INTRODUCTION

Lung cancer, characterized by its high incidence and mortality rates globally, has smoking as one of its primary risk factors. The American Cancer Society predicts that lung cancer will soon become the leading cause of cancer-related deaths among both genders, imposing a considerable impact on society and the economy. Lung cancer is categorized into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC constitutes approximately 80% of lung cancer cases. Despite advancements in screening methodologies and diagnostic technologies, early symptoms of lung cancer often remain inconspicuous. Consequently, the majority of patients with lung cancer are diagnosed at an advanced stage, resulting in a low survival rate (1, 2). In recent years, the elucidation of the relationship between the immune system and cancer has led to significant clinical advancements in immunotherapy. Among the pathways explored, the programmed death ligand 1 (PD-1)/programmed death ligand 1 (PD-L1) pathway has been the subject of extensive research. According to a study, in ovarian cancer, interferon-γ (IFN-γ) secreted by CD8+ T cells within the tumor stroma can elevate the expression of PD-L1 on the surface of tumor cells (3). PD-L1 is expressed on the surface of a wide range of tumor cells. In the absence of immunotherapy, patients with lung cancer and high levels of PD-L1 expression tend to have a poor prognosis, and there is a significant correlation between PD-L1 expression and therapeutic outcomes (4, 5).

Iron is an important element in a variety of biochemical reactions. Compared with normal cells, tumor cells have a high demand for iron, and are vulnerable to iron metabolism disorders, and there is a risk of ferroptosis when intracellular iron is high (6). Existing studies have established that immune cells are susceptible to the phenomenon of ferroptosis. Upon activation, T cells lacking glutathione peroxidase 4 (GPX4) undergo rapid alterations in lipid metabolism, which subsequently precipitates the onset of ferroptosis. In contrast, a deficiency in acyl-CoA synthetase long-chain family member 4 (ACSL4), which sensitizes cells to ferroptosis, acts as a protective mechanism for CD8+ T cells, safeguarding them against the detrimental effects of ferroptosis (7, 8). Therefore, iron metabolism disorders in patients with cancer can affect immune cells, with the compromised activity and function of CD8+ T cells impacting the expression of PD-L1 in tumor cells. This indicates that the iron metabolism levels in patients with cancer may be associated with...
the expression of PD-L1. The aim of this study was to explore the association between iron metabolism indicators (serum iron, transferrin, ferritin) and the expression level of PD-L1 in primary lesions of advanced NSCLC.

MATERIALS AND METHODS

Study participants

This study was conducted with approval from the Ethics Committee of Anqing Medical Center of Anhui Medical University (2021-77). This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

All patients with advanced NSCLC who received their initial diagnosis from October 2022 to July 2023 and were admitted to the Department of Respiratory and Critical Care Medicine at the Anqing Medical Center of Anhui Medical University were initially considered for inclusion. Those who satisfied the enrollment criteria, which included having advanced NSCLC (encompassing stages III and IV) confirmed through imaging and pathology, possessing complete medical records, and being over 18 years of age, were ultimately included in the study.

The exclusion criteria for the study were as follows: patients who had received anti-cancer treatment, individuals diagnosed with blood system diseases, nephritis, nephrotic syndrome, and AIDS, patients who had been treated for immunosuppressive or autoimmune diseases with significant effects on immune cells, individuals with other malignant tumors, patients with a history of hyperthyroidism, hypothyroidism, hypertension, diabetes, chronic gastritis or gastric ulcer, individuals undergoing iron therapy, and patients who, for various reasons, could not undergo PD-L1 expression testing. The specific inclusion process is shown in Fig. 1.

Detection methods

Four to five milliliters of blood were collected from each patient following an 8-hour fasting period. The serum was then separated through centrifugation at 2500 rpm for 5 minutes. Serum levels of iron, transferrin, and ferritin were determined using an automatic biochemical analyzer, with assays using a BIOSTEC reagent (Roche, Basel, Switzerland), and employing electrochemical luminescence methods. Indicator detection of PD-L1 expression: the Dako22C3 reagent antibody, supplied by Lotus Bioscience (Hong Kong, China) was employed for the detection of PD-L1 expression, ensuring the availability of at least 100 viable tumor cells for each assessment. A tumor cell was classified as positive for PD-L1 expression when more than or equal to 1% of its membrane exhibited staining (whether partial or complete), regardless of the staining intensity.

