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ORIGINAL ARTICLE

Novel risk stratification of de novo diffuse large B cell lymphoma based on tumour-infiltrating T lymphocytes evaluated by flow cytometry

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Abstract

The prognostic value of tumour-infiltrating T lymphocytes (TIL-Ts) has been demonstrated in many solid tumours but remained unclear in diffuse large B cell lymphoma (DLBCL). We conducted a retrospective cohort study reviewing the TIL-Ts proportion and CD4:CD8 of 66 de novo DLBCL by flow cytometry to construct a risk stratification based on TIL-Ts-related prognostic factors. In univariate analysis, low TIL-Ts (< 14%) was significantly related to shorter survival (HR = 2.58, 95% CI 1.11–5.99, $p = 0.028$). In multivariate analysis, low TIL-Ts (HR = 6.48, 95% CI 2.16–19.46, $p = 0.001$) and high CD4:CD8 (> 1.2) (HR = 4.22, 95% CI 1.43–12.35, $p = 0.009$) were independent risk factors. For the risk stratification, three groups were defined based on TIL-Ts-related risk factors: low-risk group (high TIL-Ts and low CD4:CD8), intermediate risk group (low TIL-Ts, low CD4:CD8 or high TIL-Ts, high CD4:CD8) and high-risk group (low TIL-Ts and high CD4:CD8). The patients in high-risk group have significantly shorter survival than that in intermediate risk group ($p = 0.025$) and low-risk group ($p = 0.002$). This new risk stratification which is independent of performance status and age of the patients could hint the prognosis and may guide treatment of DLBCL.

Keywords DLBCL · TIL-Ts · CD4:CD8 · Risk stratification

Introduction

Diffuse large B cell lymphoma (DLBCL) accounts for 25–30% of adult non-Hodgkin lymphomas in western countries [1] and 35.9% in southwest China [2]. Many studies investigated the prognostic markers of DLBCL and demonstrated that many biomarkers and genetic changes have essential prognostic value [3–5]. However, most of these studies focused on the tumour cells per se and the tumour microenvironment were not investigated clearly. Recently, the tumour microenvironment which was mainly constituted by lymphocytes, macrophages, plasma cells, granulocytes and microvessels has been researched in classical Hodgkin lymphoma, follicular lymphoma and other B cell lymphoma which suggested that tumour-infiltrating T lymphocytes (TIL-Ts) had essential impact on prognosis [6–8]. Nevertheless, the results were paradoxical in limited studies when investigated in DLBCL [9, 10].

The TIL-Ts consist of cytotoxic T cells, regulatory T cells (T-regs), follicular helper T cells (Tfhs), etc., and it is still unclear what kind of role they had played in the tumour microenvironment [6]. Yang et al. [11] reported that infiltrating T-regs mediated the suppression of infiltrating CD4+ T cells in B cell non-Hodgkin lymphoma while Hasselblom et al. [12] suggested that the number of tumour-infiltrating TIA-1+ cytotoxic T cells but not T-regs predicted outcome in DLBCL which implied that the proportion and cross-talk of different subsets of TIL-Ts might lead to the controversial results. Besides, most of the previous research was based on immunohistochemical technique which has some limits of quantitative assessment [13]. Flow cytometry is especially suitable for cell classifying and counting analysis with the characteristics of multi-parameter, good specificity, high sensitivity and convenient operation, which can be an appropriate approach to investigate TIL-Ts [14].

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To further characterize the distribution and correlative quantity of TIL-Ts to biological and clinical features of DLBCL, we performed a retrospective study of 66 patients with de novo DLBCL to investigate the prognostic value of the TIL-Ts proportion of tumour microenvironment based on flow cytometry.

Methods

Patients

The de novo DLBCLs from January 2014 to April 2017 were identified from the database of the Clinical Flow Cytometry Laboratory at the Department of Pathology, West China Hospital, Sichuan University. All the cases met the criteria for the diagnosis of diffuse large B cell lymphoma, not otherwise specific (DLBCL-NOS) based on WHO classification of the tumour of hematopoietic and lymphoid tissue, 4th edition (2016 revision). DLBCL-NOS with MYC+ (≥ 40%) and BCL-2+ (≥ 50%) in immunohistochemical staining was identified as double expresser lymphoma (DEL). The exclusion criteria were (1) recurrent DLBCL and (2) DLBCL transforming from small B cell lymphoma. This research has been approved by the Ethics Committee of West China Hospital, Sichuan University. Relevant clinical information, including age, gender, symptoms, extranodal involvements, disease stage, routine blood test, biochemical examination, revised international prognostic index (R-IPI), performance status (PS) and treating tactics, was obtained by electric medical records and follow-up information was collected from telephone interview and/or electric medical records in July 2018. Survival time was calculated from the date of pathological diagnosis to the date of death or last follow-up.

