig. S2 B, Table 4). Surprisingly, the new prognostic index combining IMR with SII and TNM score was significantly correlated with OS, with c index of 0.602 and 0.562, respectively, which improved survival prediction and risk stratification (Fig. 3B; Supplementary Material, Fig. S2 D ， Table 4, respectively). No significant differences were found between prognostic indices RET and OS, with a C-index of 0.544 (Supplementary Material, Fig. S1A, Table 4). The new prognostic index combining IMR with RET score did not improve survival prediction and risk stratification with a C-index of 0.505 (Supplementary Material, Fig. S2 C, Table 4).

### 3.6. New index improves survival prediction and risk stratification in patients with type I-II SCLC

Further analysis of the stratification of patients with stage I-II SCLC was performed to explore the effect of IMR on TNM staging, SII, and RET scores. The new index combined with the RET and IMR scores (C-index is 0.523 and 0.630, respectively) improved survival prediction and risk stratification of patients with stage I-II SCLC, but was not statistically significant ($P = 0.99$, $P = 0.34$, Fig. 4A and C, respectively). However, The new index combined with the SII and IMR scores ， the C-index is 0.614 and 0.630, respectively. It can significantly improve the survival prediction and risk stratification of patients with stage I-II SCLC, with statistical significance ($P < 0.05$, Fig. 4B and D, respectively). It is a pity that the new index combined with IMR and TNM score failed to significantly stratify patients with stage I-II SCLC ($P > 0.05$ ， Supplementary Material, Fig. S3 D).

## 4. Discussion

This retrospective study included 192 patients with SCLC to clarify the prognostic value of baseline IMR in patients with SCLC. The results of our study showed that the high IMR group had higher mortality than the low IMR group (74.7 %, and 55.9 %, respectively), which was positively correlated with the overall survival rate of patients with small cell lung cancer. IMR emerged as an independent prognostic factor for OS in SCLC patients, surpassing IRF in distinguishing outcomes in low and high-risk groups ($P < 0.05$). In addition, combining IMR with TNM staging scores can improve survival prediction and risk stratification. The new index combining the IMR and RET scores significantly improved the stratification of stage I-II small cell lung cancer. This study represents the first report on the prognostic value of IMR in SCLC patients.

In this study, we not only demonstrated that IMR is an independent prognostic factor for OS in SCLC patients, we further compared this indicator with other prognostic indicators: IRF, MRF, RET, SII, and TNM staging system. It was confirmed that IRF and clinical TNM stage system [7] were independent factors affecting the survival time of patients, which was consistent with the results of previous studies. RET and SII [14,34] prognostic parameters did not show significant prognostic value, which is different from the results of other studies, and further studies are needed to confirm this.

