The Addition of Ferritin Enhanced the Prognostic Value of International Prognostic Index in Diffuse Large B-Cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is a highly heterogeneous non-Hodgkin lymphoma, and the prognosis of DLBCL patients is widely affected by multivariables. Clinical-factors-based prognostic systems stratify the prognosis of DLBCL with certain limitations, and the value of ferritin on the prognosis of DLBCL is unclear. In this study, 225 cases were retrieved from 4 centers of Huaihai Lymphoma Working Group (HHLWG) as the derivation cohort, and 66 cases were from the other 6 centers of HHLWG as external validation cohort. X-Tile program divided ferritin into three groups when applying 175.00 and 391.90 mg/L as the optimal cutoff points. Based on multivariable analysis, ferritin appeared to be a stronger predictor. A total of three variables (ferritin, age, and lactate dehydrogenase) were included for the development of the nomogram. The C-indexes were 0.73 and 0.70 in the derivation and validation cohort, and the calibration curve showed the consistency between the nomogram prediction and the actual observation. In conclusion, Ferritin-based nomogram enhanced the prognostic value of IPI in DLBCL.

Keywords: ferritin, DLBCL, International Prognostic Index, prognosis, nomogram
INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL) with high heterogeneity (1, 2). Cell origin, gene-based molecular subtype of NHL with high Diffuse large B-cell lymphoma (DLBCL) is the most common INTRODUCTION

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determine the optimal threshold, and a nomogram was
calculated with bootstrap method according to the regression results, and validation was carried out in parallel (21). The number of self-sampling B was 500. The closer the concordance index (C-index) to 1, the better the prediction performance. Statistical analysis was conducted with SPSS software [version 19.0 (IBM, NY, USA)] and R software (version 4.2.0; http://www.Rproject.org).
RESULTS

Clinical Characteristics
The characteristics of the patients are detailed in Table 1. The follow-up deadline was July 15, 2021, and the median follow-up time was 36.7 months in derivation cohort, and 23.1 months in validation cohort. At the end of follow-up, a total of 119 (40.90%) deaths occurred. The median age at diagnosis was 62 years, with 159 (54.60%) male and 168 (57.70%) patients were older than 60 years. Ann Arbor stage III/IV accounted for 66.60%.

The Cutoff Points of Ferritin and Other Continuous Variables Calculated by X-Tile
Based on the X-Tile program, the maximum chi-squared points 22.13 and 25.39 were reached when applying 175.00 and 391.90 mg/L as the optimal cutoff points (p < 0.0001, Figure 1). Therefore, the DLBCL patients were divided into three subgroups for further analyses by using these cutoff points. Similarly, the optimal cutoff points for age, Alb, WBC, HGB, PLT, and LDH were 75 years, 40.30 g/L, 8 × 10^9/L, 97 g/L, 205.50 × 10^9/L, and 248 U/L.

Prognostic Value of Ferritin With Clinicopathological Patterns in DLBCL
Mann–Whitney U-test was conducted for exploring continuous ferritin values on different subtypes, and the results suggested that ferritin levels between Ann Arbor I/II, III/IV and IPI LR/LIR, HIR/HR groups were significantly different (p < 0.01), but no significant difference was found in BCL-2, BCL-6, and CD5 groups.

In the whole cohort, patients with high ferritin level (>391.90 μg/L) had poor survival with 5-year OS of only 28.2% (Figure 2A). Using cutoff values of 50% positive tumor cells for BCL-2 and BCL-6, 92 (31.60%) cases were positive for BCL-2, and 93 (31.90%) cases were positive for BCL-6. Thirty-six (12.4%) patients were with high Ki-67 score (≥0.9), and 81 (27.80%) were non-GCB. Kaplan–Meier analysis found that levels of ferritin could not re-stratify BCL-2, CD5, and Eastern Cooperative Oncology Group (ECOG) scores (≥2), and Ann Arbor Stage (I/II) (p > 0.05). However, the levels of ferritin could re-stratify the prognosis in BCL-2+, BCL-6+, CD5+, cell of origin, ECOG score (<2), Ann Arbor Stage (III/VI), and IPI (p < 0.05, Figure 2).

