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# Expression and prognostic significance of CD66b in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma in the world. It exhibits high heterogeneity and invasiveness and is prone to developing treatment resistance. Therefore, there is an urgent need for good prognostic evaluation indicators and therapeutic targets. In recent years, immunotherapy has become a research hotspot for DLBCL. Tumor-associated neutrophil (TAN) is widely expressed in various tumors and is an important component of the immune microenvironment. However, there have been few studies on the role of TAN in DLBCL. This study has demonstrated that CD66b, which is a marker of TAN, is a good prognostic marker of DLBCL and its expression is related to the prognosis of DLBCL patients. The expression level of CD66b is also closely correlated with the objective response rate of the R-CHOP treatment regimen in DLBCL patients with non-GCB subtype. The expression level of CD66b has a high reference value for the determination of the treatment plan. The combined detection of CD66b and PD-L1/PD-L2 is of significance to predict the prognosis of DLBCL patients.

*Key words: diffuse large B-cell lymphoma; tumor-associated neutrophil; CD66b; immune microenvironment; immunotherapy*

Diffuse large B-cell lymphoma (DLBCL) is a common hematological malignancy in adults, accounting for about 30% of new cases of non-Hodgkin lymphoma each year [1]. The characteristic of DLBCL is its strong heterogeneity and invasiveness. DLBCL is mainly divided into two subtypes: germinal center B cell-like (GCB) and activated B cell-like (ABC) [2]. Currently, R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) is the first-line treatment regimen for DLBCL, which can lead to remission in 70–80% of patients. However, nearly 40% of patients still fail to achieve complete remission or experience recurrence after treatment [3].

To solve the problem of resistance to the R-CHOP regimen, currently in clinical practice, second- and third-line treatment regimens are mostly adopted, including epigenetic therapy, targeted therapy, immunotherapy, chimeric antigen receptor T-cell immunotherapy (CAR-T), immune checkpoint inhibitors, Bcl-2 inhibitors, Bruton's tyrosine kinase (BTK) inhibitors, etc. These new treatment regimens have achieved good therapeutic effects [4, 5].

The tumor microenvironment (TME) is an important factor that mediates the occurrence and development of tumors. In different types of tumors, there are significant differences in the immune microenvironment, and the expression patterns of immune cells also vary [6]. Studies have shown that in numerous tumors, including hematological tumors, immune cells are present in the TME and play a crucial regulatory role [7]. The infiltration level of immune cells such as $CD163^+$ TAMs and $FoxP3^+$ Tregs is closely related to the classification, differentiation, and prognosis of tumors [8]. At present, immunotherapy for tumors is receiving increasing attention and is emerging as a promising target for tumor treatment. The TME plays a very important regulatory role in the occurrence and development of DLBCL. In-depth study of the TME of DLBCL is of great significance for exploring its pathogenesis and researching treatment strategies [9].

Neutrophils are the most abundant circulating leukocytes and immune and inflammatory cells infiltrating the TME. It plays a crucial role in tumor development [10]. As an impor-

tant component of the immune microenvironment, tumor-associated neutrophils (TAN) play a crucial regulatory role in the occurrence, development, and immune evasion processes of tumors [11, 12]. Under the influence of molecules such as CEACAM1, TAN migrates to the tumor [13]. TAN can be divided into two phenotypes, N1 and N2, which respectively play the roles of inhibiting and promoting tumor progression [14, 15]. Relevant studies have shown that these two phenotypes of TAN may respectively exert their effects in the early and late stages of the tumor. In the stimulation of cytokines such as TGF-β and IL-8, the two phenotypes can transform into each other. Tumor cells can also act on TAN and alter its differentiation, thereby promoting the growth of the tumor [16]. CD11b, CD16, and CD66b are all markers of mature neutrophils, while CD66b is usually used to identify neutrophils in cancer tissues [15]. Meanwhile, the infiltration of $CD66b^+$ TAN is closely related to the expression of numerous molecules in the tumor, such as EFHD1 and SCARB2 [10]. Studies have also indicated that, as an important immune cell in the TME, the expression of tumor-infiltrating neutrophils is closely related to immune checkpoints, such as LAG3, PD-1, and PD-L1, thereby affecting patient survival. Conducting a combined analysis also helps to make a comprehensive judgment on the progression of the tumor and the prognosis of the patients [17, 18].

CD66b has been demonstrated to be expressed in non-Hodgkin lymphoma. However, there have been few studies on the role of TAN in DLBCL at present [19]. In this article, we investigated the relationship between TAN and the prognosis, clinical indicators of DLBCL patients, and expounded its significance in DLBCL.

## Patients and methods

Patients and specimens. A total of 168 cases of DLBCL, diagnosed between January 2012 and December 2018 at the Affiliated Tumor Hospital of Nantong University, were included in this study. Slides of all cases were reviewed according to the 5th edition of the World Health Organization (WHO) diagnostic criteria.

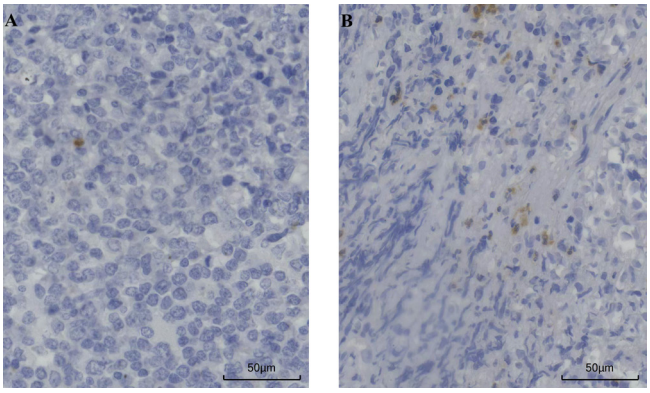


**Figure 1. Representative images of the immunohistochemistry results of CD66b. A) CD66b Low; B) CD66b High.**

The inclusion criteria for this study were as follows: 1) The patients were newly diagnosed DLBCL patients who had not received any treatment; 2) The patients had complete clinical and pathological data. The exclusion criteria for this study were: 1) The patients had other malignant tumors; 2) The patients had severe organ dysfunction, such as severe liver and kidney failure or respiratory failure. Taking the date of patient diagnosis as the starting point of the study, and the patient's death or the termination of follow-up as the end point of the study (Supplementary Figure S1). There were no cases of patient dropout. This work was approved by the ethics committee of Affiliated Tumor Hospital of Nantong University (Approval number: 2023-044) and adhered to the Declaration of Helsinki.

