Clinical Cancer Research

Molecular Profile and FDG-PET/CT Total Metabolic Tumor Volume Improve Risk Classification at Diagnosis for Patients with Diffuse Large B-Cell Lymphoma

Anne-Ségolène Cottereau[1,2], Hélène Lanic[3,4], Sylvain Mareschal[4,5], Michel Meignan[6], Pierre Vera[1,2], Hervé Tilly[3,4], Fabrice Jardin[3,4], and Stéphanie Becker[1,2]

Abstract

Purpose: The prognostic impact of total metabolic tumor volume (TMTV) measured on pretreatment ^{18}F-FDG PET/CT and its added value to molecular characteristics was investigated in patients with diffuse large B-cell lymphoma (DLBCL).

Experimental Design: For 81 newly diagnosed patients with DLBCL treated with rituximab and CHOP/CHOP-like regimen, TMTV was computed using the 41% SUV_{max} thresholding method. According to the gene expression profile, determined using DASL (cDNA-mediated Annealing, Selection, Ligation and extension) technology, a subset of 57 patients was classified in germinal center B (GCB) or activated B-cell (ABC) subtypes and *MYC* or *BCL2* overexpressed.

Results: Median follow-up was 64 months. Five-year progression-free survival (PFS) and overall survival (OS) were 60% and 63% in the whole population. Median pretherapy TMTV was 320 cm^3 (25th–75th percentiles 106–668 cm^3). With a 300 cm^3 cutoff, patients with high TMTV ($n = 43$) had a 5-year PFS and OS of 43% and 46% compared with 76% and 78% for patients with a low TMTV ($P = 0.0023$, $P = 0.0047$). ABC status, *MYC*, or *BCL2* overexpression and both overexpression ("dual expressor," DE) were significantly associated with a worse PFS and OS. TMTV combined with molecular data allowed a significant better risk substratification of ABC/GCB patients, on PFS and OS. High TMTV individualized in molecular-low-risk patients a group with a poor outcome (*MYC*, PFS=51%, OS=55% *BCL2*, PFS=49%, OS=49% or DE PFS=50%, OS=50%) and a group with a good outcome (*MYC*, PFS=93%, OS=93% *BCL2*, PFS=86%, OS=86%, or DE PFS=81%, OS=81%).

Conclusions: The combination of molecular and imaging characteristics at diagnosis could lead to a more accurate selection of patients, to increase tailor therapy. *Clin Cancer Res; 22(15); 3801–9.* *©2016 AACR.*

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most frequent non-Hodgkin lymphoma (NHL), making up about 30% to 40% of diagnoses of NHL worldwide. The addition of rituximab (R) to the CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) backbone has improved the outcome of patients with DLBCL, from 45% 5-year progression-free survival (PFS) to 60% (1–3). However, despite these improvements, over 30% of patients treated with R-CHOP or R-CHOP–like chemotherapy will not respond or will relapse with resistant disease (4), with a majority of patients succumbing to their disease (1–3). These patients with a high risk of treatment failure or relapses cannot be selected accurately by the classic prognostic factors and new prognostics factors are under investigation at diagnosis, to identify these high-risk patients, which could benefit from an alternative therapeutic strategy.

FDG-PET/CT is now recommended as the best imaging tool in DLBCL and is used routinely for staging and response assessment (5). Prospective (6) and retrospective (7–9) studies have suggested that end treatment and early PET could be used as good prognosticators of outcome. New promising PET metrics derived from baseline PET, allowing an estimate of tumor burden and metabolism, are proposed as biomarkers: the total metabolic tumor volume (TMTV) which is the sum of the regions of the local tumors with FDG uptake; the total lesion glycolysis (TLG) which is the sum of these regions weighted by the intensity of their FDG uptake. Retrospective studies have at least shown that a high TMTV was significantly associated with a worse PFS and/or overall survival (OS) in DLBCL (10, 11) but also in other type of lymphoma, in primary mediastinal large B-cell lymphoma (12), in Hodgkin lymphoma (13), and in peripheral T-cell lymphoma (14).

The gene expression profiling (GEP) of DLBCL tumors has also been reported as a prognostic factor by identifying two

[1]Nuclear Medicine Department, Henri Becquerel Cancer Center and Rouen University Hospital, Rouen, France. [2]QuantIF–LITIS (EA 4108-FR CNRS 3638), Faculty of Medicine, University of Rouen, Rouen, France. [3]Hematology Department, Centre Henri Becquerel, Rouen, France. [4]UMR INSERM U918, Centre Henri Becquerel, Rouen, France. [5]Bioinformatics, University of Rouen, Mont Saint-Aignan, France. [6]Nuclear Medicine Department, Hôpital Henri Mondor, Créteil, France.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Anne-Ségolène Cottereau, Henri Mondor Hospital, 51 avenue du Maréchal de Lattre de Tassigny, Créteil 94010, France. Phone: 337-8671-1628; Fax: 331-4981-2794; E-mail: annesegolene.cottereau@aphp.fr

