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ORIGINAL ARTICLE

Role of 18F-FLT PET/CT in suspected recurrent or residual lymphoma: final results of a pilot prospective trial

Lucia Zanoni[1] · Alessandro Broccoli[2] · Alessandro Lambertini[1] · Cinzia Pellegrini[2] · Vittorio Stefoni[2] · Filippo Lodi[1] · Cristina Fonti[1] · Cristina Nanni[1] · Pier Luigi Zinzani[2] · Stefano Fanti[1]

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Abstract

Purpose To evaluate the role of F-18-Fluorothymidine (FLT) PET/CT in lymphoma patients with suspected recurrent or residual disease.

Methods Adult lymphoma patients presenting with positive or equivocal F-18-FDG PET/CT at end-treatment or follow-up were prospectively addressed to an additional F-18-FLT-PET/CT. SUV max and tumour-to-background ratios (TBRs) were recorded for the most avid lesion. Biopsy or, when not available, clinical or imaging assessment were employed as standard of reference.

Results Overall 52 patients were recruited. Histology was available in 20/52 patients (38%), proliferation-index (Ki-67) in 14/20. Disease was excluded in 13/52 patients (25%) (one reactive follicular hyperplasia, five reactive-inflammatory tissues, four reactive nodes, two nodal sarcoid-like and one non-specific peri-caecal finding). FDG and FLT scans were concordant in disease restaging in 34/52 patients (65%), whereas in 18/52 cases (35%) relevant discrepancies were recorded. SUV max and TBR were significantly higher in the disease versus the disease-free group, with both tracers ($p = 0.0231$ and 0.0219 for FDG; $p = 0.0008$ and 0.0016 for FLT). FLT-SUVmax demonstrated slightly better performance in discriminating benign from malignant lesions (ROC-AUC: 0.8116 and 0.7949 for FLT-SUV max and TBR; 0.7120 and 0.7140 for FDG). Optimal FLT-SUV max cut-offs were searched: three would lead to 95% sensitivity, 81% accuracy, and 39% specificity, whereas seven led to 100%, 41%, and 56% respectively. No statistically significant correlation was observed between the two FLT indices and Ki-67.

Conclusions According to our results in a clinical setting of recurrent or residual lymphoma, FLT is not significantly superior to FDG and it is unlikely that it will be employed independently. FLT may be restricted to a few specific cases, as complementary to standard FDG imaging, to confirm a diagnosis or to define a better target to biopsy. However, due to FLT suboptimal performance, many findings would remain inconclusive, requiring further diagnostic procedures and reducing the effectiveness of performing an additional FLT scan.

Keywords Positron emission tomography · Lymphoma · F-18-FLT · F-18-FDG · Relapse

Introduction

PET/CT is extensively used in the assessment of patients with lymphoma, as Fluoro-18-deoxyglucose (FDG) is routinely employed to detect areas of active disease. Although FDG is a sensitive tracer, its distribution is not tumour-specific, since activated macrophages and other inflammatory cells may present increased glucose metabolism, and has been reported in aseptic inflammations (healing, burns) or septic bacterial, viral, or mycotic infections [1].

This is especially relevant in the setting of suspected recurrence or in follow-up, since several authors reported a high rate of false-positive (FP) results. For instance, Rhodes et al.

✉ Lucia Zanoni
lucia.zanoni@aosp.bo.it

[1] Nuclear Medicine, Azienda Ospedaliero-Universitaria di Bologna, Policlinico S.Orsola-Malpighi, Via Albertoni 15, 40138 Bologna (BO), Italy