**Immunohistochemistry and assessment of immunohistochemistry.** Immunohistochemistry was carried out as described previously [20]. Briefly, sections were deparaffinized in xylene and rehydrated through descending percentages of ethanol to water. Antigen retrieval, elimination of the endogenous peroxidase activity, and blocking were carried out according to standard protocols. The sections were incubated with primary antibodies at 4°C overnight. Primary antibodies used include: 1:2,000 diluted CD66b (BioLegend, #305102) and 1:50 diluted MPO (Fuzhou Maixin Biotech Co., Ltd, #RAB-0379). Following the incubation, the sections were washed with PBS 3 times, and then incubated with HRP-conjugated secondary antibody (Proteintech Group, #PK10006) for 30 mins. Antigen-antibody interaction was visualized using the chromogenic substrate 3,3' diaminobenzidine (DAB) substrate (Proteintech Group, #PK10006), and the sections were lightly counterstained with hematoxylin.

The number of positively stained cells in the paraffin section was counted, and the number of positively stained cells per square millimeter of the tissue was calculated based on the area of the tissue. The optimal cut-off points of CD66b were calculated using X-tile software (Yale University). Under these conditions, samples with a number of positively stained cells per square millimeter of tissue ≤3.18 and >3.18 were classified as low and high expression of CD66b, respectively.

**Statistical analysis.** The associations between CD66b expression and clinicopathologic parameters were evaluated by Pearson's chi-square test or Fisher's exact test. The probability of differences in overall survival (OS) was ascertained by the Kaplan-Meier method, with a log-rank test for significance. The reverse Kaplan-Meier method was used to evaluate the censored data pattern and the median follow-up time. Statistical analysis was performed using SPSS 23.0 and GraphPad Prism 10 software.

## Results

**Expression of CD66b in DLBCL.** Expression analysis of CD66b was conducted with primary tumors from 168 DLBCL patients by immunohistochemistry. Among the positive tissues, CD66b was found in the membrane and in

the cytoplasm. CD66b was highly expressed in 45 patients and lowly expressed in 123 cases. Among DLBCL patients with the GCB subtype, CD66b was highly expressed in 11 patients and lowly expressed in 29 patients. Among DLBCL patients with the non-GCB subtype, CD66b was highly expressed in 31 patients and lowly expressed in 87 patients (Figure 1). MPO staining corresponding to high CD66b expression is presented in Supplementary Figure S2.

**Relationship between expression of CD66b and clinicopathological characteristics.** As shown in Table 1, there was no significant correlation between the expression of CD66b and the clinicopathological parameters, including age, gender, Hans classification, B symptom, Ki-67 expression, extranodal invasion, ECOG PS score, IPI score, and serum LDH level (all p>0.05). Further analysis showed that in DLBCL patients with the non-GCB subtype, CD66b expression status was correlated with Ki-67 expression (p=0.0167), while there was no correlation between these two indicators in patients with the GCB subtype (Tables 2, 3). Taking age into account, among patients over 60, CD66b expression status was correlated with IPI score (p=0.0032); among patients under 60, CD66b expression status was correlated with Ki-67 expression (p=0.0070; Tables 4, 5).

**Prognostic significance of CD66b expression in DLBCL.** Kaplan-Meier survival analysis showed that OS was significantly prolonged in patients with high CD66b expression (p=0.0015; Figure 2A). The median follow-up time for patients with high CD66b expression and those with low expression was 88 months and 97 months, respectively (p=0.319).

Taking the subtype of disease into account, it was found that the expression of CD66b in patients with the GCB subtype was related to the prognosis (p=0.0194), while in patients with the non-GCB subtype, the expression of CD66b was not related to the prognosis of the patients (p=0.0935; Figures 2B, 2C).

Taking age into account, among patients over 60, CD66b expression status was correlated with the patient's prognosis (p<0.001); among patients under 60, CD66b expression status was not correlated with their prognosis (p=0.4013; Figures 2D, 2E).

Cox analysis results suggested that age (p<0.001), Hans classification (p=0.018), B symptoms (p=0.004), extranodal invasion (p=0.020), ECOG PS score (p<0.001), IPI score (p<0.001), serum LDH level (p<0.001) and CD66b expression (p=0.003) were correlated with OS. Statistically significant variables in the Cox univariate analysis were included

**Table 1. Relationship between CD66b expression levels and clinicopathological features of 168 patients with DLBCL.**

| Clinicopathological parameter | n | CD66b | | p-value |
|---|---|---|---|---|
| | | Low | High | |
| Age | | | | 0.1961 |
| <60 | 50 | 40 | 10 | |
| ≥60 | 118 | 83 | 35 | |
| Gender | | | | 0.9950 |
| male | 71 | 52 | 19 | |
| female | 97 | 71 | 26 | |
| Hans classification[a] | | | | 0.8792 |
| GCB | 40 | 29 | 11 | |
| non-GCB | 118 | 87 | 31 | |
| B symptom | | | | 0.3920 |
| absence | 147 | 106 | 41 | |
| exist | 21 | 17 | 4 | |
| Ki-67[b] | | | | 0.0728 |
| <75% | 60 | 39 | 21 | |
| ≥75% | 104 | 81 | 23 | |
| Extranodal invasion | | | | 0.4998 |
| 0–1 | 105 | 75 | 30 | |
| ≥2 | 63 | 48 | 15 | |
| ECOG PS score | | | | 0.2872 |
| <2 | 97 | 68 | 29 | |
| ≥2 | 71 | 55 | 16 | |
| IPI score | | | | 0.1232 |
| 0–2 | 66 | 44 | 22 | |
| 3–5 | 102 | 79 | 23 | |
| LDH | | | | 0.2028 |
| normal | 44 | 29 | 15 | |
| high | 124 | 94 | 30 | |