Univariable and Multivariable Analysis of DLBCL Patients
The effects of different clinical variables on OS were analyzed by univariable and multivariable analyses, and the results showed that the level of ferritin appeared to be a stronger predictor. Univariable analysis exhibited that Alb, HGB, PLT, and age were prognostic predictors (p < 0.05, Table 2). Following the model iterations in multivariable analysis, the final prognostic index consisted of three factors, as shown in Table 2. Ferritin was proved to be an adverse factor for the survival of DLBCL.
patients. Nevertheless, WBC in the current multivariable model was observed to be not predictive \( p = 0.07, \text{HR} = 1.52, 95\% \text{CI} (0.97–2.37) \).

### Development of Ferritin-Based Prognostic Nomogram and Validation

Based on multivariable analysis, a prognostic nomogram was developed to predict 1-, 3-, and 5-year OS of DLBCL patients (Figure 3), and the Harrell’s concordance index and brier score (C-index = 0.73, Brier score = 0.17) were calculated between the predicted and the real outcome of the model for internal validation.

We further validated this nomogram externally by the calibration curve and computed the C-index and Brier score in an independent validation cohort of 66 patients (C-index = 0.70, Brier score = 0.22) in the external validation. The calibration

### TABLE 2 | Prognostic factors of OS in the derivation cohort.

| Variables             | Univariable analysis |          |          |          |          |          | Muivariable analysis |          |          |
|-----------------------|----------------------|----------|----------|----------|----------|----------|----------------------|----------|----------|
| Fer                   | HR                   | 95% CI   | p        | Fer                   | HR                   | 95% CI   | p        |
| <175                  | 1                    |          |          | <175                  | 1                    |          |          |
| 175–391.9             | 3.77                 | 2.23–6.22| <0.01    | 175–391.9             | 2.05                 | 1.17–3.59| <0.01    |
| >391.9                | 2.05                 | 1.19–3.53| <0.01    | >391.9                | 2.89                 | 1.68–4.96| <0.01    |
| LDH                   | 3.56                 | 2.24–5.65| <0.01    | Age                   | 2.68                 | 1.63–4.43| <0.01    |
| Alb                   | 0.38                 | 0.22–0.65| <0.01    | <75                   | 2.14                 | 1.28–3.57| <0.01    |
| HGB                   | 0.48                 | 0.32–0.73| <0.01    | ≥75                   | 1.50                 | 0.89–2.53| 0.13     |
| Ann Arbor Stage       | 2.31                 | 1.43–3.72| <0.01    | WBC                   | 1.50                 | 0.89–2.53| 0.13     |
| Age                   | 2.12                 | 1.32–3.42| <0.01    | ≥248                  | 3.03                 | 1.69–5.43| <0.01    |
| WBC                   | 1.92                 | 1.25–2.94| <0.01    | <8                    | 1.52                 | 0.97–2.37| 0.07     |
| ECOG                  | 2.54                 | 1.28–5.14| 0.01     | ≥8                    | 1.50                 | 0.89–2.53| 0.13     |
| PLT                   | 0.64                 | 0.43–0.96| 0.03     | Ann Arbor Stage       | 1.50                 | 0.89–2.53| 0.13     |
| Ki-67                 | 3.83                 | 0.76–19.24| 0.10    | II/III                | 1.50                 | 0.89–2.53| 0.13     |
| BCL-2                 | 0.67                 | 0.32–1.39| 0.28     |                      |                      |          |          |
| Gender                | 0.83                 | 0.55–1.25| 0.37     |                      |                      |          |          |
| COO                   | 0.92                 | 0.58–1.47| 0.73     |                      |                      |          |          |

Fer, ferritin; Alb, albumin; LDH, lactate dehydrogenase; HGB, hemoglobin; PLT, platelet; WBC, white blood cell count; COO, cell of origin.
curves were close to the ideal curves, suggesting that the predicted result and the actual outcome had a good consistency (Figure 4).

