Prognostic relevance of tumor-infiltrating CD4$^+$ cells and total metabolic tumor volume-based risk stratification in diffuse large B-cell lymphoma

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Received: January 10, 2024.
Accepted: March 28, 2024.
Early view: April 4, 2024.

https://doi.org/10.3324/haematol.2024.285038

Abstract

In order to elucidate the relationship between pretreatment radiomic parameters and the proportions of various tumor-infiltrating (TI) cells, we retrospectively analyzed the association of total metabolic tumor volume (TMTV) and TI cells on biopsied tumor lesions in 171 patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL). The surface markers of TI cells were analyzed by multicolor flow cytometry using a dissected single-cell suspension. In examining the correlation between TI cells and positron-emission tomography-derived parameters (maximum standardized uptake value [SUV_{max}], total metabolic tumor volume [TMTV], and total lesion glycolysis), intratumoral cell types minimally influenced the results, except for a weak negative correlation between CD4$^+$ cells and SUV_{max} (R=-0.16, *P*=0.045). Even for the lesion fluorodeoxyglucose uptake at the biopsied site, CD19$^+$ cells (indicative of malignant burden) showed only a weak correlation with the highest SUV (R=0.21, *P*=0.009), whereas CD3$^+$ (R=-0.25, *P*=0.002) and CD4$^+$ cells (R=-0.29, *P*<0.001) demonstrated a similarly weak inverse correlation. High TMTV and low TI CD4$^+$ cells were independently associated with poor prognosis and their combination identified the most adverse population (3-year progression-free survival: 32.3%, 95% confidence interval [CI]: 19.4-53.7; 3-year overall survival: 48.4%, 95% CI: 33.6-69.6). Moreover, radiomic parameters incorporating the international prognostic index significantly improved the 3-year survival prediction (area under the curve: 0.76, *P*<0.05) compared to their standalone use. This study underscores the prognostic impact of TI CD4$^+$ cells on DLBCL and suggests that integration of TMTV and TI cell analysis enhances the accuracy of prognostic prediction.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a predominantly aggressive non-Hodgkin lymphoma (NHL) affecting adults.[1] DLBCL is biologically heterogeneous,[2] exhibiting variable responses to curative-intent chemotherapy. Approximately one-third of patients relapse after first-line treatment,[1] leading to dismal outcomes despite advances in salvage therapeutic strategies, including chimeric antigen receptor (CAR) T-cell therapies and bispecific antibodies.[3,4] In light of these challenges, there is an urgent need to develop more sophisticated risk stratification models at diagnosis beyond the conventionally adopted International Prognostic Index (IPI)-based system.[5-7] Despite recent progress in understanding the genomic landscape,[8] the implementation of molecular profiling into clinical practice, such as double-hit signatures[9,10] and genetic clustering,[2,11] has been limited to a few advanced facilities.

Fluorine-18 fluorodeoxyglucose-positron emission tomography/computed tomography (^{18}FDG-PET/CT) is recommended as part of the diagnostic work-up for precise staging.[12] Notably, multiple functional radiomic features can be extracted from PET/CT. Total metabolic tumor volume (TMTV) represents the estimated tumor burden, while total lesion glycolysis (TLG) considers both tumor volume and metabolic activity. Recently, metabolic heterogeneity, denoting intratumor heterogenous ^{18}FDG uptake, has been reported to have prognostic value in DLBCL[13,14] and different solid tumors.[15] Among these parameters, TMTV stands out as the most extensively validated for its prognostic utility in

various study settings.[16] Notably, due to its ease of acquisition from PET/CT, minimal interobserver variation, and high reproducibility with full or semi-automated segmentation software,[17] TMTV has been accepted as a biomarker, either on its own[18,19] or in combination with other indicators.[20-23] However, PET/CT cannot differentiate between the ^{18}FDG uptake of different intratumoral cellular components and genuine tumor uptake. Consequently, TMTV may not accurately represent the actual tumor burden of malignant B cells. Moreover, numerous studies have characterized the DLBCL tumor milieu at the cellular level,[24-28] showing that an enrichment of tumor-infiltrating (TI) T cells is associated with a more favorable prognosis.[29-33] Although TI T cells may reflect the FDG-uptake at the baseline PET/CT, their role as a pretreatment PET/CT parameter in DLBCL remains undefined. To date, there is no data on the simultaneous quantification of tumor volume and cell content in DLBCL. Thus, this study aimed to elucidate the association between lymphocytes infiltrating the tumor tissue, including their subset, and TMTV measured by PET/CT in DLBCL. We anticipate that elucidation of this association could potentially refine the current risk stratification of patients with DLBCL by PET/CT and IPI.

Methods

Patient cohort and study design

We conducted a retrospective analysis of newly diagnosed patients with histologically confirmed DLBCL who were treated with rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or R-CHOP-like chemotherapy at Kameda Medical Center between 2006 and 2020. Primary exclusion criteria were based on availability, specifically the absence of pretreatment PET/CT with ^{18}FDG-avid lesions and the insufficient flow cytometric (FCM) data from biopsied lymphoma lesions. Patients with high-grade BCL harboring concurrent rearrangements of MYC and BCL2 and/or BCL6 were also excluded. The present study was conducted in accordance with the Declaration of Helsinki and was approved by our institutional review board (approval number: 22-095).

Measurement of positron emission tomography/computed tomography-derived parameters

All PET/CT images were acquired according to our institution's standardized protocol.[34] TMTV was defined as the sum of the volume of visually identified lymphoma lesions with a standardized uptake value (SUV) of ≥ 4.0 as the absolute threshold. TLG was calculated by multiplying TMTV by the mean SUV. For tumor delineation and calculation of these radiomic features, a semi-automatic computer-aided analysis of PET/CT images was performed using the open-source software Metavol (Hokkaido University, Sapporo, Japan). Considering the spatial heterogeneity, the lesion PET/CT parameters obtained from biopsied sites were also measured (Figure 1A).

Quantification of intratumoral cell populations

Briefly, sampled lymph nodes (LN) or extranodal (EN) lesions were mechanically dissociated into cell suspensions, then washed, incubated, and stained with a panel of antibodies including CD45-KrO, CD19-APC, CD3-FITC, CD4-FITC, CD8-PE, and CD56-PE (if available). Proportions of CD19+, CD3+, CD4+, CD8+, and CD56+ cells within the acquired monoclonal cells were quantified after gating based on the characteristic forward and side scatter patterns and CD45 positivity specific to lymphocytes (Figure 1B). While the tissue sampling manner was not strictly limited to an excisional biopsy, a minimum of 10,000 mononuclear cells per sample was required, based on a previous study.[31] Data were acquired on a Navios flow cytometer using the Kaluza software (Beckman Coulter, CA, USA).