Flow cytometry

Biopsy samples with a medium size of 1 cm × 1 cm × 1 cm were put into a tube with RPMI 1640 medium (Gibco by Life Technologies, UK) for flow cytometry analysis. The specimen was gentle minced in 2 mL of RPMI using scissors and ground for 5 min. Then, the cell suspension was filtered through a 40-μm filter and centrifuged at 1000 rpm for 5 min. The pelleted cells were washed for three times with phosphate-buffered saline (PBS) and then resuspended in 1 mL of RPMI-1640. After counting with a hemocytometer, the 2×10^5 cells per tube was stained with appropriately antibodies as the following panels (Fitc/PE/PerCP/PE-Cy7/APC/APC-Cy7): (1) CD7/CD3/CD4/CD8/CD45 and (2) lambda(sIg)/kappa(sIg)/CD19/CD10/CD38/CD20. Cell suspensions were incubated in the dark for 5 min at room temperature (according to the instruction) and then lysed with 0.15 mol/L of ammonium chloride for 5 min, thereafter washed with 2 mL of PBS and resuspended with 0.2 mL of PBS prior to analysis. These data were obtained by BD FACSCartoII with two lasers (blue and red) and analyzed with FACSDiva Software. The fluorescent-labeled antibodies were from BD.

At least 20,000 leukocytes in each sample and 50,000 events were acquired. Total cell numbers of lymphocytes were determined by the surface markers of CD45. CD19 and CD20 were used to identify and gate the B lymphocytes; meanwhile, the ratio of kappa and lambda population was confirmed in the tumour population of B cell. CD3 was used to identify and gate the TILs, and the ratio of TIL subsets (CD4:CD8) was tabulated for statistical analysis. Compensation control tubes were used in each assay.

Statistics

Statistical analysis was performed by SPSS 19.0 software (SPSS Corp, Chicago, IL, USA). Continuous data was analyzed by independent sample *t* test while categorical data was compared by chi-square test. The Ridit analysis was applied for comparison of ranked data [15]. The cut-off of TIL-Ts proportion and CD4:CD8 ratio was defined as the point where the sensitivity plus specificity were maximized in the ROC curves for predicting overall survival. The survival time was calculated by the Kaplan–Meier analysis, and the log-rank test was used to evaluate the association with potential variables and survival time [16]. The Cox's hazard regression model was applied to estimate independent prognostic factors. Due to the sample size restriction, four factors of the lowest probability value were included in multivariate analysis. Data were recorded as the mean ± standard deviation for continuous variables or number (%) for categorical data or ranked data. Tests were considered significant at two-sided probability value less than 0.05 ($P < 0.05$).

Risk stratification

TIL-Ts-related risk factors were considered into risk stratification. Specific cut-off values were defined for each risk factor based on the receiver operator characteristic (ROC) curves. We identified low-risk group of patients with no risk factor, intermediate risk group with one risk factor and high-risk group with two risk factors.

Results

Patient characteristics

Among 83 identified cases, 14 recurrent DLBCL and 3 with evidence of transforming from small B cell lymphoma were excluded. Of the eligible 66 patients in the cohort, the median age was 61-year-old (range, 8–83), with a male to female ratio

of 3:2. More than half of the patients (53.5%) were at early stage (I/II) with good general condition (PS, 0–1). R-IPI was evaluated to reveal that 34 (61.8%) patients were at the group of very good or good. For extranodal involvement, 14 (23.3%) patients were found to have more than 2 extranodal sites of involvement but bone marrow infiltration was detected in only 3 (9.7%) patients. Besides, elevated lactate dehydrogenase (LDH) was evaluated in 38 (65.5%) patients. For pathologic features, most of the cases (81.5%) were identified as non-germinal centre B cell (N-GCB) by Hans algorithm. DEL was detected in 35.7% of the cases. Most of the patients received chemotherapy (84.6%) with R-CHOP or R-CHOP-like regimen while part of the patients (11.5%) underwent other proper therapy including surgery and radiotherapy. A few patients (1.9%) received supportive care only because of advanced stage with bad general condition and chemotherapy intolerance. Besides, one patient denied treatment after the diagnosis. Moreover, 65.9% of the patients that received chemotherapy achieved complete remission. All the basic information is summarized in Table 1.