Comparison of the Current Nomogram With IPI

In this study, all cases had complete data for all the variables required to calculate the IPI score. We analyzed the effect of ferritin levels on the prognosis in different IPI risk groups, and Kaplan–Meier analysis results are shown in Figure 5. Abnormal ferritin level was an adverse factor for patients in LIR/LR and HIR/HR groups in global comparisons ($p <0.05$, Figure 5). The 5-year OS for patients in different levels of ferritin in the LIR/LR group were 80.50%, 63.10%, and 51.20%, respectively (Figure 5A), and the 3-year OS in the HIR/HR group were
57.90%, 46.50%, and 21.50%, respectively (Figure 5B). Compared with IPI, the nomogram showed better accuracy in predicting survival of patients in both groups.

**DISCUSSION**

In this study, we assessed the level of ferritin to evaluate its prognostic value on DLBCL. Our research showed that patients with high level of ferritin had a poor prognosis. Based on model iteration, we established a prognosis nomogram for DLBCL patients, and the relative importance of the predictors could be determined by the length of the lines in the nomogram (23).

Nutritional markers affect the prognosis and survival of cancer patients. Indices such as the prognostic nutritional index (PNI) (24), albumin, BMI, and obesity have been well studied, and there is considerable evidence for their prognostic effects (25–29). Ferritin, an important indicator of metabolic level and nutrition, is associated with prognosis in end-stage liver disease, which can satisfactorily predict 11- and 90-day mortality (30).

A previous study in South Korea revealed that age, obesity, drinking habits, and glucose levels were significantly associated with women’s serum ferritin levels (31). Ferritin is detected at higher levels in the sera of many cancer patients, and the higher levels relate to aggressive disease and poor clinical outcome (32). In this study, we evaluated ferritin level using X-Tile program to seek more accurate cutoff point, and the results showed that the maximum chi-squared points of 22.13 and 25.39 were reached when applying 175.00 and 391.90 mg/L as the optimal cutoff points. Univariate Cox analysis showed that ferritin was a strong prognostic predictor of DLBCL. Elevated ferritin level (Fer ≥ 391.90 μg/L) was significantly correlated with prognosis. In addition, we used X-Tile program rather than the usual criteria to divide the age into two groups to achieve precise stratification. Multivariable analysis showed that when patients ≥75 years, the 3-year OS was only 28.10%.

R-CHOP plus X regiments failed to improve OS in those patients with MYC and BCL-2/BCL-6 double expression/hit, activated B cells, and CD5-positive subtype. Previous studies have shown that different pathological immunophenotypes have an impact on outcomes (33, 34). In this study, ferritin level could re-stratify patients of CD5-positive group, and high levels of ferritin had a poor prognosis in both negative and positive BCL-6 groups (p < 0.05). High levels of ferritin in the GCB group also had a significant impact on the prognosis, but accurate stratification of ferritin in prognosis was not achieved in CD5- and BCL-2-negative groups.

The nomogram model demonstrated in this study included three variables: ferritin, age, and LDH. The specific DLBCL prognostic nomogram aimed to estimate the probability of 1-, 3-, and 5-year OS based on multivariable Cox proportional hazard models. Bootstrap resampling, C-index, and calibration curve were used to validate the model. The C-index was 0.73 between the predicted outcome and the real outcome of the model. The predicting clinical factors of the nomogram and original IPI were similar, with the former applying a refined categorization of age by X-Tile program. The C-index of the nomogram was higher than that of IPI, demonstrating a more obvious advantage than IPI. In conclusion, the addition of ferritin enhanced IPI for the prognosis of DLBCL. However, due to the inherent flaw of the retrospective design, further prospective studies need to be explored.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the independent Ethics Committees of each center.
in HHLWG and met Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

WS and SH: designed this study. ZS and CH: analysis and interpretation. SZ, MZ, LH, QS, DY, JY, HZ, WG, YM, QL, CO, JZ, CW, and TZ: acquisition of data. CO provided the advices of this study. All authors contributed to the article and approved the submitted version.

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