Statistical analysis

Statistical analyses were performed with R version 4.1.1 (R Foundation, Vienna, Austria). Pearson correlation analysis was employed to examine the relationships between radiomic features and cellular content within tumors. In order to maximize the predictive power, optimal cut-off values were determined using receiver operating characteristic (ROC) curves for 3-year progression-free survival (PFS) and 3-year overall survival (OS). The PFS and OS were estimated using the Kaplan–Meier methods and compared with the log-rank test. Univariate and multivariate analyses were conducted using Cox proportional hazards models to assess the factors affecting PFS and OS. Statistical significance was defined as a two-sided *P* value of <0.05. Methods are further detailed in the *Online Supplementary Appendix*.

Results

Overall patient characteristics

In total, 171 of 518 patients were included in the analysis (*Online Supplementary Figure S1*) based on the inclusion and exclusion criteria. Regarding subtypes, 156 (91.2%) were DLBCL-not otherwise specified, eight (4.7%) were DLBCL histologically transformed from follicular lymphoma, and seven (4.1%) were Epstein-Barr virus (EBV)-positive DLBCL. The median age was 71 years (interquartile range [IQR]: 61-79). Approximately half of the patients (48.0%) were categorized as IPI 3-5. Using the Hans algorithm,[35] cell of origin (COO) was determined in 137 patients (80.1%), 48 (35.0%) of whom were germinal center B (GCB) type and 89 (65.0%) were non-GCB type. This higher proportions of non-GCB type may reflect the geographical distribution of activated B-cell-like DLBCL in Asian countries.[36,37]

Most patients (87.1%) received R-CHOP, with a complete remission rate of 81.3% at the end of induction according

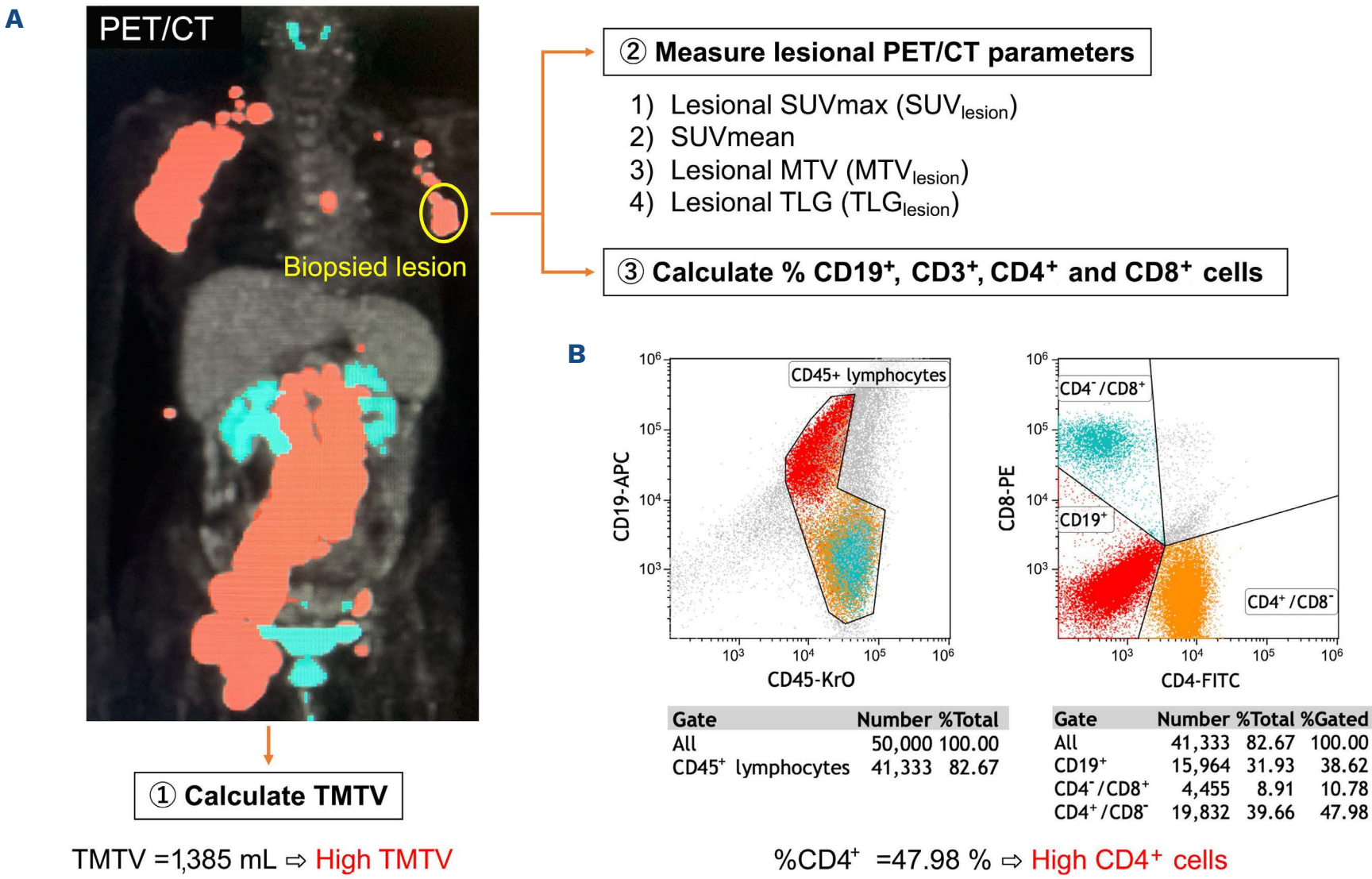

Figure 1. Schematic representation of the calculation of functional positron emission tomography/computed tomography parameters and the gating strategy for intratumoral cellular contents. (A) An example of a patient with a high tumor burden. The red areas represent lymphoma lesions used for calculating the total metabolic tumor volume (TMTV). Physiological uptakes, such as those in the tonsils and urinary tract, are manually excluded and marked in blue. The yellow circle indicates the biopsied lymph node from which lesion radiomic parameters and cellular composition are subsequently measured. (B) The gating strategy for quantifying cellular content within biopsied samples. The left panel shows the distinction between CD19⁺ and CD19⁻ lymphocytes, while the right panel identifies the CD4/CD8 expression pattern. SUV: standardized uptake value; TLG: total lesion glycolysis.

to the Lugano 2014 criteria. During the median observation period of 51 months, 68 patients (39.8%) died and 81 (47.4%) experienced PFS events, resulting in the estimated median OS and PFS of 140 months (95% confidence interval [CI]: 96-not reached [NR]) and 81 months (95% CI: 38-NR), respectively.