TIL-Ts proportion and correlation with clinicopathological features

The mean proportion of TIL-Ts in de novo DLBCL was 27.1% (range, 0.5–76.2%). All patients were divided into two groups (high TIL-Ts vs low TIL-Ts) with the cut-off value as 14% by ROC curves. The correlation between TIL-Ts proportion and clinicopathological features is shown in Table 2. No obvious difference was detected in age, gender, pathologic features, stage or R-IPI at diagnosis between these two groups.

CD4:CD8 ratio and correlation with clinicopathological features

The mean CD4:CD8 ratio of TIL-Ts was evaluated as 1.4 (range, 0.03–8.6).Two groups were defined based on CD4:CD8 ratio with the cut-off value of 1.2. No obvious difference was detected in age, gender, pathologic features, stage or R-IPI at diagnosis between these two groups; however, extranodal involvements were more likely appeared in patients with CD4:CD8 ratio ≤ 1.2 (Table 3).

Survival and prognostic value

Follow-up ranged from 0.5 to 46 months, the median overall survival of those patients with de novo DLBCL was 25 months (95% confidence interval (CI) 10.4–39.6 months; Fig. 1a). The univariate and multivariate analyses for overall survival are displayed in Table 4. On univariate analysis, low TIL-Ts (< 14%) ($p = 0.028$) and DEL ($p = 0.023$) were significant risk factors of overall survival while stage, R-IPI, extranodal involvement and Han's algorithm had no obvious impact on overall survival. However, in multivariate analysis, apart from low TIL-Ts (Fig. 1b; $p = 0.001$) and DEL ($p = 0.004$), high CD4:CD8 (> 1.2) (Fig. 1c; $p = 0.009$) was also found to be an independent prognostic factor of patients with de novo DLBCL.

Table 1 The baseline of patients with de novo DLBCL

Characteristics	No. in group (%)
Age	56.3 ± 16.38 (8–83) years
Gender	
Male	41 (62.1)
Female	25 (37.9)
PS	
0–1	30 (50.0)
2–5	30 (50.0)
Stage	
I/II	30 (53.6)
III/IV	26 (46.4)
R-IPI	
Very good or good	34 (61.8)
Poor	21 (38.2)
Extranodal involvement	
None	23 (38.3)
1 site	23 (38.3)
≥ 2 sites	14 (23.3)
BM infiltration	
Yes	3 (9.7)
No	28 (90.3)
Elevated LDH	
Yes	38 (65.5)
No	20 (34.5)
Han's algorithm	
GCB	12 (18.5)
N-GCB	53 (81.5)
DEL	15 (35.7%)
TIL-T (%)	27.1 ± 20.4 (0.5–76.2)
CD4:CD8	1.4 ± 1.4 (0.03–8.6)
Treatment	
Chemotherapy	44 (84.6)
Other therapy	7 (13.5)
No treatment	1 (1.9)
Response	
CR	29 (65.9)
PR	13 (29.5)
NR	2 (4.5)

Chemotherapy: R-CHOP or R-CHOP-like regimen; Other therapy: including surgery and radiotherapy

BM bone marrow, *CR* complete remission, *PR* partial remission, *NR* no response, *GCB* germinal centre B cell, *N-GCB* non-germinal centre B cell, *DEL* double expresser lymphoma, *R-IPI* revised international prognostic index