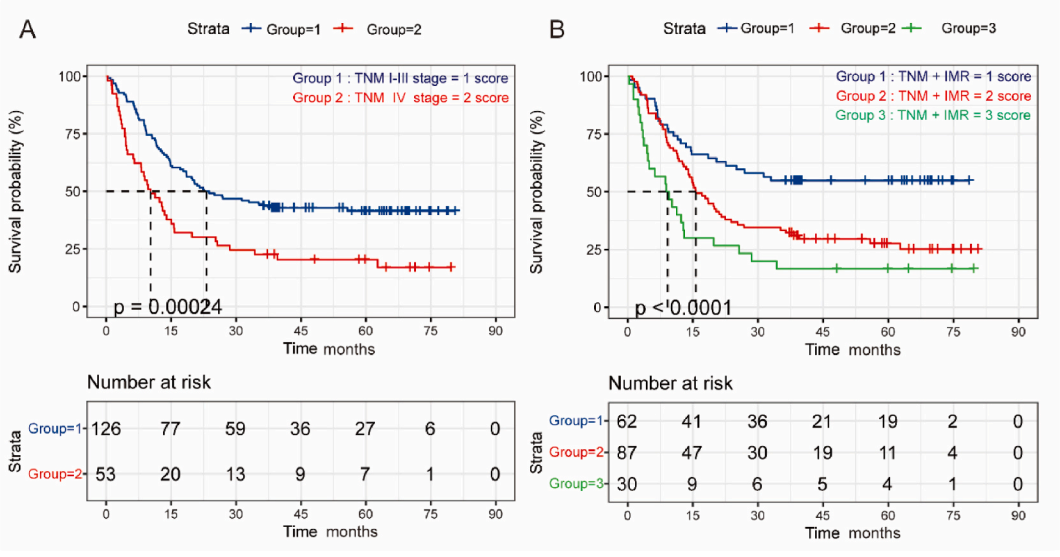


**Fig. 3. Kaplan–Meier plots of overall survival (OS). (A)** TNM stage (group1: I-III stage = 1 score; group 2: IV stage = 2 score);**(B)** The new index combining IMR and TNM stage (group1: TNM + IMR = 1 score; group2: TNM + IMR = 2 score; group 3: TNM + IMR = 3 score).

**Table 4**
C-index for discriminatory values on survival.

| | C-index for OS | C-index for OS (stage I-II SCLC) |
|---|---|---|
| RET | 0.544 | 0.513 |
| RET + IMR | 0.505 | 0.630 |
| SII | 0.535 | 0.614 |
| SII + IMR | 0.562 | 0.630 |
| TNM | 0.579 | 0.559 |
| TNM + IMR | 0.602 | 0.601 |

Abbreviations: IMR, Immature reticulocyte fraction to mature reticulocyte fraction ratio; RET, Reticulocyte count; SII, systemic immune-inflammation index.

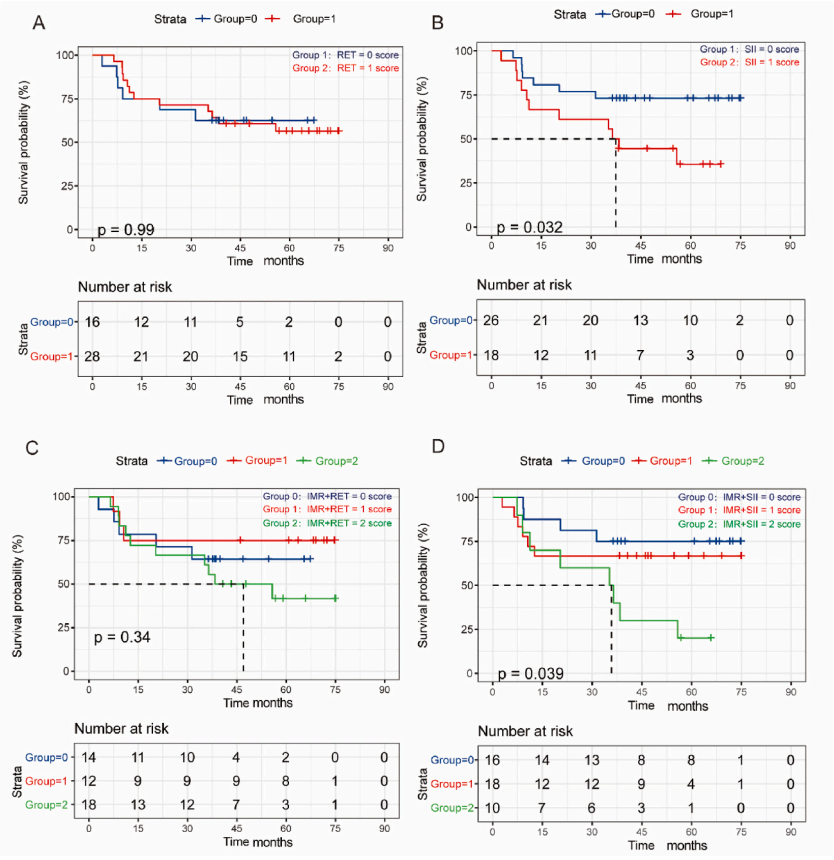


**Fig. 4. Kaplan–Meier plots of overall survival (OS) for stage I-II SCLC patients. (A)** RET index (group 0: RET = 0 score; group 1: RET = 1 score); **(B)** SII index (group 0: SII = 0 score; group 1: SII = 1 score); **(C)** The new index combining IMR and RET; **(D)** The new index combining IMR and SII (group 0: IMR + RET/SII = 0 score; group 1: IMR + RET/SII = 1 score; group 2: IMR + RET/SII = 2 score); A score less than or equal to the median is 0, and a score greater than the median is 1.