Correlation between tumor-infiltrating cells and positron-emission tomography-derived parameters

One hundred forty-four patients (84.2%) were biopsied at a LN while the remaining 27 (15.8%) were biopsied at an EN lesion. The biopsy sites matched the lesions with SUV_{max} in 89 (52.0%) patients. The median percentage of CD19⁺, CD3⁺, CD4⁺, CD8⁺ and CD56⁺ cells (N=120) in the biopsied lesion were 41.5% (IQR, 20.5-60.3), 49.8% (IQR, 29.5-67.6), 26.3% (IQR, 13.8-42.7), 16.2% (IQR, 9.5-23.1) and 0.6% (IQR, 0.4-1.2), respectively.

The median SUV_{max}, TMTV, and TLG were 20.8 (IQR, 14.9-26.8), 177.8 mL (IQR, 47-560), and 1,683 (IQR, 351.9-5,557.4), respectively. The correlation between quantified cellular components in biopsied lesions and functional PET-derived parameters is shown in Figure 2. Intriguingly, SUV_{max}, TMTV, and TLG were not significantly influenced by intratumoral cell types except for a weak negative correlation between CD4⁺ cells and SUV_{max} (R=-0.16, *P*=0.045) (Figure 2A-C). Even for the lesion ^{18}FDG uptake, CD19⁺ cells indicative of the malignant cell burden exhibited only a weak correlation with the highest SUV (SUV_{lesion}) and SUV_{mean} within the biopsied site (R=0.21, *P*=0.009), while CD3⁺ and CD4⁺ cells exhibited a correspondingly inverse weak correlation (CD3⁺ R=-0.25, *P*=0.002 and CD4⁺ R=-0.29, *P*<0.001) (Figure 2D). Neither the overall nor the lesion radiomic parameters were correlated with CD8⁺ cell proportions.

Determination of optimal cut-off values

ROC analysis for determining the optimal cut-off of TI-cell populations and radiomic features is displayed in *Online Supplementary Figure S2*. CD4⁺ T cells had the highest area under the curve (AUC) for both 3-year PFS (AUC: 0.61;

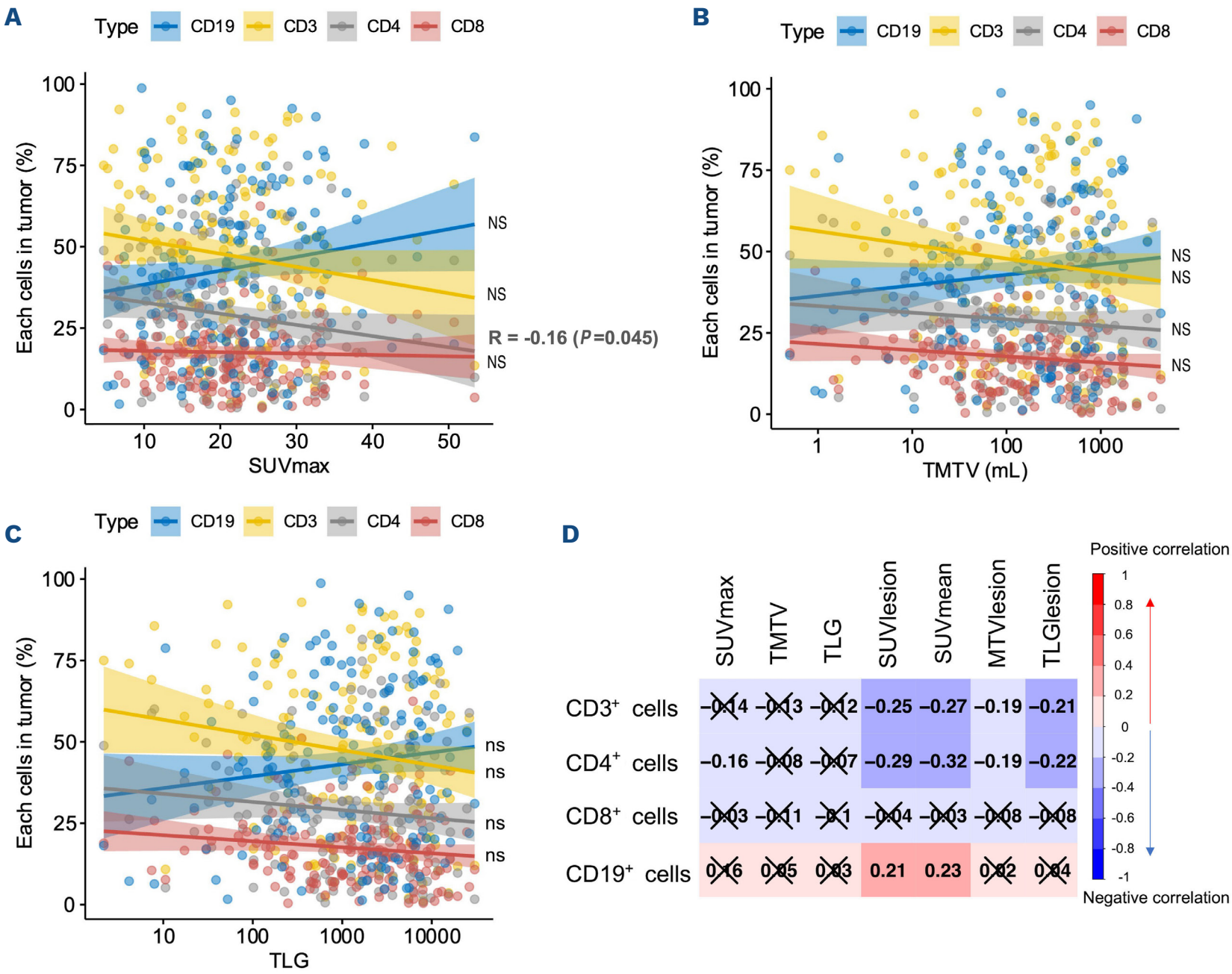

Figure 2. Relationship between functional positron emission tomography/computed tomography parameters and the proportions of intratumoral cellular components. The correlation between the proportions of intratumoral CD19⁺, CD3⁺, CD4⁺, and CD8⁺ cells (represented by blue, yellow, grey, and brown dots, respectively) and (A) maximum standardized uptake value (SUV_{max}), (B) total metabolic tumor volume (TMTV), and (C) total lesion glycolysis (TLG) is illustrated with regression lines and a 95% confidence interval (shaded area). (D) The heatmap representing correlation plots between radiomic and cellular parameters. Each square represents the correlation coefficients (R values) between the variables on its axes, with a cross mark indicating no statistical significance. NS: not significant.

specificity [Sp]: 0.69, sensitivity [Se]: 0.48) and OS (AUC: 0.62; Sp 0.6, Se 0.61), with corresponding cut-off values of 22.1% and 24.6%, respectively. For simplicity, CD4⁺ <24% was defined as low CD4⁺ cell infiltration for further analysis. In contrast, CD8⁺ and CD19⁺ cells demonstrated notably lower predictive value, with AUC approaching 0.5.