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Translational Relevance

The present study underlines the interest of combining functional imaging data (FDG-PET/CT) and biological molecular data obtained at diagnosis to improve risk stratification of patients with diffuse large B-cell lymphoma (DLBCL). This integrative procedure allows a more accurate classification of patients than each method. Although several prognostic models are based on surrogate of tumour burden this study uses as a prognostic parameter the total metabolic tumor volume (TMTV) measured on PET/CT. The results suggest that high TMTV is a negative prognosticator and that combined with molecular data (GCB/ABC phenotypes, MYC and BCL2 overexpression), it identifies in low-molecular-risk patients a subset of patients with poor outcome. This model used in DLBCL could be generalized to other lymphoma subtypes and other tumors.

main subtypes (15): those with gene expression reminiscent of germinal center B (the GCB group) and those with gene expression similar to activated B cells (the ABC group). The cell-of-origin (COO) phenotype has been demonstrated as a strong prognostic biomarker by different assays. Patients with GCB subtype have significantly better clinical outcome than those with an activated B-cell phenotype (15–17). Similarly, DLBCL with *MYC* and *BCL2* overexpression termed Dual expressors (DE) is usually considered as a subgroup with a poor outcome (18–20). Therefore molecular data, even if they are not used routinely, are potentially predictive biomarkers. The combination between these two prognosticators, FDG-PET/CT and gene expression profiling, has been recently investigated and our group has suggested that the response to treatment evaluated by interim PET combined with molecular profile could gain some interest in risk stratification (21).

The aim of the current study was to investigate the prognostic impact of baseline PET/CT metrics, including TMTV in patients with newly diagnosed DLBCL and their added value to molecular characteristics, including ABC/GCB status, *MYC*, and *BCL2* overexpression.

Materials and Methods

Study population

This retrospective analysis included consecutive patients diagnosed with DLBCL between October 2004 and January 2009, in the Centre Henri Becquerel (Rouen, France), with the following inclusion criteria: underwent a baseline FDG PET/CT, treated by front-line R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine, prednisolone) or R-ACVBP regimen (doxorubicine, vindesine, cyclophosphamide, bleomycin, prednisolone), DLBCL confirmed in all patients by a histopathologic review and tissue sample available from baseline biopsy.

The expression profile of 18 GCB/ABC–related genes, previously defined by Wright and colleagues (22), were determined, when frozen tissues were available, using DASL (cDNA-mediated Annealing, Selection, Ligation and extension) technology. According to the GEP, patients were classified as GCB or ABC subtype, as previously reported (21). *MYC* and *BCL2* mRNA expression were also determined by DASL technology.

The study was conducted in accordance with the precepts of the Helsinki declaration and received approval by our Institutional review board with a waiver of informed consent due to its retrospective nature.

PET/CT parameters

All patients underwent FDG-PET before the onset of chemotherapy. They were asked to fast for at least 6 hours before the injection of ^{18}F-FDG and to have blood glucose under 11 mmol/L. PET data were acquired for the mid-thigh toward the base of the skull, 60 to 70 minutes after injection of a weight-adjusted dose of 4 to 5 MBq/kg, on the Biograph 16 Siemens integrated PET CT scanner in the nuclear medicine department.

Quantitative parameters were computed by a nuclear medicine physician (A.-S. Cottereau) blinded to patient outcome, on semi-automatic software, Planet Onco, version 2.0; DOSISoft. Lesions were identified by visual assessment with PET/CT images scaled to a fixed SUV display and color table. Several parameters were measured: (i) TMTV was obtained by summing the metabolic volumes of all nodal and extranodal lesions. This method used the 41% SUV_{max} threshold method, as recommended by European Association of Nuclear Medicine (EANM), and because of its high interobserver reproducibility already described in lymphoma (23). A volume of interest was set around each lesion (node or organ involvement) as described previously (10, 13, 23). Bone marrow involvement was included in volume measurement only if there was focal uptake. Spleen was considered as involved if there was focal uptake or diffuse uptake higher than 150 % of the liver background; (ii) the TLG was the sum of the product of the metabolic volume of each local tumor times its SUV_{mean} ($TLG = \Sigma MTV_L \times SUV_{mean_L}$); (iii) the patient SUV_{max} was the highest SUV_{max} measured in the tumor sites.

Statistical analysis

The threshold to determine optimal cutoff values of the quantitative parameters for survival prediction was tested by Receiver Operating Curve (ROC) and by X-stile analysis. OS and PFS were defined according to the revised NCI criteria (24). Survival functions were estimated using the Kaplan–Meier (KM) method and compared using the log-rank test. Multivariate analyses were performed using a Cox proportional hazards model. Characteristics of population were compared between groups, using Fisher or χ^2 test appropriately. Differences between the results of comparative tests were considered significant if the two-sided *P* value was less than 0.05. Statistical analysis was conducted using MedCalc (MedCalc Software), and S-Plus7 software (Insightful).