Table 2 Patient characteristics by TIL-Ts proportion

	TIL-Ts < 14% (*N*, %)	TIL-Ts ≥ 14% (*N*, %)	*P* value
Mean age (years)	56.6 ± 17.0	56.2 ± 16.2	0.912
Gender			0.284
Male	16 (72.7)	25 (56.8)	
Female	6 (27.3)	19 (43.2)	
Stage			0.408
I/II	9 (45.0)	21 (58.3)	
III/IV	11 (55.0)	15 (41.7)	
R-IPI			0.386
Very good or good	10 (52.6)	24 (66.7)	
Poor	9 (47.4)	12 (33.3)	
Extranodal involvement			0.052
None	4 (18.2)	19 (50.0)	
1 site	12 (54.5)	11 (28.9)	
≥ 2 sites	6 (27.3)	8 (21.1)	
Han's algorithm			
GCB	3 (14.3)	9 (20.5)	0.737
N-GCB	18 (85.7)	35 (79.5)	
DEL	7 (43.8)	8 (30.8)	0.511
Response			
CR	7 (77.8)	22 (62.8)	0.377
PR	2 (28.6)	11 (31.4)	
NR	0 (0)	2 (5.7)	

CR complete remission, *PR* partial remission, *NR* no response, *GCB* germinal centre B cell, *N-GCB* non-germinal centre B cell, *DEL* double expresser lymphoma, *R-IPI* revised international prognostic index

Risk stratification analysis

On implementation of risk stratification, the patients were classified into three groups based on TIL-Ts-related risk factors: low-risk group (high TIL-Ts and low CD4:CD8), intermediate risk group (low TIL-Ts, low CD4:CD8 or high TIL-Ts, high CD4:CD8) and high-risk group (low TIL-Ts and high CD4:CD8) (Fig. 2). The clinicopathological comparison of three groups is summarized in supplementary table (Table s1). However, the survival curves of the three groups were dramatically different ($p = 0.006$). The patients in high-risk group have significantly shorter survival than that in intermediate risk group ($p = 0.025$) and low-risk group ($p = 0.002$). Moreover, the patients in low-risk group also tend to have better prognosis ($p = 0.180$) than that in intermediate risk group (Fig. 1d).

Discussion

Immunotherapy, which has been a research hotspot since it was proposed, has begun to revolutionize tumour treatment by introducing therapies that regulate the host immune system in order to kill tumour cells [17, 18]. Thus, the immune microenvironment of tumours is considered as an essential target since it affects the tumour in development, progression and metastasis directly. In addition, tumour-infiltrating lymphocytes (TILs) as the significant component of immune microenvironment of tumours have been demonstrated to have prognostic value in gastric cancer, breast cancer and other solid tumours, implying that TILs may actively promote or depress tumour growth [19–22]. Besides, TILs have also been investigated to affect prognosis of patients with DLBCL in limited research, while the conclusions were inconsistent and the mechanism has not been revealed. Therefore, more work should be done to find the significance of TILs in DLBCL's microenvironment and throw light on exploiting novel immunotherapy of DLBCL.

Our study focused on TIL-Ts mainly because previous studies investigating lymphoma and other solid tumours demonstrated that TIL-Ts accounted for the majority percentage of TILs [16, 19, 23]. Moreover, this phenomenon was also observed in our clinical practice and verified by statistics method of this cohort (supplementary data, Table s2). Besides, TIL-Ts were regarded as effector cells of the novel immunotherapeutic drugs and antibody blockade of immune checkpoints. Accordingly, we designed this study to investigate TIL-Ts in

Table 3 Patient characteristics by CD4:CD8 ratio

	CD4:CD8 ≤ 1.2 (*N*, %)	CD4:CD8 > 1.2 (*N*, %)	*P* value
Mean age (years)	55.1 ± 17.8	58.1 ± 14.1	0.463
Gender			
Male	23 (59.0)	18 (66.7)	0.610
Female	16 (41.0)	9 (33.3)	
Stage			0.408
I/II	21 (58.3)	9 (45.0)	
III/IV	15 (41.7)	11 (55.0)	
R-IPI			0.779
Very good or good	21 (60.0)	13 (65.0)	
Poor	14 (40.0)	7 (35.0)	
Extranodal involvement			0.002
None	9 (23.7)	14 (63.6)	
1 site	17 (44.7)	6 (27.3)	
≥ 2 sites	12 (31.6)	2 (9.1)	
Han's algorithm			0.102
GCB	10 (26.3)	2 (7.4)	
N-GCB	28 (73.7)	25 (92.6)	
DEL	11 (42.3)	4 (36.4)	0.506
Response			0.537
CR	20 (69.0)	9 (60.0)	
PR	8 (27.6)	5 (33.3)	
NR	1 (4.4)	1 (6.7)	

CR complete remission, *PR* partial remission, *NR* no response, *GCB* germinal centre B cell, *N-GCB* non-germinal centre B cell, *DEL* double expresser lymphoma, *R-IPI* revised international prognostic index

DLBCL to reveal the prognostic value and build the foundation for further study of immunotherapy.