[2] Hematology "L. e A. Seràgnoli", Azienda Ospedaliero-Universitaria di Bologna, Policlinico S.Orsola-Malpighi, Bologna, Italy

described an FDG-positive predictive value (PPV) of 53% in the follow-up of paediatric lymphoma patients [2]. Other authors reported significant FDG FPs in surveillance, with a PPV as low as 23% [3] and 19% [4] in a setting of adult diffuse large B cell lymphoma (DLBCL), while another group observed a PPV approaching 21% in aggressive non-Hodgkin lymphoma (NHL) [5]. A prospective study investigating the value of serial FDG-PET over time, in 421 lymphoma patients (160 Hodgkin lymphoma HL, 183 aggressive NHL, and 78 indolent follicular NHL) who achieved the first complete remission, demonstrated that, in the particular signs of FDG-PET positivity, histological confirmation by biopsy is mandatory because at least one-third of such patients can present benign conditions [6]. A more selected series suggested that a mediastinal FDG positivity should not be considered sufficient for diagnostic purposes in view of its lack of discrimination (actually not confirmed by histology in approximately 40%) [7].

Being aware of FDG limitations, F18–3′-fluoro-3′ deoxythymidine (FLT) was developed as a PET proliferation tracer, an attractive biological target in cancer imaging being a distinctive feature of tumour cells and a key component of tumour development and growth [8]. Thymidine is a native nucleoside which is utilized by proliferating cells for DNA replication during the S-phase of the cell cycle. FLT is handled by cytosolic thymidine kinase 1 (TK1), the salvage pathway key enzyme, whose activity is cell cycle-dependent and three-to-four times higher in malignant cells than in benign cells. Thus, FLT uptake is not related to glucose metabolism, and reflects essentially the activity of cytosolic TK1 [9]. Preclinical studies have shown a clear relationship between FLT maximum standard uptake value (SUVmax) and cancer proliferative activity measured by biochemical indices of proliferation [proliferating cell nuclear antigen (PCNA) immunostaining, Ki-67/MIB-1 or S-phase fraction) [10–13]. Given the high uptake of FLT in normal bone marrow and the high frequency of marrow involvement in lymphoma, and given its lower tumour-to-normal-tissue contrast than FDG outside the brain, the clinical usefulness of FLT-PET in staging lymphoma is limited [14]. On the other hand, one of the main advantages of FLT relies on its potential as a proliferation tracer, with an already established role in the monitoring of other primary neoplasms including breast, lung, and colorectal cancers and hepatocellular carcinoma [15]. A few studies have also underlined its capability to discriminate cancer from inflammation, i.e., in pancreatic, gastric, and lung malignant lesions, although other authors have observed significant FLT uptake in non-metastatic reactive lymph nodes, mostly due to reactive B lymphocyte proliferation [16]. Also, clinical studies have suggested a higher specificity and a relatively lower sensitivity for malignant cells, compared to the standard tracer FDG [17].

Wang et al. reported, in a setting of mantle cell lymphoma, a better performance for FLT than FDG in assessing disease and in performing an early treatment evaluation [18]. On the contrary, in a prospective head-to-head comparison of FDG and FLT in patients with follicular lymphoma (FL) and transformed FL (TF), Wondergem et al. documented that FDG PET was a better biomarker than FLT [19]. In a series of 34 malignant lymphomas, FLT SUV correlated with both tumour grading and proliferation fraction (Ki-67); thus, FLT was suggested to be a superior PET tracer in the detection of malignant lymphoma, especially in organs with high physiologic FDG uptake. In addition, a cut-off value of SUV = 3 was shown to accurately discriminate between indolent and aggressive lymphoma [14]. Later, FDG and FLT PET results were compared for the assessment of lymphoma residual mass: patients with FDG-positive and FLT-negative findings had a better outcome than those with both positive scans [20]. FLT was also successfully employed for early monitoring of response to treatment: in 22 high-grade NHL patients, an early decrease in lesion FLT uptake was detected after the administration of R-CHOP/CHOP but not after rituximab alone, indicating no early anti-proliferative effect of immunotherapy [21]. Other authors reported a higher specificity of FLT over FDG in distinguishing residual lymphoma from post-treatment inflammation, in a setting with at least a residual FDG-avid lesion after treatment [22]. The potential use of FLT-PET to implement risk-adapted treatment was suggested by a prospective study on 66 NHL patients treated with R-CHOP, where initial mean SUV appeared to be a negative predictor of response and correlated with the international prognostic index (IPI) score [23]. Finally, within a very small but prospective subset of mantle cell lymphoma, FLT presented a high sensitivity and a strong positive correlation with Ki-67 expression [24].