Results

Eighty-one patients with a median age of 66 years (range, 22–87 years) were enrolled: 80% had stage III/IV, 73% an elevated LDH, and 2/3 (68%) an aa-IPI (age-adjusted International Prognostic Index) greater than 1. Median follow-up was 64 months (7–129 months). Relapse or progression occurred in 34 patients at a median of 11 months after diagnosis, and 31 patients died at a median of 17 months. The 5-year PFS was 60%, and the 5-year OS was 63% in the whole population. Sixty patients were treated by R-CHOP chemotherapy regimen (including 5 R-mini CHOP) and 21 by R-ACVBP. Fifty-seven

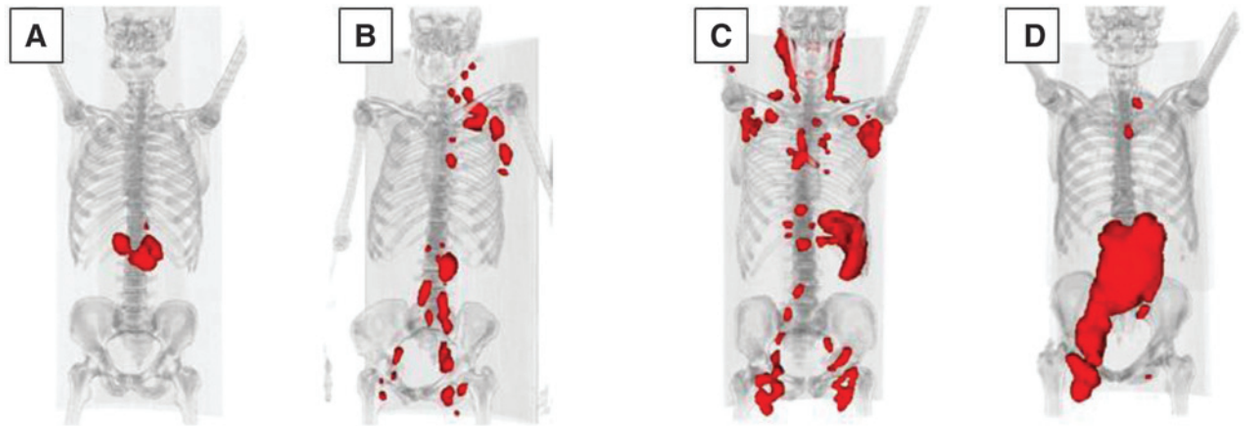

Figure 1.
Examples of patients with low TMTV; patient **A** (137 cm^3), patient **B** (280 cm^3), and patients with high TMTV; patient **C** (1,103 cm^3), patient **D** (1,363 cm^3).

patients had frozen material allowing molecular analysis. Their characteristics did not differ in age stage, aa-IPI, and treatment allocation from the whole population (Supplementary S1).

Quantitative PET parameters

In the whole population, the mean TMTV was 471 cm^3 (median 320 cm^3, 25th–75th percentiles 106–668 cm^3). There was a continuous increased of risk with TMTV for PFS and OS with a Cox model ($P = 0.043$ and $P = 0.031$ respectively). X-tile and ROC analysis revealed that the optimal TMTV cutoff value was 300 cm^3 for both estimating PFS and OS (Fig. 1). Areas under the raw ROC curves were 0.69 ($P = 0.0012$) for PFS and 0.68 ($P = 0.0037$) for OS. The 300 cm^3 cutoff value had a sensibility and a specificity of 73.5% and 63.8% for PFS and 74.2% and 62% for OS respectively. Kaplan–Meier curves show that TMTV, using this cutoff, was a strong prognostic factor of both PFS and OS. A high TMTV (TMTV> 300 cm^3, $n = 43$) was predictive of both PFS and OS at univariate level, and also for OS at multivariate level when TMTV was adjusted for baseline aa-IPI risk groups (Table 2). The 5-year estimates of PFS was 42% in the high-metabolic-burden group compared with 75% in the low-metabolic-burden group ($P = 0.0023$; hazard ratio [HR], = 3.0). Patients with a high TMTV had a 5-year OS of 46%, whereas 78% for patients with a low TMTV ($P = 0.0047$; HR, 3.0; Fig. 2). The 550 cm^3 cutoff, already proposed in DLBCL (10) was also able to divide our population on PFS and OS. Even its specificity was better (79% and 76%), its sensitivity was lower than the sensitivity of the 300 cm^3 threshold (50% for PFS and 48% for OS). Moreover, the separation of two groups of patients with different PFS and OS was less significant (HR, 2.59 and HR, 2.33, respectively).

Patients with a high TMTV had a more advanced disease, with significantly more advanced stage and Bulky disease (defined by a long axial nodal mass greater than 10 cm). A strong correlation was observed between aa-IPI and TMTV: more than 90% of patients with high TMTV had an aa-IPI >1 (Table 1). No significant difference of ABC/GCB subtypes, *MYC* or *BCL2* overexpression, or chemotherapy regimens have been observed between low or high TMTV groups (Table 1). Unlike TMTV, bulk measurement was not predictive of outcome. Age was strongly associated with bad prognosis ($P = 0.001$ on PFS and $P = 0.0035$ on OS using Cox model). TMTV and age were two independent prognostic factors of PFS and OS, without significant interaction on multivariate analysis, treating these variables either as continuous ($P = 0.03$ and $P = 0.024$ for TMTV; $P = 0.001$ and $P = 0.0034$ for age respectively) or dichotomized ($P = 0.0033$ and $P = 0.0075$ for TMTV > 300 cm^3, respectively; $P = 0.0038$ and $P = 0.0065$ for age >60-year-old).