For other solid tumours, the standardized method of assessing tumour-infiltrating lymphocytes is immunohistochemical staining which has been recommended by International Immunooncology Biomarkers Working Group [24, 25]. However, such method was not suitable for lymphomas because of the subjectivity and low precision in our practical experience. Although digital image processing technique has been applied to investigate TIL-Ts [16], it was not widely used because of the low cost-effectiveness. To solve this dilemma, we assessed TIL-Ts based on flow cytometry technique. Biomarker, CD3, was applied to identify T lymphocytes, while CD4 and CD8 were applied for subdividing the subsets. Comparing to the previous studies based on immunohistochemical technique [13], flow cytometry is much more suitable for cell classifying and counting analysis with the characteristics of multi-parameter, good specificity, high sensitivity and convenient operation [14]. Accordingly, the TIL-Ts proportion and CD4:CD8 ratio calculated in our study was more precise.

TIL-Ts proportion of DLBCL in our research was a significant risk factor of overall survival ($p = 0.028$) in univariate analysis, which remained significant in multivariate analysis ($p = 0.001$). Consistent with previous reports, it is suggested that higher TIL-Ts proportion predicted better prognosis in DLBCL [9]. However, the cut-off of TIL-Ts was defined in a different way. In contrast to the former study which defined the cut-off value by examining the *P* values of a series of survival curves, we performed the ROC curves to determine the point (14%) where the sensitivity plus specificity were maximized [16]. According to literature, host immune defense against neoplastic cells depends on the action of TILs [26], and the TILs are mainly composed of T cells [27]. In this respect, TIL-Ts may inhibit and/or kill the tumour cells via specific pathways [28]. When the number of T cells was decreased, this meant fewer T cells could participate in anti-tumour activity and the prognosis was poor. Therefore, the number of TIL-Ts was essential to the prognosis of DLBCL.

Interestingly, our result implied that high CD4:CD8 (CD4:CD8 > 1.2) was an independent risk factor of overall survival ($p = 0.009$), which contradicted a previous study investigating DLBCL [9]. However, this result consisted with other studies investigating solid tumours such as breast cancer, cervical carcinoma and pancreatic ductal adenocarcinoma [29–31]. Moreover, although other studies investigating DLBCL reported that high CD4+TIL-Ts correlated with favourable outcomes [32, 33], these studies did not investigate