Regarding PET/CT findings, TMTV and TLG exhibited nearly overlapping ROC curves, with TMTV having a slightly higher AUC (3-year PFS AUC: 0.64, Sp: 0.73, Se 0.55 for TMTV *vs*. AUC: 0.63, Sp: 0.64, Se: 0.57 for TLG; 3-year OS AUC: 0.62, Sp: 0.68, Se: 0.57 for TMTV *vs*. AUC: 0.61, Sp: 0.61, Se: 0.57 for TLG). The cut-off value of TMTV for predicting both 3-year PFS and OS was determined to be 314 mL. Using these thresholds, 76 (44.4%) patients with low TI-CD4⁺ cells and 66 (38.6%) patients with high TMTV were identified.

Survival outcomes according to intratumoral CD4⁺ cell levels and tumor burden

Patient characteristics according to TI-CD4⁺ cell proportions are shown in Table 1. Comparable values for TMTV, baseline IPI components, IPI category, and COO were observed between groups with high and low intratumoral CD4⁺ cell burden, except for a lower prevalence of Eastern Cooperative Oncology Group performance status (ECOG PS) score ≥2 with marginal significance (14.5% *vs*. 27.4%, *P*=0.065) in those with low intratumoral CD4⁺ cells. Reflecting the smaller number of patients with impaired PS, those with low TI-CD4⁺ cells were significantly more likely to receive standard R-CHOP or more intensive chemotherapy than those without (98.7% *vs*. 91.6%, *P*=0.044). Notably, none of the patients with EBV-positive DLBCL were classified in the low TI-CD4⁺ cell group. Compared to EBV-negative DLBCL,

Table 1. Clinical characteristics of included patients according to tumor-infiltrating CD4⁺ cell levels.

Characteristics	Total N=171 (100%)	Patients with high TI-CD4⁺ cells N=95 (55.6%)	Patients with low TI-CD4⁺ cells N=76 (44.4%)	*P*
Age in years, median (IQR)	71 (61-79)	70 (62-78)	71 (61-79)	0.856
>60, N (%)	130 (76.0)	72 (75.8)	58 (76.3)	1
Female, N (%)	80 (46.7)	43 (45.3)	37 (48.7)	0.758
ECOGPS ≥2, N (%)	37 (21.4)	26 (27.4)	11 (14.5)	0.065
LDH >UNL, N (%)	116 (67.8)	64 (67.4)	51 (67.1)	1
Ann Arbor stage ≥3, N (%)	101 (59.1)	60 (63.2)	41 (53.9)	0.533
Number of EN lesions ≥2, N (%)	38 (22.2)	21 (22.1)	17 (22.4)	1
IPI, N (%)				
0-2	89 (52.0)	47 (49.5)	42 (55.3)	-
3-5	82 (48.0)	48 (50.5)	34 (44.7)	0.549
Disease type, N (%)				
DLBCL, NOS	156 (91.2)	82 (86.3)	74 (97.4)	0.013
t-FL	8 (4.7)	6 (6.3)	2 (2.6)	0.302
EBV-positive DLBCL	7 (4.1)	7 (7.4)	0 (0)	0.017
COO according to Hans algorithm, N (%)				
GCB type	48 (28.1)	25 (26.3)	23 (30.3)	0.647
non-GCB type	89 (52.0)	51 (53.7)	38 (50.0)	0.61
Missing	34 (19.9)	19 (20.0)	15 (19.7)	1
TMTV mL, median (IQR)	177.8 (47-560)	170.7 (29.5-536)	188 (73.1-646.4)	0.169
Cellular contents in tumor %, median (IQR)				
CD19⁺ cells	41.5 (20.5-60.3)	25.9 (12.7-40.5)	60.7 (46-74.9)	<0.001
CD3⁺ cells	49.8 (29.5-67.6)	65.4 (51.3-77.5)	23.1 (13.1-39.7)	<0.001
CD4⁺ cells	26.3 (13.8-42.7)	39.3 (31.7-52)	11.8 (5.9-19.2)	<0.001
CD8⁺ cells	16.2 (9.5-23.1)	18.2 (11.5-25)	12.4 (6.8-20.5)	0.002
Induction regimen, N (%)				
R-CHOP	149 (87.1)	82 (86.3)	67 (88.2)	0.82
More intensive chemotherapy	13 (7.6)	5 (5.2)	8 (10.5)	0.25
Less intensive chemotherapy	9 (5.3)	8 (8.4)	1 (1.3)	0.044
CR achievement at end of induction, N (%)	139 (81.3)	81 (85.3)	58 (76.3)	0.168

TI: tumor-infiltrating; IQR: interquartile range; ECOGPS: European Cooperative Oncology Group Performance Status; LDH: lactate dehydrogenase; UNL: upper normal limit; EN: extranodal; IPI: International Prognostic Index; DLBCL, NOS: diffuse large B-cell lymphoma, not otherwise specified; t-FL DLBCL transformed from follicular lymphoma; EBV: Epstein-Barr virus; COO: cell of origin; GCB: germinal center B-cell; TMTV: total metabolic tumor volume; R-CHOP: rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone; CR: complete remission.