The median TLG was 3677 (25th–75th percentiles 1066–6096). The ROC curve analysis showed an optimal cutoff value

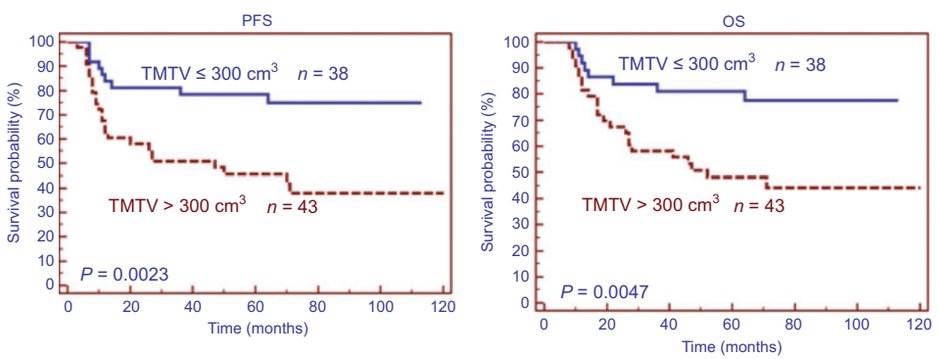

Figure 2.
Kaplan–Meier estimates of PFS and OS according to the baseline TMTV.

Table 1. Patient clinical characteristics for the whole population and stratified according to pretreatment TMTV with the 300 cm^3 cutoff.

Characteristics	Total population $n = 81$	Low tumor burden TMTV $\leq$ 300 cm^3 ($n = 38$)	High tumor burden TMTV > 300 cm^3 ($n = 43$)	*P*
Age >60 years	51 (63%)	24 (63%)	27 (63%)	NS
Female sex	37 (46%)	21 (55%)	16 (37%)	NS
ECOG 2–3	18 (22%)	3 (8%)	15 (35%)	0.008
Ann Arbor stage III-IV	65 (80%)	22 (58%)	43 (100%)	<0.0001
Bone marrow biopsy involved[a]	14 (18%)	4 (11%)	10 (23%)	NS
Serum lactate Dehydrogenase >1N	59 (73%)	20 (53%)	39 (91%)	0.0004
Bulky disease (>10 cm)	32 (40%)	4 (10%)	28 (65%)	<0.0001
aa-IPI 2-3	55 (68%)	15 (39%)	40 (93%)	<0.0001
R-CHOP	60 (74%)	28 (74%)	32 (74%)	NS
ABC subtype[a]	27 (47%)	11 (42%)	16 (52%)	NS
MYC overexpression[a]	26 (45%)	10 (38%)	16 (51%)	NS
BCL2 overexpression[a]	12 (21%)	5 (19%)	7 (22%)	NS

[a]BMB data available for 77 patients and GEP for 57 patients.

of 3,904, for both PFS ($P = 0.0007$) and OS ($P = 0.0042$). A high TLG ($n = 36$) was predictive of a higher disease progression rate (5-year PFS=41% vs 72% for patients with low TLG, $P = 0.0016$) and a worse survival (5-year OS 45% vs 73%, $P = 0.014$). However, TLG appeared less predictive of PFS and OS in univariate analysis compared with TMTV (Table 2), and did not retain prognostic value in multivariate analysis including aa-IPI as covariate.

Patient SUV_{max}, with a median of 18 (range, 4.6–45), was not related with outcome. No significant difference of SUV_{max} distribution has been found between patients with a high TMTV (median of 19.6; range, 7.3–36.3) and patients with a low TMTV (17, 4.6-45).

Molecular analysis

In the subset of patients where analysis of tumor RNA was available, the GCB/ABC phenotype was predictive of the outcome. As expected, patients with ABC subtype ($n = 27$) had a lower PFS than patients with GCB subtype ($n = 30$; 5-year PFS 38% vs 69%, $P = 0.019$) and a worse OS (5-year OS: 37% vs 73%, $P = 0.0046$; Fig. 3A; Table 2).

The optimal cutoffs determined by ROC and X-tile analysis for *MYC* and *BCL2* overexpression were 4,800 and 11,800, respectively, very close from the median (4,660 and 10,948, with a range of 1,656–12,249 and 3,448–12,985, respectively). Patients with overexpression of *MYC* ($n = 26$) had an increased risk of relapse or progression ($P = 0.0032$) and a reduced

Table 2. Prognostic impact of TMTV, ABC/GCB phenotype, MYC or BCL2 overexpression, and "DE" on PFS and OS.