We constructed a new index to assess the impact of IMR on these prognostic indicators by combining IMR with the RET, SII, and TNM stage system scores. The new prognostic indices, including IMR combined with the SII score and IMR combined with the TNM score, showed greater predictive power. At the same time, TNM staging system as an independent prognostic factor for SCLC, we found that there was no significant risk stratification in stage I-II patients. It is worth noting that the course of small cell lung cancer progresses rapidly, and early stratification is very valuable for later treatment selection. Therefore, we further used the new index of IMR combined with RET, SII, and TNM scores to analyze patients with stage I-II SCLC. Surprisingly, the prognostic index formed by the combination of SII and IMR was significantly stronger than the original prognostic index in predicting the prognosis of patients with stage I-II small cell lung cancer ($P < 0.05$). The new index combined was able to improve survival prediction and risk stratification in patients with stage I-II SCLC, but it was not statistically significant. As an independent prognostic factor for small cell lung cancer, IMR can not only improve the survival prediction ability and risk stratification of other prognostic indicators but also have unexpected effects in early patients. It is well known that IMR is an off-the-shelf parameter on automated hematology analyzers, does not require

additional cost and effort, and is a simple and economical prognostic factor. Therefore, the prognostic value of IMR in SCLC is worthy of further prospective and multicenter studies to verify.

The IMR prognostic index was the ratio of the immature reticulocyte fraction to the mature reticulocyte fraction. In contrast to the prognostic indicators of SCLC in other studies, the IMR index is derived from blood analysis. It is easier to obtain, can be put into clinical use immediately, does not require long-term verification such as scientific research experiments, and greatly reduces the cost of testing and many other advantages. IRF refers to the proportion of young reticulocytes, which reflects erythropoietic activity and is the first sign of blood recovery [35,36]. The quantitative and morphological characteristics of reticulocytes are associated with the aggressiveness of the tumor and the more severe clinical status of the patient [37]. Although the mechanism by which IMR or RET are associated with cancer patient survival is unclear, one possible explanation is that it is related to the production of red blood cells. Either the disease itself or anemia caused by radiation or chemotherapy. Hypoxia, which can further be induced, enhances the progression and aggressiveness of malignancies, ultimately leading to increased resistance to therapy and poorer long-term prognosis [38–40]. Additional research is needed to explain the chemical and molecular mechanism relating to IMR and cancer patient mortality. IMR as a prognostic factor for SCLC belongs to the exploration of new uses of old parameters. Our research results prove that this kind of application is effective and feasible. In addition, the combination with the parameter can improve the risk prediction ability of other prognostic indicators. It provides more research ideas for the application of more existing blood parameters in cancer. In particular, these classical blood parameters are combined with AI through Clinlabomics methods to provide more information on clinical diagnosis, treatment, and prognosis [41].

However, this study has limitations, including its single-center nature, subjects only before surgery or treatment, and a limited sample size. The retrospective design introduces potential biases in data collection and interpretation. Confirmation of our results in larger, multicenter prospective studies is essential. In conclusion, IMR emerges as a significant predictor of outcome, with high levels correlating with poor survival in SCLC patients.

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## Data availability statement

The raw data for this study have been uploaded to https://github.com/Huaichao2018/Clabomic/blob/main/IMR%20statistics.xlsx.

## Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Sichuan Cancer Hospital & Institute (No. KY-2021-076)). All blood samples were used for this study to meet the no-informed consent application conditions.

## CRediT authorship contribution statement

**Xingmei Zhang:** Writing – review & editing, Writing – original draft, Formal analysis. **Hanxiao Ren:** Writing – review & editing, Formal analysis. **Jiangchuan Tian:** Software, Project administration. **Chaoguo Yang:** Conceptualization. **Huaichao Luo:** Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23830.