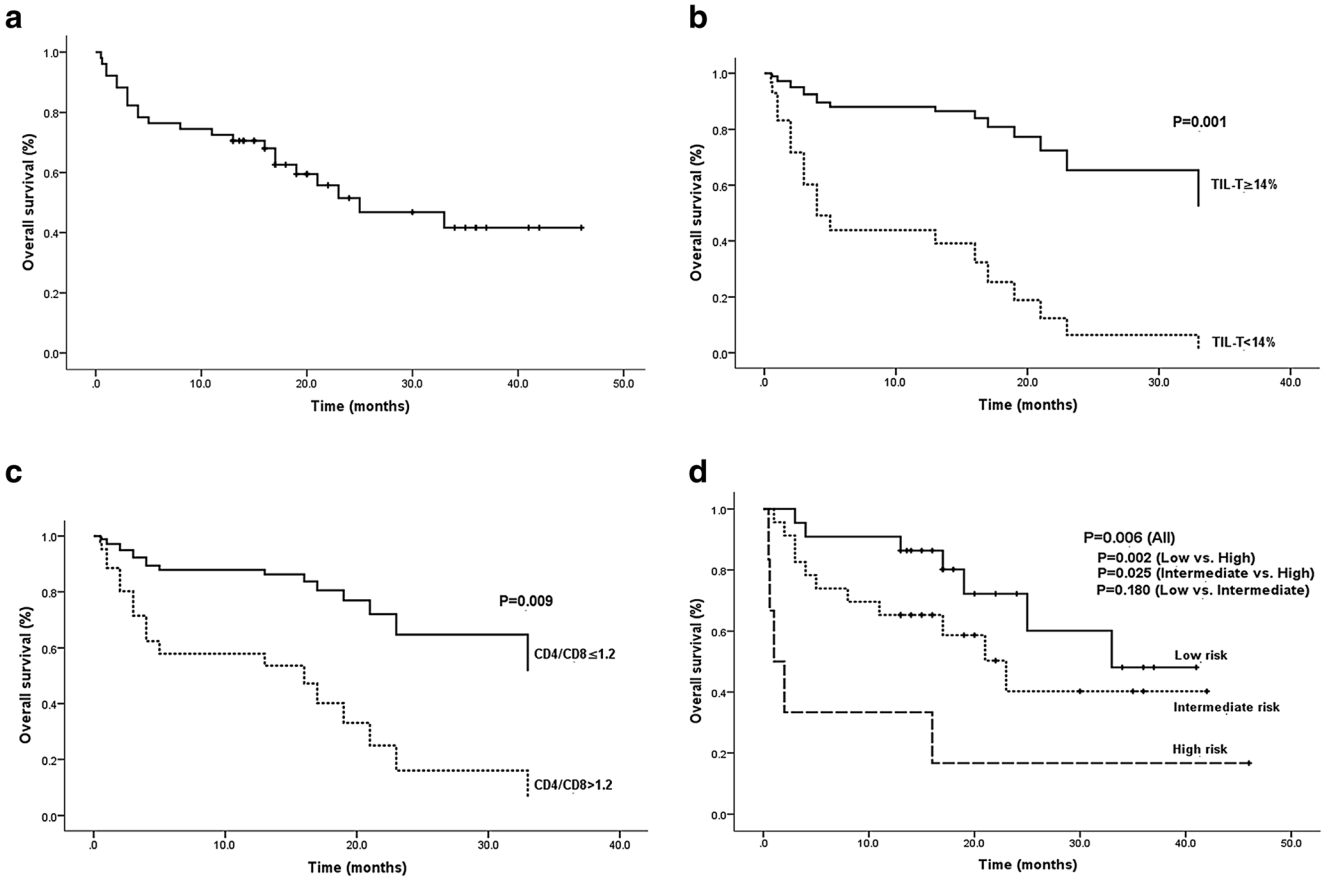

Fig. 1 Kaplan–Meier estimates of rate of overall survival for all patients (**a**), TIL-Ts proportion (**b**), CD4:CD8 ratio (**c**) and risk stratification (**d**)

the CD4:CD8 ratio. In our study, high CD4+TIL-Ts and high CD8+TIL-Ts were both found to be correlated with a good prognosis (data not shown) which consisted with the prognostic value of total TIL-Ts. However, high CD4:CD8 ratio means CD4+TIL-Ts outnumber CD8+TIL-Ts other than high CD4+TIL-Ts absolute amount. Additionally, CD8+ T cells (majority are cytotoxic T cells) have been revealed to be a key effector cell population mediating effective anti-tumour immunity [34]. Indeed, Kumagai et al. also reported that the action of cytotoxic CD8+ cells in the immune responses could inhibit the aggressiveness of malignant cells.CD8+ T cells can recognize and kill potentially tumour cells, which express peptides from oncogenic viral proteins or mutant cellular proteins [27]. Nevertheless, CD4+ T cells (part are T-regs) suppress anti-tumour T cell responses. Thus, lower CD4:CD8 ratio, which means relatively more cytotoxic T cells, implied

Table 4 Risk factors of patients with de novo DLBCL

Variable	Univariable		Multivariable	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
N-GCB	2.54 (0.59–10.85)	0.209	0.68 (0.07–6.45)	0.736
DEL	2.96 (1.16–7.58)	0.023	5.08 (1.70–15.15)	0.004
TIL-Ts < 14% (vs ≥ 14%)	2.58 (1.11–5.99)	0.028	6.48 (2.16–19.46)	0.001
CD4:CD8 ≥ 1.2 (vs < 1.2)	1.94 (0.86–4.41)	0.113	4.22 (1.43–12.35)	0.009
Stages III/IV (vs I/II)	1.12 (0.48–2.58)	0.797	–	–
R-IPI poor (vs very good or good)	1.66 (0.70–3.91)	0.251	–	–
Extranodal involvement (≥ 1 site)	1.43 (0.60–3.43)	0.420	–	–