	PFS Analysis		
	Univariate		Multivariate[a]
	HR (95% CI)	5-year PFS (95% CI)	HR (95% CI)
Low TMTV	1 (ref)	75% (67–82)	1 (ref)
High TMTV	3.06 (1.43–6.54)	42% (34–50)	1.61 (0.70–3.69)
Low TLG	1 (ref)	72% (65–79)	1 (ref)
High TLG	2.92 (1.45-5.90)	41% (33–49)	1.72 (0.18-3.62)
GCB	1 (ref)	69% (61–77)	1 (ref)
ABC	2.49 (1.13–5.50)	38% (28–48)	2.55 (1.15–5.66)
MYC negative	1 (ref)	72% (66–80)	1 (ref)
MYC positive	3.15 (1.40–7.08)	31% (22–40)	3.47 (1.53–7.8611)
BCL2 negative	1 (ref)	65% (58–72)	1 (ref)
BCL2 positive	4.40 (1.98–9.77)	9% (1–17)	2.97 (1.33–6.66)
DE negative	1 (ref)	62% (55–69)	1 (ref)
DE positive	4.50 (1.91–10.61)	0%	3.15 (1.33–7.44)
	OS analysis		
	Univariate		Multivariate[a]
	HR (95% CI)	5-year OS (95% CI)	HR (95% CI)
Low TMTV	1 (ref)	78% (71–85)	1 (ref)
High TMTV	3.01 (1.35–6.70)	46% (38–54)	3.0 (1.35–6.70)
Low TLG	1 (ref)	73% (66–80)	1 (ref)
High TLG	2.39 (1.16–4.92)	45% (36–54)	1.5 (0.68–3.28)
GCB	1 (ref)	73% (63–83)	1 (ref)
ABC	3.20 (1.37–7.50)	38% (28–48)	3.45 (1.47–8.11)
MYC negative	1 (ref)	75% (68–82)	1 (ref)
MYC positive	4.38 (1.81–10.60)	31% (21–41)	5.28 (2.15–12.97)
BCL2 negative	1 (ref)	65% (58–72)	1 (ref)
BCL2 positive	2.89 (1.22–6.86)	24% (10–38)	2.3 (0.97–5.48)
DE negative	1 (ref)	64% (57–71)	1 (ref)
DE positive	4.23 (1.70–10.52)	0%	3.71 (1.49–9.25)

Abbreviation: 95% CI, 95% confidence interval.
[a]Adjusted for aa-IPI score.

survival rates ($P = 0.0004$), with a 5-year PFS of 31% and 5-year OS of 31% in contrast 72% and 75%, respectively, for the others patients (Fig. 2B). In a subanalysis, *MYC* was predictive of outcome for AB patients (PFS 70% vs 16%, $P = 0.019$, HR, 3.8; OS 70% vs 16%, $P = 0.014$, HR, 4.1). It was not significant in GCB subtype.

Overexpression of *BCL2* ($n = 12$) was also related with a poor outcome (PFS: $P = 0.0001$; OS: $P = 0.01$; Fig. 3C).

Interestingly, *MYC* overexpression appeared to be more frequent in ABC subtype (65%) as well as *BCL2* overexpression (75%) compared with GCB subtype.

Combining *MYC* and *BCL2* expression allowed individualizing a subset of 9 patients with a double over expression (DE), with a dismal outcome: at 5 years, they relapsed and died (Fig. 3D). Eight of the nine patients had an ABC subtype.

Combining TMTV with molecular parameters

TMTV allowed a stratification of ABC/GCB patients ($P = 0.013$ for PFS and $P = 0.0036$ for OS): GCB patients with low TMTV ($n = 15$) had a 87% 5-year PFS and 87% 5-year OS compared with 53% and 60% for GCB with high TMTV ($n = 15$); values for ABC patients with TMTV ≤ 300 cm^3 ($n = 11$) were 50% and 60% compared with 30% and 23% for ABC with high TMTV ($n = 16$; Fig. 3A).

Combining *MYC* with TMTV split the population in 3 risk groups: *MYC*-negative patients with a low TMTV ($n = 16$, 5-year PFS of 93% and 5-year OS 93%); *MYC*-negative patients with a high TMTV ($n = 15$, 45% and 55%); *MYC*-positive patients whatever their TMTV0 ($n = 26$, 31% and 31%), $P = 0.0011$ and $P = 0.0005$, respectively (Fig. 2B). Similarly, TMTV allows a better risk substratification of *BCL2*-negative patients: 50% 5-year PFS and 49% 5-year OS for patients with a high tumor burden compared with 85% for both 5-PFS and OS (Fig. C).

A high TMTV individualized also in DE-negative patients a subset of patients with a worse outcome, 5-year PFS and OS of 50% and 49%, compared with 81% 5-year PFS and OS.

Discussion

The current study confirms the strong prognostic value of baseline TMTV in patients with DLBCL. Quantifying the volume rather than the single largest diameter of the mass gives a more relevant estimation of tumor burden than the bulk for prognostic purposes, particularly in advanced disease.