Observation and evaluation of indicators

For patients identified with advanced NSCLC via radiographic imaging and pathology, clinicopathological parameters, including tumor node metastasis (TNM) stage and pathological type, were assessed using chest and abdomen enhanced computed tomography (CT), brain enhanced magnetic

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**Fig. 1.** Flow chart of the patient inclusion process.
resonance imaging (MRI), and cervical lymph node color ultrasound. Before initiating any immunotherapy, fasting serum levels of iron, transferrin, and ferritin, along with the expression of PD-L1 in tumor tissues, were determined.

**Statistical methods**

Data analysis was conducted using SPSS25 statistical software. Quantitative data are presented as mean ± standard deviation (x̄ ± s) for distributions that conformed to normality and as median (P25, P75) for distributions that did not. Comparative analysis between the two groups utilized the independent sample t-test and the two independent sample rank sum test for continuous variables, alongside the chi-squared test for categorical variables in univariate analyses. Correlational assessments were performed using Pearson’s correlation analysis. Additionally, the optimal cut-off values for serum iron and transferrin to predict positive PD-L1 expression were determined using the receiver operating characteristic curve (ROC). A P-value of <0.05 was considered statistically significant.

**RESULTS**

**Analysis of baseline information and general clinical information of patients in the two groups**

There was no significant difference between the two groups in age, gender, smoking history, pathological type, clinical stage, hemoglobin concentration, and C-reactive protein (CRP) (P >0.05). Details are shown in Table 1.

**Correlation between tumor PD-L1 expression and iron metabolism indicators**

1. **Analysis of peripheral serum iron, transferrin, and ferritin levels in patients in the two groups**

Compared to PD-L1-negative patients, those who were PD-L1-positive exhibited significantly lower levels of peripheral serum iron and transferrin, with the differences being statistically significant (P<0.05). However, no significant difference was observed in ferritin levels between the two groups (P>0.05). Further details can be found in Table 2 and Fig. 2.

2. **Correlation between iron metabolism indicators and PD-L1 expression in PD-L1 positive patients**

Pearson’s correlation analysis revealed that, in patients with positive PD-L1 expression, the level of PD-L1 expression exhibited a negative correlation with serum iron and transferrin (r = -0.465, P=0.003; r = -0.447, P=0.005), and a positive correlation with ferritin (r=0.393, P=0.015). Additional details are provided in Table 3 and Fig. 3.

3. **Comparison of the predictive values of selected parameters on PD-L1 expression in patients with advanced non-small cell lung cancer**

Upon analysis, the area under the curve (AUC) values for serum iron and transferrin in patients with positive PD-L1 expression were determined to be 0.664 and 0.661, respectively, with corresponding P-values of 0.03 and 0.034. Both P-values fell below 0.05, signifying a statistically significant difference in the levels of serum iron and transferrin among patients with positive PD-L1 expression. Further details are provided in Table 4.

The optimal cut-off value for serum iron to predict positive PD-L1 expression was identified as 11.15 μmol/L, achieving a sensitivity of 83.3% and a specificity of 47.4%. For transferrin, the optimal cut-off value was established at 164.33 mg/dl, with a sensitivity of 87.5% and a specificity of 50%. Additional details can be found in Fig. 4.