N-GCB non-germinal centre B cell, *DEL* double expresser lymphoma, *R-IPI* revised international prognostic index, *HR* hazard ratio, *CI* confidence interval

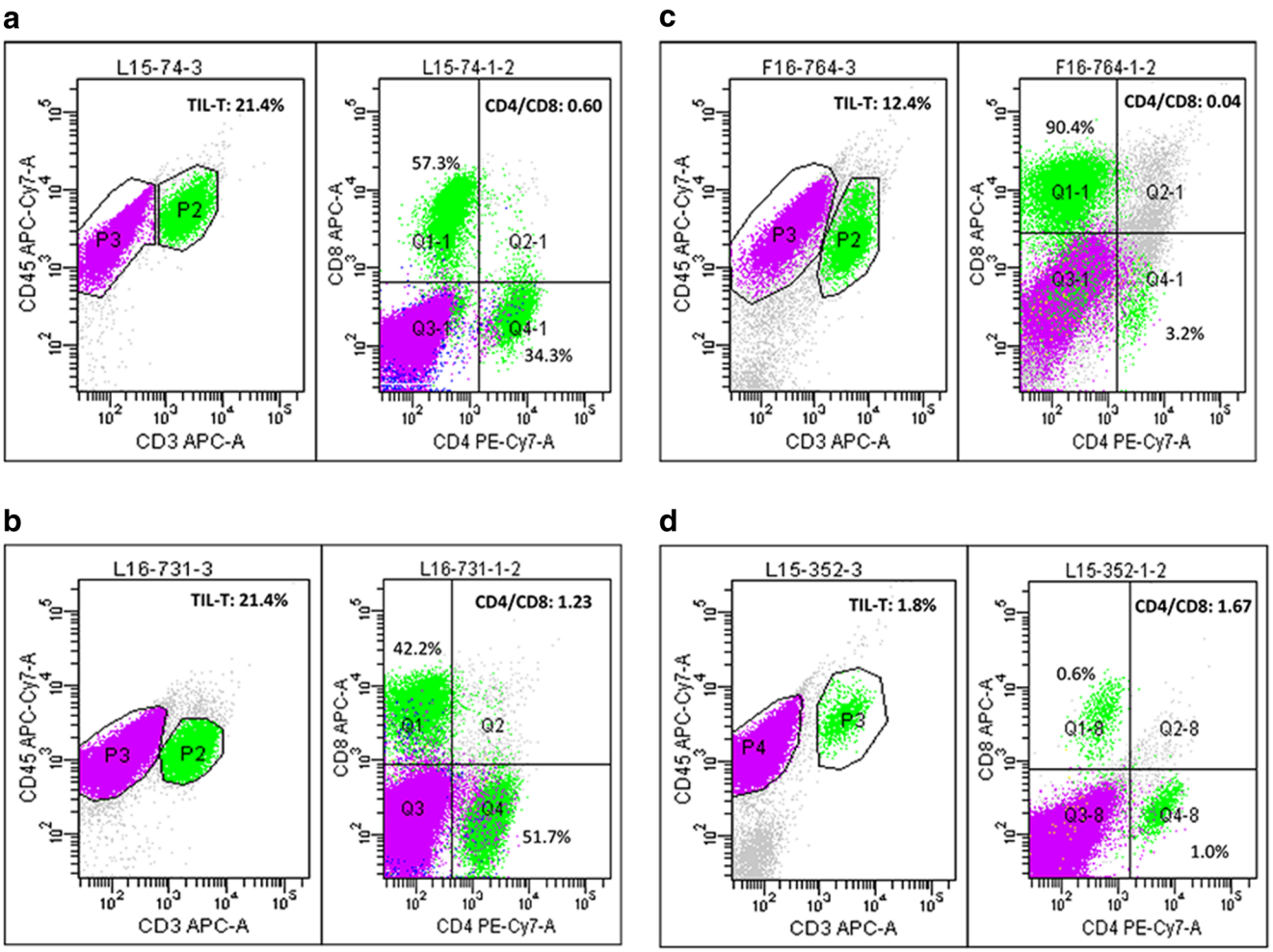

Fig. 2 Risk stratification based on flow cytometry. Green: TIL-Ts; purple: tumour cells. **a** Low-risk group (TIL-Ts ≥ 14% and CD4:CD8 ≤ 1.2). **b** Intermediate risk group (TIL-Ts < 14%, CD4:CD8 ≤ 1.2). **c** Intermediate risk group (TIL-Ts ≥ 14%, CD4:CD8>1.2). **d** High-risk group (TIL-Ts < 14% and CD4:CD8 > 1.2)