Notes: [a]10 values were missing in Hans classification; [b]4 values were missing in Ki-67 value

**Table 2. Relationship between CD66b expression levels and clinicopathological features of 40 patients with DLBCL of GCB subtype.**

| Clinicopathological parameter | n | CD66b | | p-value |
|---|---|---|---|---|
| | | Low | High | |
| Age | | | | 0.6937 |
| <60 | 11 | 9 | 2 | |
| ≥60 | 29 | 20 | 9 | |
| Gender | | | | >0.9999 |
| male | 15 | 11 | 4 | |
| female | 25 | 18 | 7 | |
| B symptom | | | | >0.9999 |
| absence | 35 | 25 | 10 | |
| exist | 5 | 4 | 1 | |
| Ki-67 | | | | 0.1582 |
| <75% | 15 | 13 | 2 | |
| ≥75% | 25 | 16 | 9 | |
| Extranodal invasion | | | | 0.7152 |
| 0–1 | 26 | 18 | 8 | |
| ≥2 | 14 | 11 | 3 | |
| ECOG PS score | | | | 0.0678 |
| <2 | 27 | 17 | 10 | |
| ≥2 | 13 | 12 | 1 | |
| IPI score | | | | 0.1530 |
| 0–2 | 17 | 10 | 7 | |
| 3–5 | 23 | 19 | 4 | |
| LDH | | | | >0.9999 |
| normal | 13 | 9 | 4 | |
| high | 27 | 20 | 7 | |

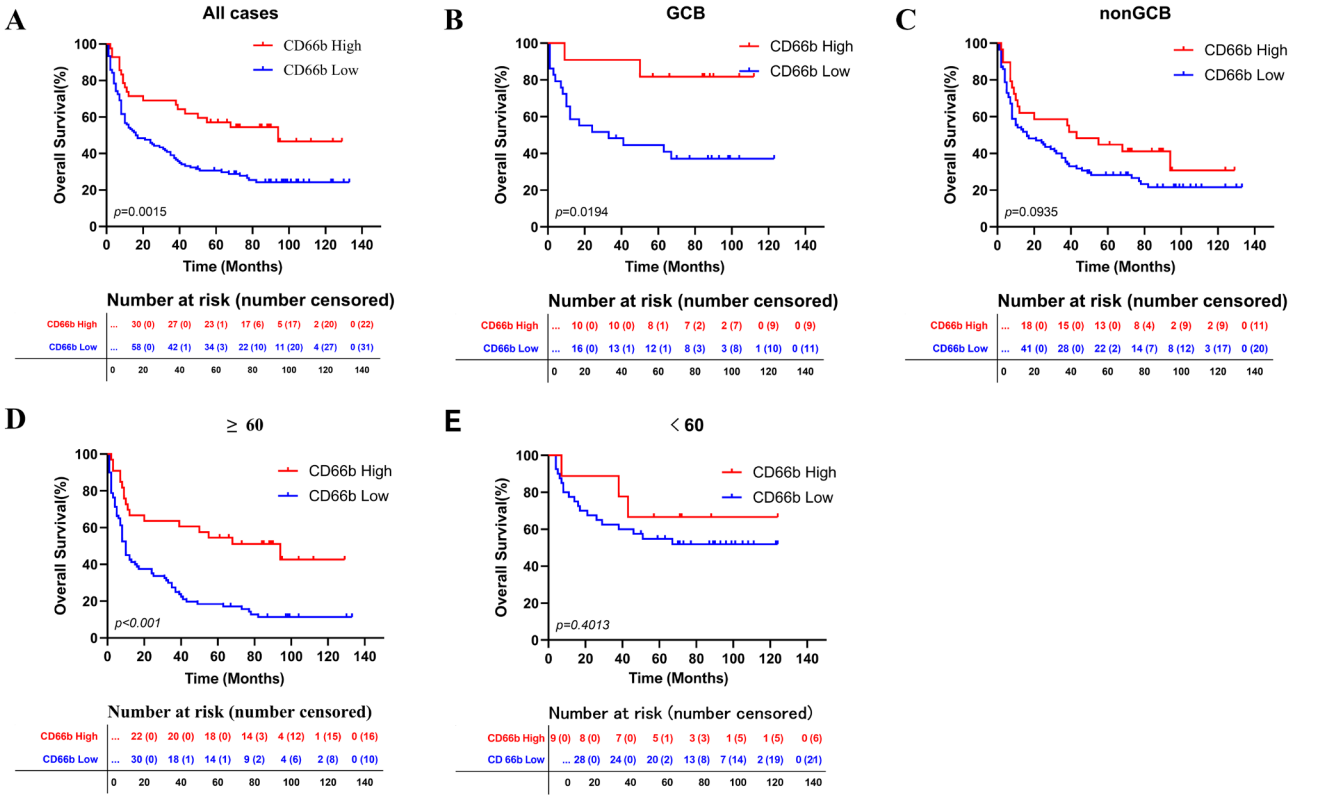


**Figure 2. Expression of CD66b in DLBCL patients. A) A survival curve was drawn according to the expression level of CD66b in tumor tissues of patients, and OS was significantly prolonged in patients with high CD66b expression. B) Survival curve was drawn according to the expression level of CD66b in tumor tissues of patients with GCB subtype, and OS was significantly prolonged in patients with high CD66b expression in patients with GCB subtype. C) Survival curve was drawn according to the expression level of CD66b in tumor tissues of patients with non-GCB subtype, and there was no significant correlation between the expression status of CD66b and patients' OS in patients with non-GCB subtype. D) Survival curve was drawn according to the expression level of CD66b in tumor tissues of patients aged 60 or above, and OS was significantly prolonged in patients with high CD66b expression in patients aged 60 or above. E) Survival curve was drawn according to the expression level of CD66b in tumor tissues of patients under the age of 60, and there was no significant correlation between the expression status of CD66b and patients' OS in patients under the age of 60.**

in the multivariate analysis. Multivariate analysis showed that IPI score (p<0.001), Hans classification (p=0.016), and CD66b expression (p=0.015) are independent prognostic factors for OS (Table 6).