Different methods have been proposed to calculate the volume-based PET parameters either in solid tumors or in different subtypes of lymphoma. We used a 41% SUV_{max} threshold as recommended by European guidelines (25), and already tested in Hodgkin lymphoma, DLBCL (23). In the current study, a tumor volume greater than 300 cm^3 was associated with a dismal outcome. Patients with TMTV > 300 cm^3 had a significantly shorter 5-year PFS (43%) and 5-year OS (46%) than those with TMTV $\leq$ 300 cm^3 (76% and 78%, respectively). This optimal threshold is different from the optimal thresholds reported in DLBCL by two other studies either using the same or different methods, that is, 550 cm^3 in 114 patients or 220 cm^3 in 169 patients (10). These different cutoffs could be attributed to differences in the distribution of patient's age, stage, and treatment included in these retrospective series. For instance, the lower optimal threshold observed in our population compared with the 550 cm^3 reported by Sassanelli and colleagues might be explained by the older age of our patients. In their study, the median age was 56 years with 21 % of patients over 60 years compared with a median of 66 years old with 63% of patients >60 years in the current study. Indeed a lower value of tumor burden is probably sufficient to discriminate patients with good or bad prognosis in elderly. The number of events was also higher in our group (30% vs 20%), most of events occurring in oldest patients. In addition in both study there is a continuous increase of risk with the increase of TMTV. The 550 cm^3 could be applied in our population with a better specificity but a much lower sensitivity and would separate the survival curves of patients with low and high volumes albeit with less significance than the 300 cm^3.

The 220 cm^3 cutoff found by Song and colleagues could be explained by the difference in the percentages of patients with advanced stage, 41% versus 80% in our series. Moreover advanced stage patients in the Song series were all stage III. In our studies, TLG was also predictive of PFS and OS, as Kim and colleagues (26) have already reported, but was less significant than TMTV.

We also confirmed in the current study that ABC phenotype (16, 17), *MYC* (27, 28), and *BCL2* (20) overexpression are negative prognosticators, in agreement with previous reported data. Patients were classified high or low *MYC* or/and *BCL2* expression, according to their level of gene mRNA which suggests that gene mRNA level from DLBCL tumors could be a biomarker as already shown by Rimsza and colleagues for MYC (29). Quantitative methods raise the problem of cutoff point determination. Rimsza and colleagues found that the most significant cutoff point was the 80th percentile of the *MYC* mRNA expression, in our series the median appeared the best, but with both a wide range of significant cutoff points. The other methods to determine COO, or MYC and BCL2 expressions such as IHC have their own limitations. For instance, in some studies, non-GCB phenotype do not correlate with bad prognosis (30–32) as confirmed by a recent meta-analysis (33) and concordance between IHC and GEP is imperfect (34). Even though concurrent overexpression of both MYC and BCL2 had been associated with a poor outcome in several studies (18, 35), the recent European Society for Medical Oncology consensus (36) does not yet recommended changing therapeutic strategy according to these results due to problems in reproducibility of manual or visual IHC scoring and lack of agreement on the optimal positivity thresholds throughout the laboratories. In this setting, development of new GEP techniques may be promising (37).

Combination of tumor cell molecular genotypes with their functional properties expressed by the tumor glucose metabolism obtained from FDG-PET seems promising (38). Lanic and colleagues (21) have associated ABC/GCB signatures with interim PET response assessment, using delta SUV_{max}. GCB-DLBCL patients with early metabolic response had a favorable outcome in contrast with slow-responding patients who were characterized by an unfavorable outcome, regardless of their molecular subtype. However, interim PET is not yet recommended by European Society for Medical Oncology guidelines for clinical practice and exclusively intended for treatment outcome prediction in the setting of clinical trials (36). Delta SUV_{max} predict the response and outcomes with a particular treatment strategy (predictive factor) while TMTV stratify outcomes for individuals with a particular disease (prognostic factor). As COO phenotype, TMTV is a "true" prognosticator as it is available at baseline and independent from treatment. Therefore, it seems relevant to combine molecular profiles obtained at diagnosis with baseline PET metrics