**DISCUSSION**

With the approval of immunosuppressant drugs as first-line and second-line treatments for advanced NSCLC, PD-1/PD-L1-based immunotherapy has expanded the treatment alternatives available to these patients. The fundamental mechanism of this immunotherapy is focused on obstructing the interaction...
between PD-L1, present on the surface of tumor cells, and PD-1, located on the surface of T cells. This blockade effectively diminishes T cell exhaustion and necrosis, while also curtailing the immune evasion abilities of tumors (9). Nevertheless, the considerable expense associated with PD-L1 testing and the extended duration required to obtain results necessitate an indicator for predicting PD-L1 expression. Currently, research has been conducted on the relationship between PD-L1 expression and metabolic parameters from 18FDG PET/CT scans (10, 11); however, the complexity of this procedure and patient reluctance to undergo such testing limit its widespread applicability. To alleviate the financial strain on patients and to approximate the expression of PD-L1 effectively, identifying a low-cost and highly efficacious biomarker is essential.

According to research findings, there is a close relationship between iron metabolism and the development of tumors (6). Iron is a crucial element for vital biological processes. In 2012, Stockwell introduced a new cellular death mechanism termed ‘ferroptosis’ (12). This unique form of cell death is primarily

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum iron (μmol/L)</th>
<th>Transferrin (mg/dl)</th>
<th>Ferritin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1(−) (n=24)</td>
<td>14.53±4.93</td>
<td>183.53±29.54</td>
<td>287.83±146.97</td>
</tr>
<tr>
<td>PD-L1(+) (n=38)</td>
<td>11.67±3.90</td>
<td>165.94±34.99</td>
<td>311.76±121.71</td>
</tr>
<tr>
<td>t</td>
<td>-2.53</td>
<td>-2.04</td>
<td>0.696</td>
</tr>
<tr>
<td>P</td>
<td>0.014</td>
<td>0.045</td>
<td>0.489</td>
</tr>
</tbody>
</table>

Table 2. Comparison of iron metabolism indicators between PD-L1-negative and PD-L1-positive patients.

Table 3. Correlation between iron metabolism indicators and PD-L1 expression level in PD-L1-positive patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PD-L1 expression level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (μmol/L)</td>
<td>-0.465</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>-0.447</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>0.393</td>
</tr>
</tbody>
</table>

Fig. 2. Comparison of serum iron and transferrin between PD-L1 (+) and PD-L1 (−).
triggered by iron-dependent lipid peroxidation, leading to distinctive changes in cell morphology. These changes encompass increased mitochondrial membrane density, diminished or absent mitochondrial cristae, and integrity of the nuclear structure. The core characteristics of ferroptosis are centered around disturbances in iron metabolism, amino acid

Fig. 3. The relationship between iron metabolism indexes and PD-L1 expression in PD-L1 positive patients. (A): Correlation analysis of serum iron and PD-L1 expression in PD-L1 positive patients ($r = -0.465$, $P=0.003$); (B): Correlation analysis between transferrin and PD-L1 expression in PD-L1 positive patients ($r = -0.447$, $P=0.005$); (C): Correlation analysis between ferritin and PD-L1 expression in PD-L1 positive patients ($r=0.393$, $P=0.015$).
metabolism, and lipid metabolism (12). Iron, transferrin, the transferrin receptor, and ferritin play pivotal roles in mediating iron metabolism. Alterations in the levels of iron metabolism are associated with ferroptosis. Transferrin, the protein responsible for transporting serum iron within the body, binds to peripheral serum iron and enters human cells via transferrin receptors, contributing to critical biological metabolic activities (6). The upregulation of PD-L1 expression within tumor cells may be influenced by disruptions in iron metabolism among patients with malignancies, thereby potentially influencing T-cell activation and functionality. Through an examination of the relationship between indicators of iron metabolism and varying levels of PD-L1 expression, our analysis revealed that patients exhibiting positive PD-L1 expression exhibited diminished levels of peripheral serum iron and transferrin compared to those demonstrating negative PD-L1 expression. Importantly, these observed distinctions bore statistical significance. Utilizing ROC curve analysis, the optimal threshold values for serum iron and transferrin in predicting positive PD-L1 expression were determined to be 11.15 μmol/L and 164.33 mg/dl, respectively. Correspondingly, the sensitivity and specificity associated with these thresholds were found to be 83.3% and 47.4% for serum iron, and 87.5% and 50% for transferrin. These findings suggest that serum iron and transferrin possess discernible reference thresholds for predicting PD-L1 expression; however, it is noteworthy that the specificity associated with these indicators is not particularly high.