**Relationship between CD66b expression and the efficacy of R-CHOP/CHOP regimen.** The majority of patients in the study were treated with CHOP/R-CHOP regimen after being diagnosed, among whom 73 patients were treated with the CHOP regimen, and 40 patients were treated with the R-CHOP regimen.

In the 73 patients treated with the CHOP regimen, the objective response rate (ORR) was 65.2% and 42% in CD66b-high and CD66b-low patients, respectively. In 40 patients treated with the R-CHOP regimen, the ORR was 58.3% and 75% in CD66b-high and CD66b-low patients, respectively (Table 7).

Taking the DLBCL subtype into account, it was found that in the patients with non-GCB subtype treated with R-CHOP regimen, the ORR was 37.5% and 84.2% in CD66b-high and CD66b-low patients, respectively (p=0.0267). While in other subtypes, no significant differences were observed between the CD66b expression and the ORR in the patients treated with CHOP/R-CHOP regimen (p>0.05; Figure 3). The above results suggested that lower CD66 expression was associated with higher ORR in the patients with non-GCB subtype treated with the R-CHOP regimen, which was helpful in making a treatment decision.

**Prognostic significance of CD66b combined with PD-L1/PD-L2 in DLBCL.** We previously reported that immune checkpoints PD-L1 and PD-L2 were a promising therapeutic target in DLBCL [21]. In this study, we analyzed the value of jointly detecting the expression levels of CD66b and PD-L1/PD-L2 in evaluating the prognosis of DLBCL patients.

When combining CD66b and PD-L1 expression, we found that patients with low CD66b expression and positive PD-L1 expression had shorter OS than other patients (p=0.034). When combining CD66b and PD-L2 expression,

**Table 3. Relationship between CD66b expression levels and clinicopathological features of 118 patients with DLBCL of non-GCB subtype.**

| Clinicopathological parameter | n | CD66b | | p-value |
|---|---|---|---|---|
| | | Low | High | |
| Age | | | | 0.2200 |
| <60 | 37 | 30 | 7 | |
| ≥60 | 81 | 57 | 24 | |
| Gender | | | | 0.7994 |
| male | 51 | 37 | 14 | |
| female | 67 | 50 | 17 | |
| B symptom | | | | 0.6610 |
| absence | 104 | 76 | 28 | |
| exist | 14 | 11 | 3 | |
| Ki-67 | | | | **0.0167** |
| <75% | 43 | 26 | 17 | |
| ≥75% | 73 | 59 | 14 | |
| Extranodal invasion | | | | 0.8974 |
| 0–1 | 75 | 55 | 20 | |
| ≥2 | 43 | 32 | 11 | |
| ECOG PS score | | | | 0.9744 |
| <2 | 65 | 48 | 17 | |
| ≥2 | 53 | 39 | 14 | |
| IPI score | | | | 0.7804 |
| 0–2 | 47 | 34 | 13 | |
| 3–5 | 71 | 53 | 18 | |
| LDH | | | | 0.0732 |
| normal | 28 | 17 | 11 | |
| high | 90 | 70 | 20 | |

**Table 4. Relationship between CD66b expression levels and clinicopathological features of 118 patients aged 60 or above.**

| Clinicopathological parameter | n | CD66b | | p-value |
|---|---|---|---|---|
| | | Low | High | |
| Gender | | | | 0.7225 |
| male | 51 | 35 | 16 | |
| female | 67 | 48 | 19 | |
| Hans classification | | | | 0.8873 |
| GCB | 29 | 20 | 9 | |
| non-GCB | 81 | 57 | 24 | |
| B symptom | | | | 0.3041 |
| absence | 102 | 70 | 32 | |
| exist | 16 | 13 | 3 | |
| Ki-67 | | | | 0.4390 |
| <75% | 35 | 23 | 12 | |
| ≥75% | 81 | 59 | 22 | |
| Extranodal invasion | | | | 0.3300 |
| 0–1 | 73 | 49 | 24 | |
| ≥2 | 45 | 34 | 11 | |
| ECOG PS score | | | | 0.0583 |
| <2 | 55 | 34 | 21 | |
| ≥2 | 63 | 49 | 14 | |
| IPI score | | | | **0.0032** |
| 0–2 | 32 | 16 | 16 | |
| 3–5 | 86 | 67 | 19 | |
| LDH | | | | 0.1098 |
| normal | 26 | 15 | 11 | |
| high | 92 | 68 | 24 | |