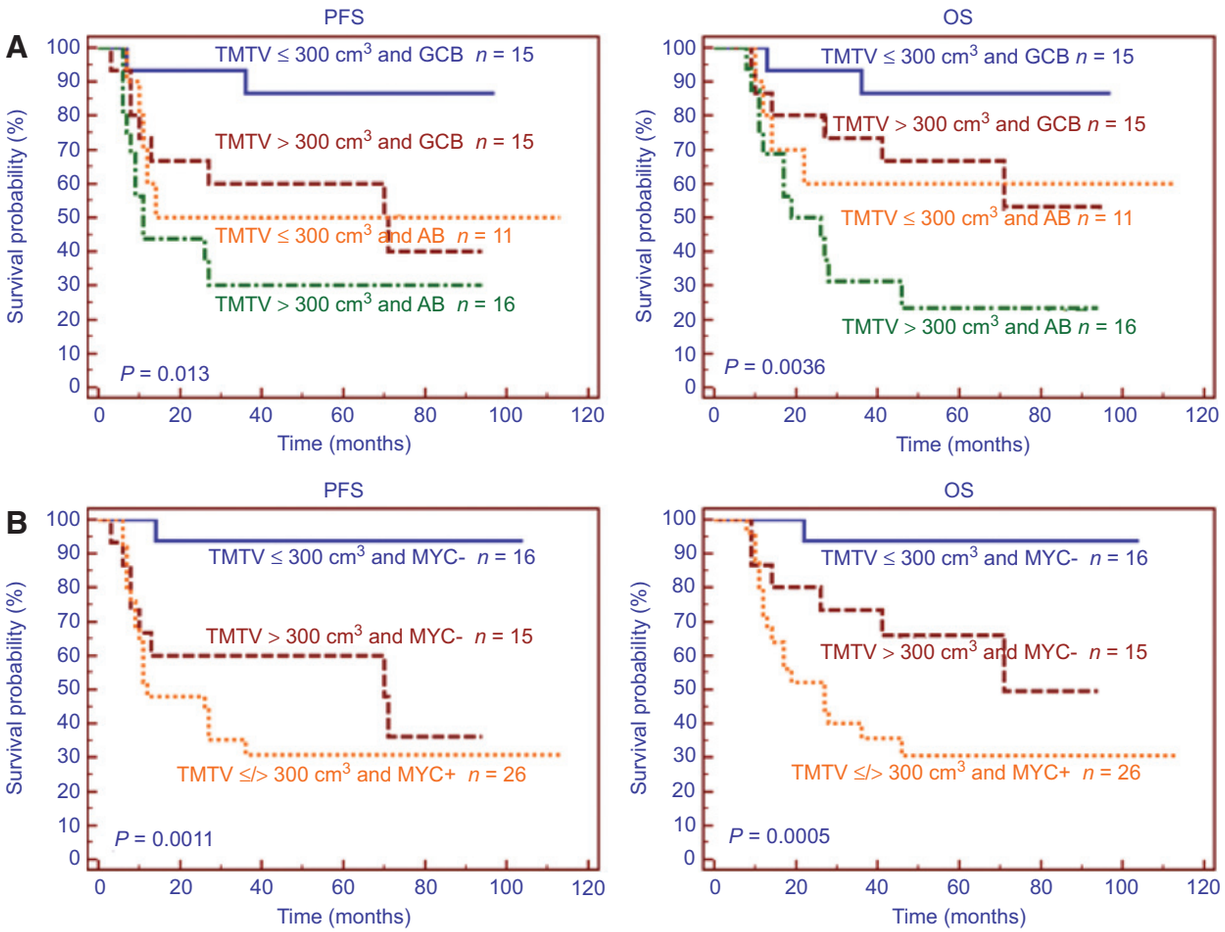

Figure 3.
Kaplan–Meier estimates of PFS and OS according to baseline TMTV with ABC/GCB phenotype (**A**), MYC (**B**), BCL2 (**C**) overexpression or "DE" (**D**). (*Continued on the following page.*)

derived from tumor metabolism, with the aim to develop new prognostic models.

Ours results suggested that TMTV gave added prognostic value on molecular risk analysis, either on ABC/GCB profiles or on negative *MYC*, *BCL2*, or double overexpression. Combining COO phenotype with TMTV stratified the population into four different prognostic groups. Among patients with GCB-DLBCL usually considered as low risk, a high TMTV brings down the 5-year PFS from 87% to 53%, the 5-year OS 87% to. 60%. Conversely, in ABC patients who are characterized by an unfavorable outcome, TMTV individualize a subset of patients with a better outcome, those with a low TMTV, with a 5-year PFS of 50% and 5-year OS of 60%. ABC patients with a high TMTV displayed a very poor outcome, with 5-year PFS of 30% and 5-year OS of 23%.

MYC expression associated with TMTV allowed a more accurate selection of patients than *MYC* alone dividing *MYC*-negative patients according to TMTV into distinct prognostic groups. In *MYC*-negative patients, representing more than half of the population (55%), a high TMTV individualized a group of patients, 26% of the whole population, with an inferior outcome than foreseen. Similarly, TMTV discriminate in *BLC2*-negative patients, which represent 79% of the population, one half of patients with a dismal outcome. The impact of TMTV is even more striking in patients without double *MYC*/*BCL2* overexpression (84% of patients) identifying 45% patients from the whole population with an inferior prognosis.

The current study highlighted the benefit of an integrative approach, including molecular data and quantitative PET metrics, at diagnosis for patients with DLBCL. TMTV could reclassify an important subset of patients: in the GCB group, in the *MYC* and *BLC2*-negative groups and in the non-DE patients. TMTV allows identifying in patients with negative molecular biomarkers a subpopulation which can no longer be considered at low risk.

The limitation of this study would be in the determination of the TMTV cutoff. The few studies (10, 11, 39) which have reported the prognostic value of TMTV on DLBCL have included a small number of patients (less than 150 patients) and are retrospective which can explain the cutoff discrepancies. It encouraged enlarging the analysis of the relevance of TMTV in a large cohort to stabilize the optimal threshold. However, the choice of the cutoff for a trial would depend on its primary objective which can be escalating of deescalating the treatment. This could be compared with the use of Deauville 5-point scale for interim PET-guided therapy. In RAPID (40) a de-escalating trial in Hodgkin lymphoma a cutoff over 2 was used to declare a patient positive to select for de-escalation patients truly negative. In contrast in RATHL