Tumor cells exhibit a pronounced requirement for iron due to their heightened metabolic demands. Paradoxically, their elevated reliance on iron, coupled with the generation of substantial lipid reactive oxygen species (ROS), renders tumor cells notably susceptible to ferroptosis (12). Previous research has demonstrated that inhibiting the biosynthetic enzymes responsible for iron-sulfur cluster formation can prompt tumor cells to accumulate significant quantities of iron, consequently triggering ferroptosis in these cells. Additionally, the ferroptosis inducer ‘Erastin’ has been observed to elevate intracellular levels of ROS, thus impeding tumor cell proliferation (13, 14). At present, the main focus of ferroptosis research is chemical therapy, drug to drug combination, and the synergistic effect of drugs with certain therapies. Studies have shown that natural compounds used in combination with traditional lung cancer treatments can combat the resistance of different cells to treatment or chemotherapy. The combination of iron death inducer and traditional lung cancer treatment can overcome cancer resistance and improve the therapeutic effect (15). Another study showed that cells can regulate autophagy through the expression of genes that make them resistant to lung cancer drugs (16). Ferroptosis is a highly iron-dependent cell death mode, and enhancing cell sensitivity to iron death by regulating intracellular iron levels may be a potential target for future lung cancer drug therapy. Consequently, investigating the mechanism underlying ferroptosis and its clinical implications in the context of lung cancer holds promise for improving patient outcomes. Furthermore, a negative correlation between serum iron and transferrin levels and PD-L1 expression was observed in patients exhibiting positive PD-L1 expression. This suggests that tumor cells with heightened PD-L1 expression tend to exhibit

### Table 4. Efficacy of each parameter in predicting PD-L1 expression.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum iron</th>
<th>Transferrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>0.664</td>
<td>0.661</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.527–0.802</td>
<td>0.524–0.799</td>
</tr>
<tr>
<td>SE</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>P</td>
<td>0.03</td>
<td>0.034</td>
</tr>
<tr>
<td>Threshold</td>
<td>11.15</td>
<td>164.33</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>83.3%</td>
<td>87.5%</td>
</tr>
<tr>
<td>Specificity</td>
<td>47.4%</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Fig. 4.** ROC curve analysis of the predictive ability of serum iron and transferrin for PD-L1 expression in advanced NSCLC.
increased iron consumption, thereby implying a greater reliance on iron and a heightened susceptibility to ferroptosis. Ferritin serves as the cellular reservoir for iron storage, and a discernible positive correlation has been observed between ferritin levels and PD-L1 expression. This suggests that tumor cells exhibiting elevated PD-L1 expression necessitate increased iron storage for metabolic processes. Such insights may prove valuable in guiding future investigations aimed at elucidating strategies to induce tumor cell death through iron-mediated mechanisms.

This study furnishes a straightforward, cost-effective, and readily accessible biomarker for predicting PD-L1 expression in advanced NSCLC. Through the assessment of serum iron, transferrin, and ferritin levels, a preliminary estimation of PD-L1 expression can be attained, thereby offering practical insights for the clinical utilization of immunosuppressive agents. Several limitations are evident in this study. Firstly, the sample size is modest, necessitating additional prospective studies to corroborate the present findings effectively. Secondly, the dynamic and spatial heterogeneity of PD-L1 expression within tumor tissue introduces potential inaccuracies (17), thus underscoring the need for extensive large-scale, multicenter studies to validate these findings comprehensively.

In summary, the levels of serum iron and transferrin in patients with advanced NSCLC offer valuable insights for predicting PD-L1 expression. Moreover, a discernible correlation exists between iron metabolism indicators and PD-L1 expression, particularly evident in PD-L1 positive patients. Furthermore, heightened PD-L1 expression corresponds to increased iron consumption, implying greater iron dependency and a heightened susceptibility for inducing ferroptosis in cells. These findings underscore the potential utility of ferroptosis-based anticancer therapies for patients with lung cancer, thus paving the way for future research endeavors in this field.

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