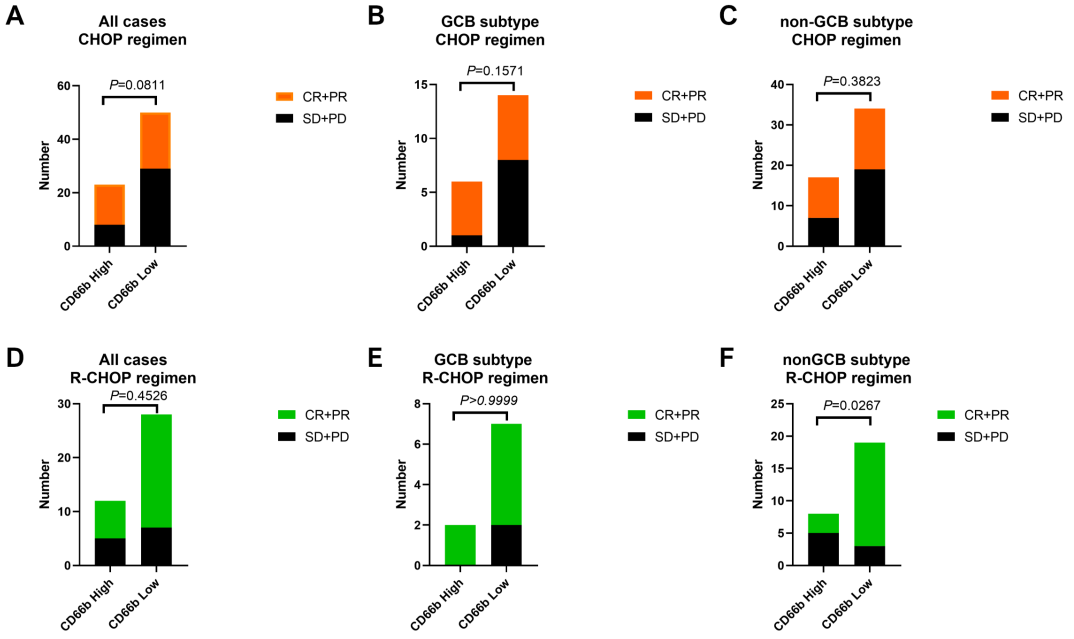


**Figure 3. Relationship between CD66b expression and clinical effect of CHOP/R-CHOP regimen. A) Objective response rates of all patients with high/low expression of CD66b receiving the CHOP regimen. B) Objective response rates of GCB subtype patients with high/low expression of CD66b receiving the CHOP regimen. C) Objective response rates of non-GCB subtype patients with high/low CD66b expression receiving the CHOP regimen. D) Objective response rates of all patients with high/low CD66b expression receiving the R-CHOP regimen. E) Objective response rates of GCB subtype patients with high/low CD66b expression receiving the R-CHOP regimen. F) Objective response rates of non-GCB subtype patients with high/low CD66b expression receiving the R-CHOP regimen.**

it was found that patients with high CD66b expression and positive PD-L2 expression had longer OS than other patients (p=0.003; Figure 4).

The above results indicated that a combined analysis of the expression levels of CD66b and PD-L1/PD-L2 was helpful in predicting the prognosis of patients with DLBCL.

**Table 5. Relationship between CD66b expression levels and clinicopathological features of 50 patients under the age of 60.**

| Clinicopathological parameter | n | CD66b | | p-value |
|---|---|---|---|---|
| | | Low | High | |
| Gender | | | | 0.4705 |
| male | 20 | 17 | 3 | |
| female | 30 | 23 | 7 | |
| Hans classification | | | | 0.9561 |
| GCB | 11 | 9 | 2 | |
| non-GCB | 37 | 30 | 7 | |
| B symptom | | | | >0.9999 |
| absence | 45 | 36 | 9 | |
| exist | 5 | 4 | 1 | |
| Ki-67 | | | | **0.0070** |
| <75% | 25 | 16 | 9 | |
| ≥75% | 23 | 22 | 1 | |
| Extranodal invasion | | | | 0.7683 |
| 0–1 | 32 | 26 | 6 | |
| ≥2 | 18 | 14 | 4 | |
| ECOG PS score | | | | 0.6997 |
| <2 | 42 | 34 | 8 | |
| ≥2 | 8 | 6 | 2 | |
| IPI score | | | | 0.5443 |
| 0–2 | 34 | 28 | 6 | |
| 3–5 | 16 | 12 | 4 | |
| LDH | | | | 0.7683 |
| normal | 18 | 14 | 4 | |
| high | 32 | 26 | 6 | |

**Table 6. Cox univariate and multivariate regression analysis.**

| Variate | OS | |
|---|---|---|
| | HR (95 % CI) | p-value |
| Cox unifactor analysis | | |
| Age (≥60 vs. <60) | 2.352 (1.486–3.721) | **<0.001** |
| Gender (male vs. female) | 1.020 (0.704–1.478) | 0.917 |
| Hans classification (non-GCB vs. GCB) | 1.802 (1.107–2.935) | **0.018** |
| B symptom (exist vs. absence) | 2.117 (1.274–3.518) | **0.004** |
| Ki-67 (≥75 % vs. <75%) | 1.421 (0.954–2.115) | 0.084 |
| extranodal invasion (≥ 2 vs. 0–1) | 1.560 (1.073–2.267) | **0.020** |
| ECOG PS score (≥ 2 vs. <2) | 4.662 (3.161–6.877) | **<0.001** |
| IPI score (3–5 vs. 0–2) | 3.634 (2.357–5.602) | **<0.001** |
| LDH (high vs. normal) | 3.347 (1.991–5.626) | **<0.001** |
| CD66b (high vs. low) | 0.491 (0.308–0.783) | **0.003** |
| Cox multivariate analysis | | |
| IPI score (3–5 vs. 0–2) | 3.116 (2.001–4.853) | **<0.001** |
| B symptom (exist vs. absence) | 1.611 (0.932–2.787) | 0.088 |
| Hans classification (non-GCB vs. GCB) | 1.830 (1.119–2.991) | **0.016** |
| CD66b (high vs. low) | 0.553 (0.342–0.893) | **0.015** |

**Table 7. Relationship between CD66b expression and clinical effect of CHOP/R-CHOP regimen.**

| | CHOP | | p-value | R-CHOP | | p-value |
|---|---|---|---|---|---|---|
| | SD+PD | CR+PR | | SD+PD | CR+PR | |
| CD66b | | | 0.0811 | | | 0.4526 |
| High | 8 | 15 | | 5 | 7 | |
| Low | 29 | 21 | | 7 | 21 | |