EBV-positive cases had significantly higher proportions of intratumoural CD3⁺ (median 81.2% *vs*. 66.8%, *P*=0.016) and CD8⁺ T cells (median 22.9% *vs*. 15.8%, *P*=0.011), as well as a non-significant trend toward increased CD4⁺ cells (median 35.7% *vs*. 26.0%, *P*=0.093), which may represent the distinct immune-evasive properties of EBV-positive DLBCL (*Online Supplementary Figure S3*).[38]

Figure 3 shows the PFS and OS according to the levels of TI-CD4⁺ cells and TMTV. Despite the above-mentioned favorable clinical factors, which were biased towards patients with low TI-CD4⁺ cells, these individuals had significantly poorer PFS and OS compared to those with high TI-CD4⁺ cells (3-year PFS: 50.7%, 95% CI: 40.6-63.4 *vs*. 64.4%, 95% CI: 55.3-75.0, *P*=0.025; 3-year OS: 64.0%, 95% CI: 54.0-75.9 *vs*. 79.6%, 95% CI: 71.9-86.2, *P*=0.002). Even when restricted to the 27 patients with biopsy sites in EN lesions, significantly worse PFS and OS persisted (3-year PFS: 13.3%, 95% CI: 3.6-48.4 *vs*. 54.5%, 95% CI: 31.8-93.6, *P*=0.006; 3-year OS: 53.3%, 95% CI: 33.2-85.6 *vs*. 72.7%, 95% CI: 13.4-50.6, *P*=0.033) (*Online Supplementary Figure S4*). Similar to low TI-CD4⁺ cells, elevated TMTV also significantly worsened PFS (3-year PFS rate: 40.3%, 95% CI: 30.0-54.2 *vs*. 69.8%, 95% CI: 61.4-79.3, *P*<0.001) and OS (3-year OS rate: 60.2%, 95% CI: 49.4-73.4 *vs*. 80.5%, 95% CI: 73.2-88.5, *P*<0.001).

Intratumoral CD4⁺ cell levels influence the total metabolic tumour volume-based stratification

We also examined the prognostic impact of TI-CD4⁺ cells on TMTV-based outcome stratification (Figure 4). Patients with low TMTV and high TI-CD4⁺ cells showed markedly improved PFS (3-year PFS: 74.4%, 95% CI: 63.9-86.5) and OS (3-year OS: 84.7%, 95% CI: 75.9-94.4) compared to those

Table 2. Univariate and multivariate analysis for predicting progression-free and overall survival.

Characteristics	PFS						OS					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age in years >60	3.12	1.6-6.07	0.001	–	–	–	2.82	1.41-5.79	0.003	–	–	–
ECOGPS ≥2	2.79	1.73-4.48	<0.001	–	–	–	3.22	1.94-5.36	<0.001	–	–	–
LDH >UNL	2.25	1.31-3.84	0.003	–	–	–	2.21	1.23-3.99	0.008	–	–	–
Ann Arbor stage ≥3	1.6	1.01-2.54	0.042	–	–	–	1.28	0.78-2.09	0.32	–	–	–
Number of EN lesions ≥2	2.25	1.4-3.63	0.001	–	–	–	2.16	1.3-3.61	0.003	–	–	–
Intensive chemotherapy	0.55	0.2-1.51	0.251	–	–	–	0.75	0.27-2.07	0.585	–	–	–
Cell of origin*, non-GCB	1.33	0.78-2.28	0.29	–	–	–	1.4	0.77-2.56	0.261	–	–	–
IPI 3-5	2.64	1.67-4.17	<0.001	1.97	1.13-3.42	0.016	2.39	1.46-3.92	0.001	1.75	0.96-3.21	0.068
Low CD4+ cells	1.64	1.05-2.54	0.027	1.69	1.09-2.62	0.018	2.09	1.29-3.4	0.003	2.15	1.32-3.5	0.002
High TMTV	2.59	1.67-4.02	<0.001	1.76	1.03-3	0.036	2.56	1.58-4.15	<0.001	1.85	1.02-3.34	0.04

*The cell of origin was evaluable in 137 patients according to the Hans algorithm. PFS: progression free survival; OS: overall survival; R-ISS: revised-international staging system; ECOGPS: Eastern Cooperative Oncology Group Performance Status; LDH: lactate dehydrogenase; UNL: upper normal limit; EN: extranodal; GCB: germinal center B-cell type; IPI: International Prognostic Index; TMTV: total metabolic tumor volume; HR: hazard ratio; CI: confidence interval.

with high TMTV and low TI-CD4+ cells. The latter group experienced unfavorable outcomes, with a 3-year PFS of 32.3% (95% CI: 19.4-53.7) and a 3-year OS of 48.4% (95% CI: 33.6-69.6). The prognostic relevance of TI-CD4+ cells was pronounced among high-TMTV cases, with significant outcome differences observed (*P*=0.033 and *P*=0.006 for PFS and OS, respectively).

On univariate analysis, low TI-CD4+ cells, high TMTV, and IPI 3-5 were significantly associated with worse PFS (low TI-CD4+ cells hazard ratio [HR]=1.64, 95% CI: 1.05-2.54, *P*=0.027; high TMTV HR= 2.59, 95% CI: 1.67-4.02, *P*<0.001; IPI 3-5 HR= 2.64, 95% CI: 1.67-4.17, *P*<0.001) and OS (low TI-CD4+ cells HR=2.09, 95% CI: 1.29-3.40, *P*=0.003; high TMTV HR: 2.56, 95% CI: 1.58-4.15, *P*<0.001; IPI 3-5 HR=2.39, 95% CI: 1.46-3.92, *P*=0.001) (Table 2). All these variables retained a prognostic significance on multivariate analysis (all *P*<0.05 for PFS and OS), except for IPI 3-5 for OS (HR=1.75, 95% CI: 0.96-3.21, *P*=0.068).

Integration of tumor volume and its content enhances risk prediction based on International prognostic Index

Based on the results of the multivariate analysis, we constructed a novel predictive model that included the variables TI-CD4+ cell level, TMTV level, and IPI category. The ROC curve showed that the combined use of these three indices as continuous variables significantly outperformed their individual use in predicting 3-year OS (combined model AUC: 0.76, Sp: 0.72, Se: 0.76; IPI only AUC: 0.7, Sp: 0.59, Se: 0.7; CD4+ cell proportion only AUC: 0.62, Sp: 0.6, Se: 0.62; TMTV only AUC: 0.61, Sp: 0.68, Se: 0.61, all *P*<0.05) (Figure 5A). Finally, patients were categorized into four groups based on the number of risk factors defined as IPI scores of 3-5, low levels of TI-CD4+ cells, and high TMTV (Figure 5B). Among 89 patients with IPI 0-2, approximately half had additional risk factors of either low TI CD4+ cells or high TMTV (N=39, 43.8%) or both low TI CD4+ cells and high TMTV (N=7, 7.9%). Meanwhile, the resting 82 patients with IPI 3-5 were divided into those with one (N=17 [20.7%]), two (N=41 [50.0%], and three risk factors (N=24 [29.3%]). This stratification system almost equally discriminated the outcomes (Figure 5C, D). Patients without any risk factors had an excellent 3-year PFS of 85.6% (95% CI: 75.5-97.0) and OS of 92.9% (95% CI: 85.5-100). In contrast, patients with all three risk factors had the worst outcomes with a 3-year PFS of 33.3% (95% CI: 18.9-58.7) and OS of 50.0% (95% CI: 33.5-74.6).