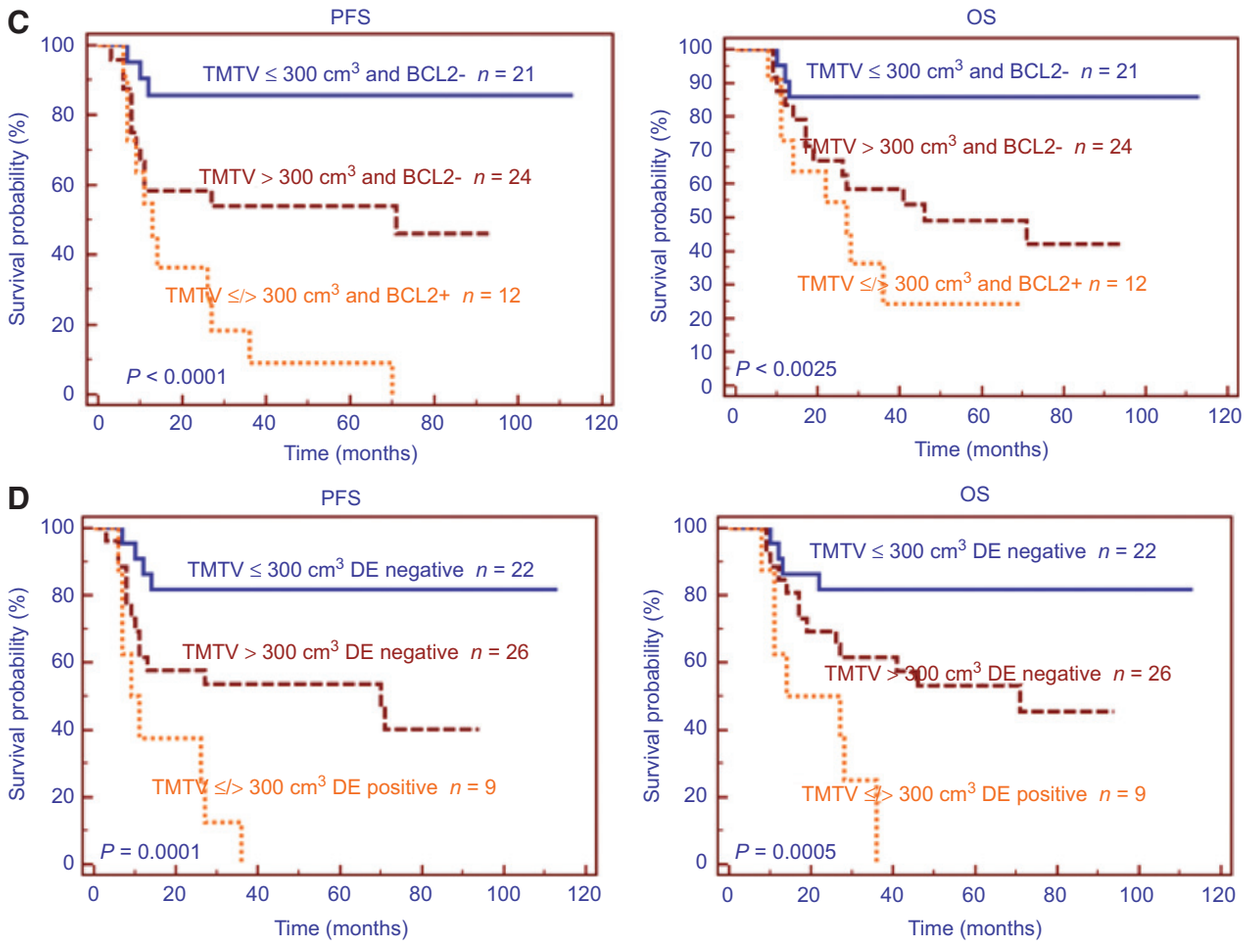

Figure 3.
(*Continued.*)

(41) an escalating trial a cutoff over 3 was used to score positivity to be more confident that patients were truly positive to escalate the treatment. Therefore, the TMTV threshold should be adjusted to the objective of the trial: lower volume threshold for getting higher sensitivity, higher volume threshold for getting higher specificity.

TMTV, as an early accessible prognostic factor, might be used to select patients for elective therapy in clinical trial. The next generation of prognostic models will probably incorporate PET scan metrics, other imaging results and sequencing data in conjunction with traditional clinical factors from the IPI index. These integrative risk models could more accurately stratify patients for novel or risk-adapted therapies, leading to a personalized medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A.-S. Cottereau, M. Meignan, F. Jardin, S. Becker
Development of methodology: A.-S. Cottereau, M. Meignan, P. Vera, F. Jardin, S. Becker
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.-S. Cottereau, H. Lanic, P. Vera, H. Tilly, F. Jardin, S. Becker
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.-S. Cottereau, S. Mareschal, M. Meignan, H. Tilly, F. Jardin
Writing, review, and/or revision of the manuscript: A.-S. Cottereau, H. Lanic, M. Meignan, P. Vera, H. Tilly, F. Jardin, S. Becker
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.-S. Cottereau, P. Vera, S. Becker
Study supervision: A.-S. Cottereau, M. Meignan, S. Becker

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