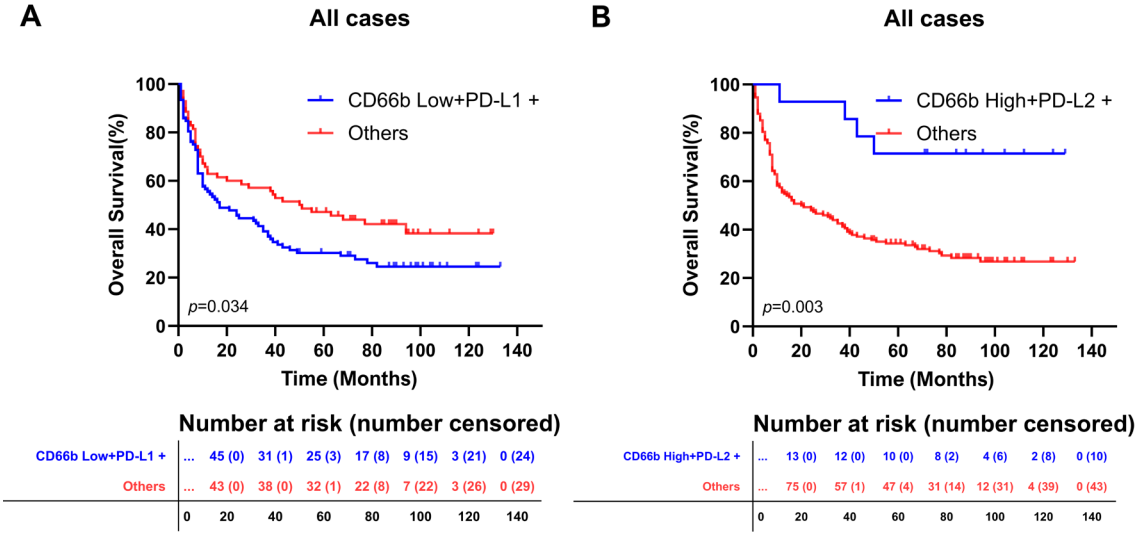


**Figure 4. Expression of CD66b and PD-L1/PD-L2 in DLBCL patients. A) Survival curve was drawn according to the expression level of CD66b and PD-L1 in tumor tissues of DLBCL patients, and OS was significantly shortened in patients with low CD66b expression and positive PD-L1 expression. B) Survival curve was drawn according to the expression level of CD66b and PD-L2 in tumor tissues of DLBCL patients, and OS was significantly prolonged in patients with high CD66b expression and positive PD-L2 expression.**

## Discussion

DLBCL is a highly aggressive disease. Currently, immunotherapy is one of the potential treatment options. Current research indicates that neutrophil infiltration plays a very important regulatory role in the occurrence and development of tumors [4, 22]. In this article, we attempt to clarify its expression and clinical value in DLBCL.

CD66b, which is also called NCA-95 and CD67, is a molecular marker of neutrophils in tumors [23]. As one of the important immune cells in the TME, TAN is expressed in numerous types of tumors, such as breast cancer, stomach cancer, tongue squamous cell carcinomas, and lung cancer [24, 25]. It is strongly associated with the prognosis of cancer, such as colorectal cancer [16, 26]. TAN inhibits tumor growth by directly killing tumor cells or secreting various cytokines such as TGF-β2, IL-1β, TNF-α, and vascular endothelial growth factor (VEGF), while it also promotes tumor growth by participating in angiogenesis and inducing epithelial-mesenchymal transition [10, 27]. Apart from the growth of tumor cells themselves, their interaction with the TME is also an important factor in mediating the occurrence and development of tumors. For instance, the infiltration situation of $CD8^+$ T lymphocytes and $FoxP3^+$ regulatory T cells (Tregs) was related to the prognosis of colorectal cancer. Highly expressed TAN in the TME has also been reported to be the driver of systemic inflammation and is closely related to the prognosis of the tumor [28]. The role of TAN in the growth of tumors is complex. In different types of tumors, it can exhibit either anti-tumor or pro-tumor effects [29]. TAN affects tumor growth by acting on the TME, which provides an effective target for tumor treatment. In different types of tumors, the treatment targeting TAN has not yielded consistent conclusions [30]. Apart from the heterogeneity of the tumors themselves, factors such as the sample size of the patients included in the study, racial differences, the method used to assess the infiltration level, and the tumor area included in the study influence the conclusions drawn.

In this study, we explored the expression of CD66b in DLBCL. In DLBCL, there is infiltration of neutrophils, and the expression level of CD66b varies among different patients. The expression of CD66b is associated with the expression of Ki-67 in DLBCL patients with the non-GCB subtype and under the age of 60. Meanwhile, the expression level of CD66b is also closely related to the prognosis of the patients, especially in the GCB subtype and patients aged 60 or above. The above results suggest that CD66b may play different roles in different subtypes of DLBCL, and it has certain value in predicting the prognosis of certain patients.

In recent years, numerous new treatment regimens for DLBCL have emerged, but R-CHOP remains the most widely used first-line treatment regimen. Our research indicates that the expression level of CD66b is closely related to the ORR of patients with the non-GCB subtype. This result indicates that the expression level of CD66b has predictive value for the treatment plan decision.