Discussion

This study elucidated the relationship between pretreatment radiomic parameters and the proportions of various tumor-infiltrating cells in DLBCL. Neither malignant B cells nor other immune cells had a substantial impact on the maximum or average ^{18}FDG uptake and tumor volume, even within locally biopsied LN. Among the non-malignant cellular components, CD4+ cells had the highest and independent prognostic value, better stratifying patients with a similar tumor burden status. Consequently, the combination of low TI-CD4+ cells and high TMTV improved the prognostic performance of the routinely used IPI system.

Over the two decades since the development of rituximab and IPI, outcomes and classification of patients with DLBCL have been improved but remains unsatisfactory. Recently, two large studies from clinical trials (the REMARC [N=1305] and GOYA [N=301] studies) highlighted TMTV as a robust prognosticator, with the determined cut-off values ranging from 220 mL (41% SUV_{max} threshold method)[19] to 366 mL (a tumor threshold of 1.5 times the mean SUV of the liver +2 standard deviations).[18] The new International

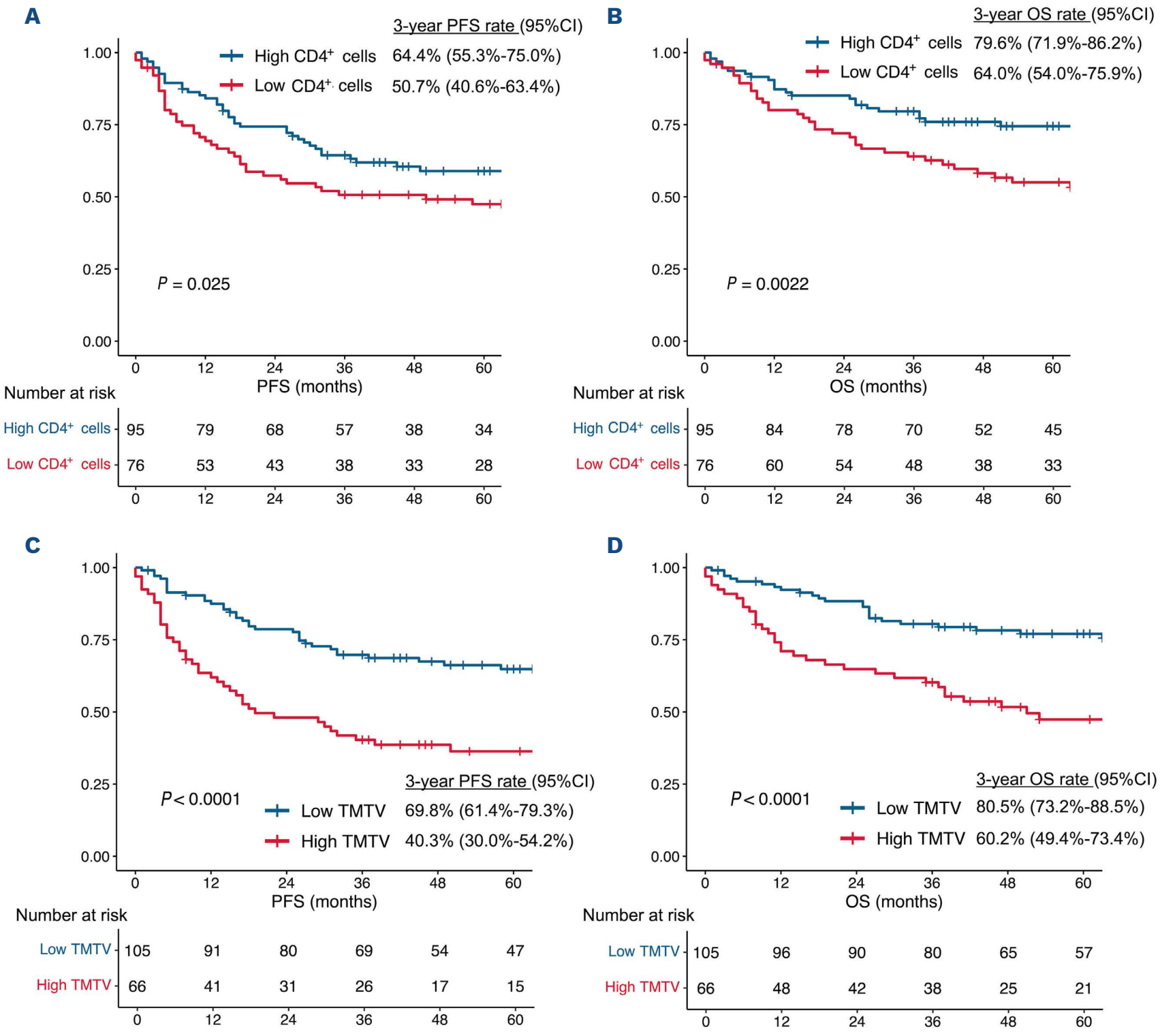

Figure 3. Kaplan–Meier estimates of progression-free and overall survival based on intratumoral CD4⁺ cell levels and total metabolic tumor volume values. (A) Progression-free survival and (B) overall survival (OS) according to intratumoral CD4⁺ cell levels. (C) PFS and (D) OS according to total metabolic tumor volume (TMTV) values. CI: confidence interval.

Metabolic Prognostic Index integrates TMTV as a continuous variable with age and stage, enabling robust and personalized patient outcome predictions.[39] Earlier studies suggested that the high baseline tumor burden leads to reduced exposure to rituximab and contributed to poorer outcomes.[40,41] Moreover, there has been growing interest in quantitative volumetric assessment with the discovery that high pretherapy TMTV is a major risk factor for early treatment failure of CAR T-cell therapies.[42,43]

Despite the limitation that the ^{18}FDG tracer detects glucose uptake by all viable cells, potentially diminishing the prognostic utility of PET/CT, the influence of intratumoral components on metabolic activities and tumor burden is scarcely studied in NHL. As of writing, the only existing study pertains to follicular lymphoma, where the malignant B-cell burden within individual lymphoma lesions was the main determinant of TMTV, and tumor-infiltrating T-lymphocytes positively influenced SUV_{max}.[44] Intriguingly, our DLBCL cohort presented contrasting results: only intratumoral CD4⁺ cells weakly contributed to a lower SUV_{max}, but not to the TMTV. Therefore, while the metabolic profile of the tumor is complex and might differ at the single-cell level,[45] our findings suggest that the total amount of intratumoral B and T cells plays a less significant role in the pre-treatment ^{18}FDG-PET/CT metrics in DLBCL.