## References

[1] K.D. Miller, et al., Cancer treatment and survivorship statistics, 2022, CA A Cancer J. Clin. 72 (5) (2022) 409–436.

[2] X. Zhao, et al., Combined small cell carcinoma of the lung: is it a single entity? J. Thorac. Oncol.: Official Publication of the International Association For the Study of Lung Cancer 13 (2) (2018) 237–245.
[3] A.Y. Deneka, et al., Tumor-targeted drug conjugates as an emerging novel therapeutic approach in small cell lung cancer (SCLC), Cancers 11 (9) (2019) 1297.
[4] J. Qi, et al., The addition of peripheral blood inflammatory indexes to nomogram improves the predictive accuracy of survival in limited-stage small cell lung cancer patients, Front. Oncol. (2021) 4094.
[5] J.U. Lim, H.S. Kang, A narrative review of current and potential prognostic biomarkers for immunotherapy in small-cell lung cancer, Ann. Transl. Med. 9 (9) (2021).
[6] L.A. Byers, C.M. Rudin, Small cell lung cancer: where do we go from here? Cancer 121 (5) (2015) 664–672.
[7] W. Gu, X. Zhong, Efficacy of surgery combined with chemoradiotherapy in treating limited-stage small cell lung cancer and prognosis analysis, J BUON 26 (3) (2021) 812–818.
[8] J.K. Hwang, et al., Validation of the eighth edition TNM lung cancer staging system, J. Thorac. Oncol.: Official Publication of the International Association For the Study of Lung Cancer 15 (4) (2020) 649–654.
[9] R. Tay, et al., Prognostic value of circulating tumor cells in limited-stage small-cell lung cancer: analysis of the concurrent once-daily versus twice-daily radiotherapy (CONVERT) randomized controlled trial, Ann. Oncol. 30 (7) (2019) 1114–1120.
[10] X. Fu, et al., Pd-L1 predicts poor prognosis in surgically resected limited-stage small-cell lung cancer, Cancer Manag. Res. 12 (2020), 10939.
[11] P. Mondelo-Macía, et al., Plasma Cell-free DNA as a Prognostic Biomarker in Small Cell Lung Cancer Patients, medRxiv, 2022.
[12] C. Xu, et al., Serum pleiotrophin as a diagnostic and prognostic marker for small cell lung cancer, J. Cell Mol. Med. 23 (3) (2019) 2077–2082.
[13] F. Najjar, et al., Circulating endothelial cells and microparticles as diagnostic and prognostic biomarkers in small-cell lung cancer, Lung Cancer 124 (2018) 23–30.
[14] Y. Zhou, C. Shen, Y. Sun, Prognostic significance of the systemic immune-inflammation index (SII) in patients with small cell lung cancer: a meta-analysis, Front. Oncol. 12 (2022) 220.
[15] S. Wang, et al., Current diagnosis and management of small-cell lung cancer, in: Mayo Clinic Proceedings, Elsevier, 2019.
[16] T. Adane, F. Asrie, Clinical utility of immature reticulocyte fraction, J Clin Chem Lab Med 4 (9) (2021).
[17] M.L. Grazziutti, et al., Recovery from neutropenia can be predicted by the immature reticulocyte fraction several days before neutrophil recovery in autologous stem cell transplant recipients, Bone Marrow Transplant. 37 (4) (2006) 403–409.
[18] S. Yesmin, et al., Immature reticulocyte fraction as a predictor of bone marrow recovery in children with acute lymphoblastic leukemia on remission induction phase, Bangladesh Med. Res. Counc. Bull. 37 (2) (2011) 57–60.
[19] E. Piva, et al., Clinical utility of reticulocyte parameters, Clin. Lab. Med. 35 (1) (2015) 133–163.
[20] I. Morkis, et al., Assessment of immature platelet fraction and immature reticulocyte fraction as predictors of engraftment after hematopoietic stem cell transplantation, Int. J. Lit. Humanit. 37 (2) (2015) 259–264.