favourable prognosis via enhanced anti-tumour responses [34]. A recent study investigating colorectal cancers demonstrated that CD4+ T cell subsets distinctly control the prognosis—$CD4^{+}FOXP3^{high}$ T-regs indicated poor prognosis while $CD4^{+}FOXP3^{low}$ non-suppressive T cells implied favourable outcomes [35]. Notably, both the former research [9, 32, 33] and our study did not further subdivide the subsets of CD4+ T cells due to the material restriction, and the proportion of CD4+ T cells subsets might be the cause of the paradoxical results in DLBCL. So, further studies are needed to investigate the TIL-Ts based on more precise subdivisions.

In addition, in contrast to the previous studies which only investigated the risk factors individually, our results implied the interaction of risk factors related to TIL-Ts would play a very important role in prognosis of DLBCL. We proposed the risk stratification based on TIL-Ts-related risk factors (TIL-Ts < 14%, CD4:CD8 > 1.2) and revealed the prognostic value. The patients within high-risk group have significantly worse overall survival than the patients within low-risk group or intermediate risk group. This risk stratification is independent of the general condition and the age of the patients; other than performance statue-related risk stratification, the patients in high-risk group who are always in old age and bad general condition could not tolerate intensive chemotherapy, which could only hint the overall survival but not guide treatment [36–38]. Our risk stratification makes it possible for providing intensive therapy for the patients with good performance status in high-risk group to improve the overall survival. In addition, this result revealed that some factors hidden in the tumour microenvironment may also play an essential role in prognosis and might throw the light on immunotherapy. The risk stratification we proposed is worthwhile, further validation for its significant prognostic value.

Our research suggested that the patients with low CD4:CD8 ratio (CD4:CD8 ≤ 1.2, $p = 0.003$) are more likely to present with extranodal lesions. This phenomenon was also detected in previous DLBCL cohort and other extranodal B cell lymphoma [9, 23, 39], while other research has reported

that CD8+ T cells outnumber CD4+ T cells that is an intrinsic property of extranodal organs such as the brain and thyroid gland [40, 41]. In addition, both our study and the previous research reported that extranodal lesion was not a prognostic factor [9]. Accordingly, we considered lower CD4:CD8 ratio as specific characteristics of extranodal DLBCL.

In conclusion, we performed the retrospective study based on 66 de novo DLBCL patients to investigate the prognostic value of TIL-Ts through flow cytometry technique. Our results demonstrated that high TIL-Ts proportion (TIL-$T \geq 14\%$) and low CD4:CD8 ratio of TIL-Ts (CD4:CD8 ≤ 1.2) are correlated with better patient survival. In addition, our proposed risk stratification which is based on TIL-Ts-related risk factors provided dramatic predictive value of overall survival. The patients within high-risk group have significantly worse overall survival than the patients within low-risk group or intermediate risk group. Further prospective studies with large sample size are in need to confirm our current conclusions for its significant prognostic value and reveal its possible mechanism.

Authors' contributions ZHC and SZ conceived and designed the study. XQD and SZ contributed to the flow cytometry diagnosis and literature searches. WYZ, YXY and WPL made pathological diagnosis of the patients. ZHC analyzed the data and SZ rechecked it. ZHC wrote the initial draft of the manuscript. SZ and LMG revised it. All authors read and approved the final manuscript.

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Data availability All the data supporting the findings are presented within the manuscript and supplementary data.

Compliance with ethical standards

Ethics approval and consent to participate As it is a retrospective study, ethics approval is not necessary after consulting the Ethics Committee of West China Hospital.

Consent for publication Written consent for publication was obtained from the patient's parent.

Competing interests The authors declare that they have no competing interests

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