Cell-mediated immunity plays a crucial role in controlling tumor growth and progression, including in DLBCL.[46] In line with our study, previous studies utilizing FCM-based

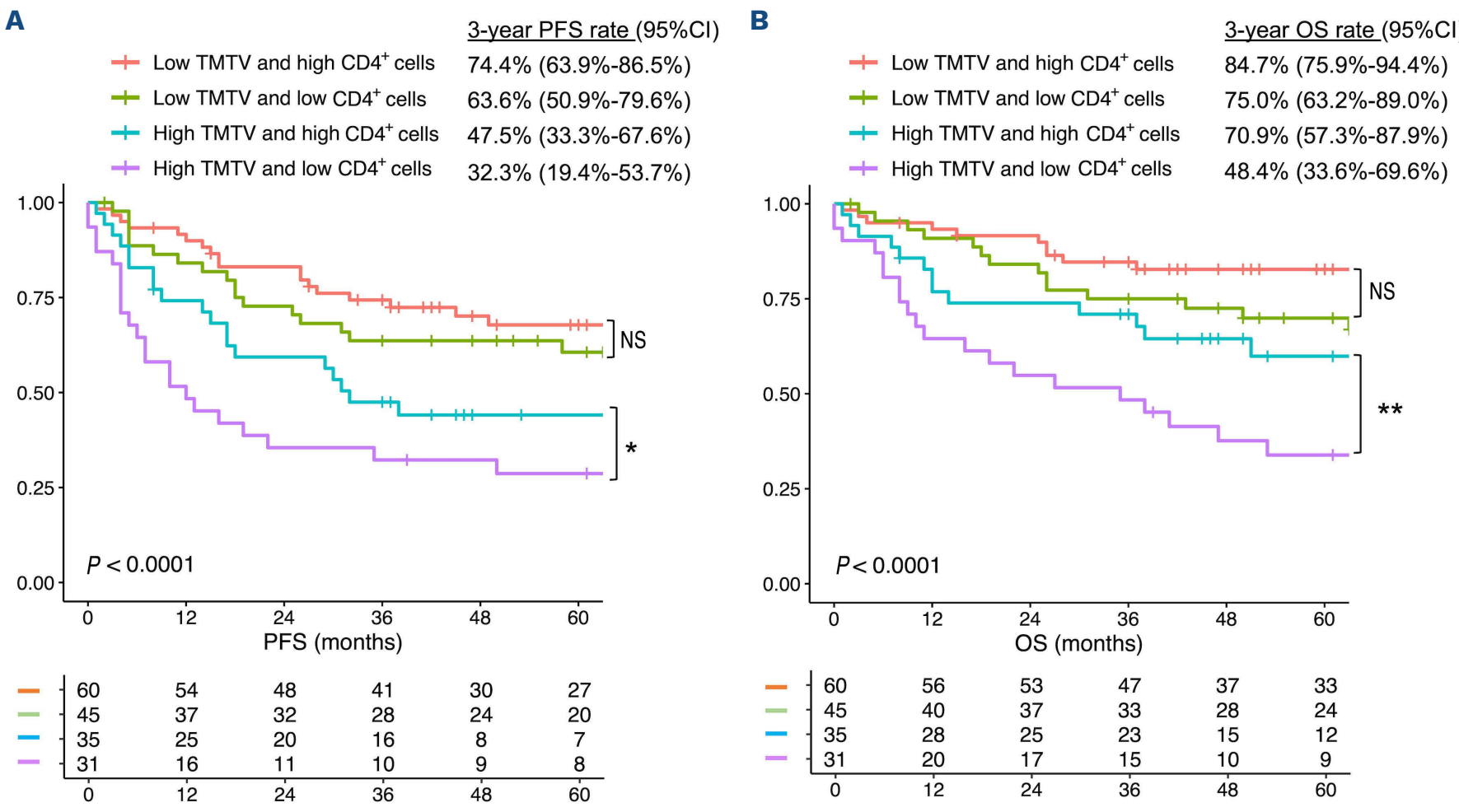

Figure 4. Survival analysis stratified by the combination of tumor-infiltrating CD4⁺ cells and total metabolic tumor volume. Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival (OS) using the combined model. * and ** represent *P* values of <0.05 and <0.01, respectively. TMTV: total metabolic tumor volume; NS: not significant; CI: confidence interval.

quantification methods also showed higher levels of CD4⁺ cell infiltration, but not CD8⁺ cell infiltration, which is associated with favorable outcomes in DLBCL.[31,33] Furthermore, the determined cut-off value of TI CD4⁺ cells (24%) in the current study is approximate to that of previous studies (20% and 23%). However, the reason why an abundance of CD4⁺ cells is associated with a good prognosis and which functional subpopulation mainly contributes to a favorable prognosis is not fully understood. One study discussed the responsibility of memory CD4⁺/CD45RO⁺ T cells,[33] whereas others demonstrated the beneficial impact of higher intratumoral CD4⁺/FOXP3⁺ regulatory T cells on DLBCL.[47] CD4⁺ T cells primarily activate other immune cells including CD8⁺ T cells rather directly eradicate tumor cells as effector T cells.[48] Given this hierarchical role in immune interaction, one possible explanation is that the prevalence of CD4⁺ cells within tumors may simply serve as a surrogate marker for a more efficient local immune surveillance.[33]

The strength of our study lies in the use of diagnostic modalities with easily derived parameters, allowing for rapid and straightforward implementation in clinical practice. However, several limitations need to be addressed in subsequent studies. First, the retrospective nature of the study design and the treatment heterogeneity (R-CHOP or R-CHOP-like regimen) in patients precluded us from drawing definitive conclusions. Second, the cohort size was relatively small, and only cases with sufficient tissue sampling were analyzed, which may have contributed to bias in the selection of cases. It should be noted that a large number of patients who faced challenges obtaining sufficient biopsy samples, especially those with predominantly extranodal lesions, were excluded from our cohort. Third, the phenotypic and functional diversity of intratumoral cells was not evaluated. Given the heterogeneity of CD4⁺ T cells, which can exhibit either suppressive or promotive effects on tumor immunity, a sole reliance on quantitative T-cell analysis may not yield a comprehensive understanding. The dysfunctional state known as T-cell exhaustion, marked by increased inhibitory receptors and decreased effector cytokines,[49] has been increasingly recognized for its prognostic significance in DLBCL.[25,26,29] Other immune cells, such as tumor-associated macrophages and dendritic cells, appear to establish a cross-talk with neoplastic cells and the tumor microenvironment.[24,50] Finally, the cell infiltration ratio derived from cell suspensions may not accurately represent the actual cell ratio observed by immunohistochemistry and could be overestimated.[51] Nevertheless, immunohistochemistry-based quantification is time-consuming, costly, and importantly method-dependent, which may lead to a higher risk of conflicting results.[52] From this perspective, easily obtainable and reproducible cell counting through FCM is suitable for routine use to complement TMTV-based risk prediction.