[21] A.K. Gupta, S.B. Kumar, Reticulocytes-Mother of Erythrocytes, 2022.
[22] R.-Z.A. Raja-Sabudin, et al., Immature reticulocyte fraction is an early predictor of bone marrow recovery post-chemotherapy in patients with acute leukemia, Saudi Med. J. 35 (4) (2014) 346–349.
[23] A. Geldard, D. Tobin, A. Cuthbert, Immature reticulocyte fraction as a useful parameter for blood transfusion assessment in anemia, Br. J. Biomed. Sci. 66 (2) (2009) 98–101.
[24] R. Sindhu, S. Behera, D. Mishra, Role of immature reticulocyte fraction in evaluation of aplastic anemia in cases of pancytopenia, Int J Basic Appl Med Sci 5 (2016) 619–624.
[25] D.H. Hufnagel, et al., Prevalence of anemia and compliance with NCCN guidelines for evaluation and treatment of anemia in patients with gynecologic cancer, J. Natl. Compr. Cancer Netw. 19 (5) (2021) 513–520.
[26] S. Vignjević Petrinović, et al., Targeting stress erythropoiesis pathways in cancer, Front. Physiol. 13 (2022), 844042.
[27] M. Gaur, T. Sehgal, Reticulocyte count: a simple test but tricky interpretation!, Pan African Medical Journal 40 (1) (2021).
[28] A.K.P. Ganti, et al., Small cell lung cancer, version 2.2022, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Cancer Netw. 19 (12) (2021) 1441–1464.
[29] F.C. Detterbeck, et al., The eighth edition lung cancer stage classification, Chest 151 (1) (2017) 193–203.
[30] C.M. Rudin, et al., Small-cell lung cancer, Nat. Rev. Dis. Prim. 7 (1) (2021) 3.
[31] H. Luo, et al., Red blood cell distribution width as a predictor of survival in nasal-type, extranodal natural killer/T-cell lymphoma, Oncotarget 8 (54) (2017), 92522.
[32] F.E. Harrell, et al., Evaluating the yield of medical tests, JAMA 247 (18) (1982) 2543–2546.
[33] J. Li, et al., Hiplot: a comprehensive and easy-to-use web service for boosting publication-ready biomedical data visualization, Briefings Bioinf. 23 (4) (2022) bbac261.
[34] D. Wang, et al., The predictive effect of the systemic immune-inflammation index for patients with small-cell lung cancer, Future Oncol. 15 (29) (2019) 3367–3379.
[35] H.P. Ringoringo, et al., Reference range of complete blood count, Ret-He, immature reticulocyte fraction, reticulocyte production index in healthy babies aged 1–4 months, Sci. Rep. 13 (1) (2023) 423.
[36] S.E. Rauf, et al., Immature reticulocyte fraction and absolute neutrophil count as predictor of hemopoietic recovery in patients with acute lymphoblastic leukemia on remission induction chemotherapy, Turk. J. Haematol. : Official Journal of Turkish Society of Haematology 33 (2) (2016) 131–134.
[37] Y. Ragulin, et al., Erythropoiesis reaction to tumor growth in lung cancer patients, Siberian journal of oncology 16 (1) (2017) 53–58.
[38] Y. Kishida, et al., Myelosuppression induced by concurrent chemoradiotherapy as a prognostic factor for patients with locally advanced non-small cell lung cancer, Oncol. Lett. 2 (5) (2011) 949–955.
[39] B.L. Gaspar, P. Sharma, R. Das, Anemia in malignancies: pathogenetic and diagnostic considerations, Hematology 20 (1) (2015) 18–25.
[40] R. Shi, et al., Identification and validation of hypoxia-derived gene signatures to predict clinical outcomes and therapeutic responses in stage I lung adenocarcinoma patients, Theranostics 11 (10) (2021) 5061–5076.
[41] X. Wen, et al., Clinlabomics: leveraging clinical laboratory data by data mining strategies, BMC Bioinf. 23 (1) (2022) 1–20.