In tumors, the infiltration level of TAN is closely related to the expression levels of other immune cells in the immune microenvironment. Tumor cells also activate PD-L1 on neutrophils, thus inducing T cell immunosuppression [31]. Relevant studies have shown that the expression of PD-L1 is closely related to the prognosis of patients with DLBCL, and treatments targeting the PD-1/PD-L1 pathway have become one of the options for treating DLBCL [9]. Our previous studies also indicated that the expression of PD-2, another ligand of PD-1, was closely related to the prognosis of patients with DLBCL and was a potential therapeutic target [21]. Therefore, we conducted a combined analysis of the above-mentioned potential targets for immunotherapy. Our results indicated that the combined detection of CD66b and PD-L1/PD-L2 was helpful for evaluating patients' prognosis. The OS of patients with low CD66b expression and positive PD-L1 expression was shorter than that of other patients; while the OS of patients with high CD66b expression and positive PD-L2 expression was longer than that of other patients. In general, TAN play a very important regulatory role in DLBCL. The expression of TAN is closely associated with the prognosis of DLBCL patients. Our research also indicates that the mechanism of action of TAN may vary in different subtypes of DLBCL. Since TAN plays different roles in various tumors, the mechanism of its action in DLBCL merits further study.

**Supplementary information** is available in the online version of the paper.

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# Expression and prognostic significance of CD66b in diffuse large B-cell lymphoma

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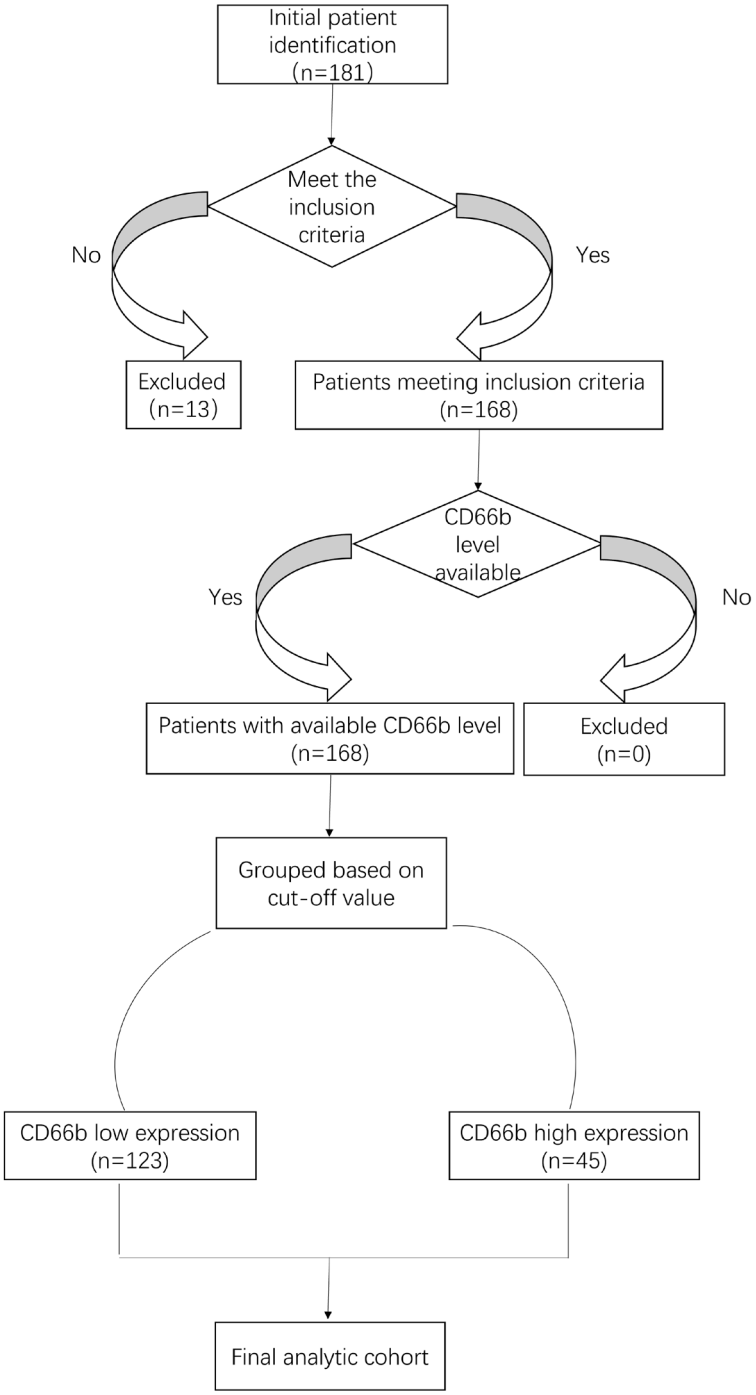
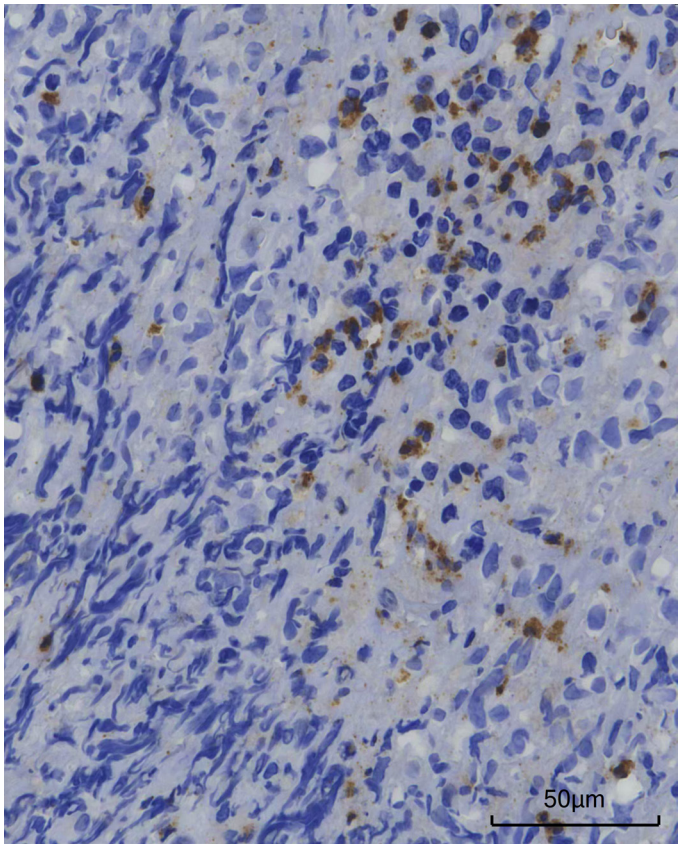
## Supplementary Information