In conclusion, our study revealed that both high TMTV and

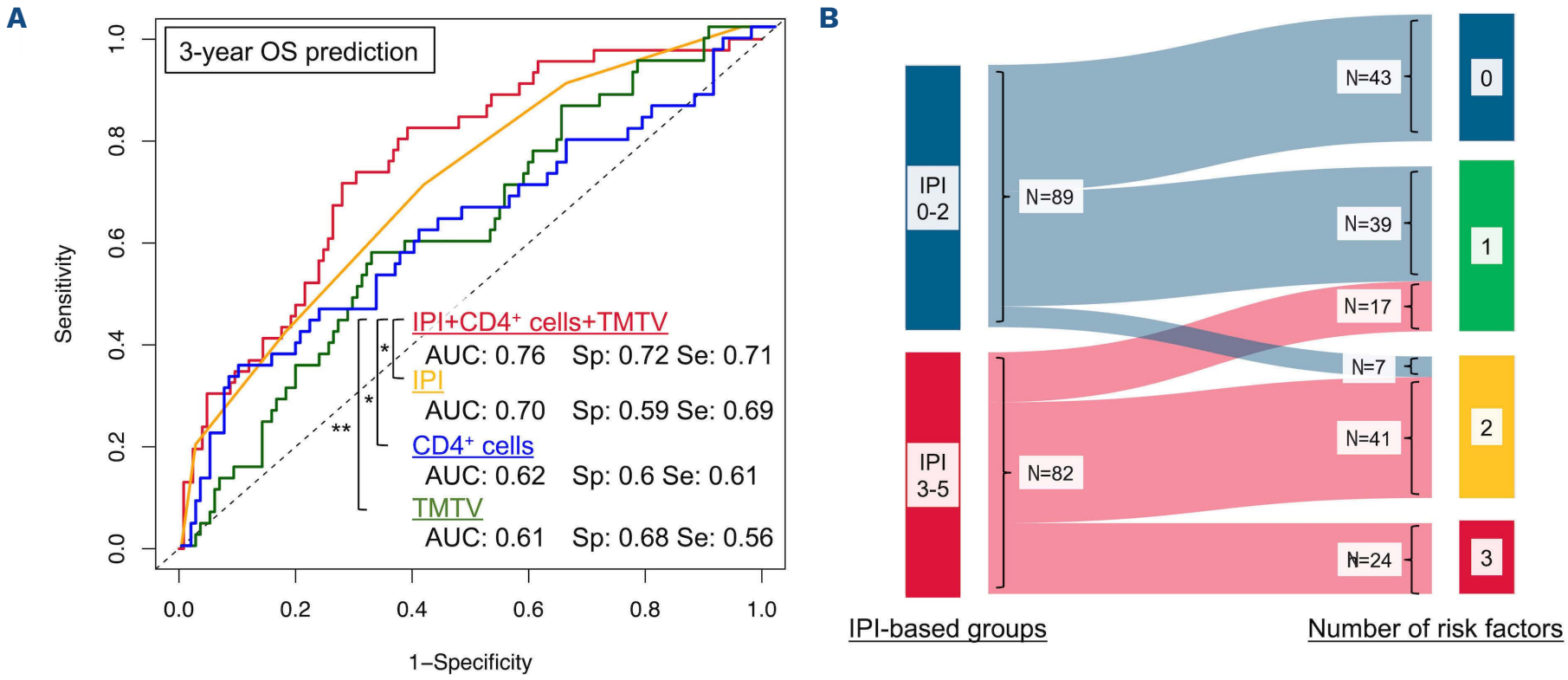

Figure 5. Integration of intratumoural CD4⁺ burden and total metabolic tumor volume into the International Prognostic Index system. (A) Receiver operating curve for predicting 3-year survival based on International Prognostic Index (IPI), CD4⁺ intratumoral cells, total metabolic tumor volume (TMTV), and their combination as continuous variables. * and ** represent *P* values of <0.05 and <0.01, respectively. (B) Alluvial diagram representing the transition of nternational Prognostic Index (IPI) category to risk factor number-based classification. (C) Progression-free survival (PFS) and (D) overall survival (OS) according to the number of risk factors (IPI 3-5, high TMTV, and low CD4⁺ cells in tumor). AUC: area under the curve; SE: sensitivity; SP: specificity; CI: confidence interval.

low TI CD4⁺ cells were independently associated with poor prognosis in newly diagnosed DLBCL. The combined use of these parameters more effectively distinguished outcomes between individuals within the same IPI 0-2 or 3-5 categories. This study underscores the need for simultaneous assessment of both tumor volume and tumor composition to augment the TMTV-based stratification systems.

Disclosures

KM received a research grant from AstraZeneca. All other authors have no conflicts of interest to disclose.

Contributions

DI and KM designed the study, interpreted the data, performed the statistical analysis, provided patient care, and

wrote the manuscript. YM interpreted the imaging findings. MO, AU, RT, KN, and MT provided patient care. All authors critically reviewed and approved the manuscript.

Acknowledgments

We would like to thank Takashi Nazuka, Tomoe Ando, and Tsubasa Murata (Haematology Section of Central Laboratory, Kameda Medical Center) for their excellent help in performing the flow cytometry analysis. We would also like to thank Dr. Hajime Senjo (Department of Hematology, Hokkaido University) and Dr. Kenji Hirata (Department of Nuclear Medicine, Hokkaido University) and Dr. Kenji Hirata (Department of Nuclear Medicine, Hokkaido University) for assisting with the use of the Metavol software. We would also like to thank Editage (www.editage.jp) for providing excellent English language editing assistance.

Data-sharing statement

The datasets generated and analyzed in the current study are available from the corresponding author upon reasonable request.

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