**Supplementary Figure S1. Flow diagram of participant selection.**




**Supplementary Figure S2. Representative images of the immunohistochemistry results of MPO.**

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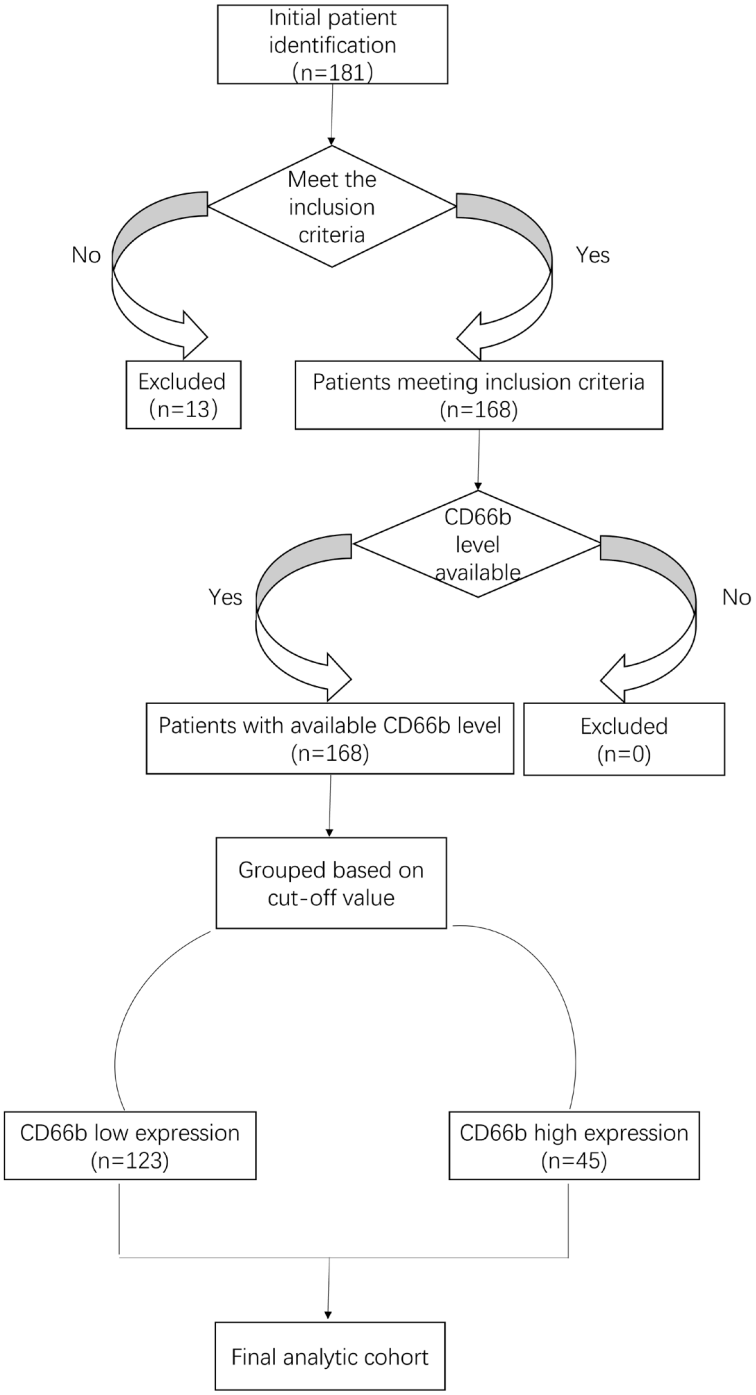
## Supplementary Information

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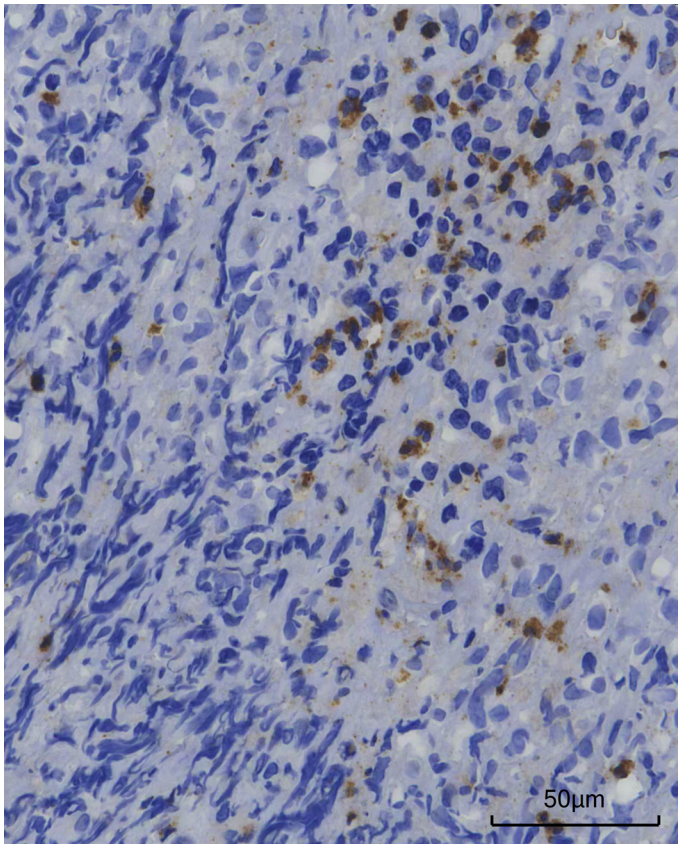


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