At the moment this study was conceived, FLT had never been prospectively investigated in the specific setting of lymphoma patients with suspected recurrence. A diagnostic tracer potentially able to identify an affected lymph node and to exclude the reactive process with high accuracy, and also to provide information on the proliferative activity of the tumour, could be of great value for treatment selection. In particular, the early and non-invasive characterization of equivocal FDG PET/CT findings might be helpful in cases of high-risk biopsy, reducing patient morbidity and discomfort. Moreover, FLT PET scanning is potentially able to evaluate whole tumour proliferation heterogeneity, which at present represents a major limitation in biopsy sampling [21].

Thus, we aimed at investigating its role in suspected lymphoma recurrence or residual disease, and whether the FLT result might be sufficient to exclude the need of biopsy, since to date pathologic confirmation is usually performed after positive FDG PET scanning, before starting treatment.

The primary aim of our research project was to evaluate the role of FLT PET/CT in lymphoma patients presenting with positive or equivocal FDG PET/CT at the end of treatment

or during follow-up. The secondary aim was to assess FLT PET/CT performance in comparison to standard FDG and biopsy performed on the suspicious area.

Materials and methods

A monocentric, prospective study, approved by the Institutional Review Board, was performed. It aimed at enrolling a consecutive cohort of adult patients, diagnosed with malignant lymphoma of all histological types, presenting with a positive or equivocal FDG-PET/CT scan at end of treatment or during follow-up. Breastfeeding patients and minors were excluded, as were those patients in life-threatening conditions, mentally unstable or refusing written consent. Written informed consent was obtained from all individual participants included in the study. All subjects were clinically followed and enrolled by the Haematology Unit of our Centre and, within approximately 3 weeks after the previous FDG PET scan, underwent a further PET/CT scan with the experimental radiotracer 18-F-FLT (FLT) as an additional diagnostic procedure, following routine examinations.

All PET/CT scans were performed in accordance with the standard EANM PET/CT acquisition guidelines [25] at the Nuclear Medicine Unit. Both the employed radiotracers (FDG and FLT) were synthesized in loco in the Radiochemistry laboratory. Initially, or in case of limited availability, FLT was purchased from a commercial provider (IASON). Scanning was performed after intravenously injecting approximately 370 MBq (10 mCi) of the selected radiopharmaceutical, with an optimal uptake time of approximately 60 min. Images were acquired on PET/CT tomographs (General Electric, Discovery STE): PET scan was acquired for 2 min for bed position, with a standard field from the base of the skull to mid-thighs (with the exception of other specific clinical indications). The emission data were corrected for scatter, random coincidence events, and system dead-time using the provided software. An iterative reconstruction (ordered-subsets expectation maximization with two iterations, followed by smoothing with a 6-mm 3D Gaussian kernel) and CT-based attenuation correction were utilized to obtain the final images.

All scans were interpreted by at least two experienced nuclear medicine physicians and classified into three distinct categories (positive, negative, or inconclusive). It should be noted that the Deauville 5-point scale for FDG uptake assessment, at the moment this study was conceived and in this specific setting, was still not yet internationally recommended.

Visual positivity was defined as a visibly higher uptake, as compared to the surrounding background, not including areas of physiological radiotracer accumulation. The scan was interpreted as negative when no pathologic tracer uptake was shown.

Areas of focal uptake were interpreted as unequivocally positive for lymphoma when they were localized at sites of previous disease (residual disease or relapse), within asymmetric lymph nodes, or within lymph nodes unlikely to be affected by inflammation (i.e., mediastinal, except for hilar, and abdominal). Sites of known physiologic uptake that showed symmetric uptake were considered non-pathologic (negative). Other sites of increased uptake were described, and attributed to a definite or possible cause. When uptake was likely to be suspicious for other diseases but unrelated to lymphoma, the report was judged as negative for lymphoma; however, if increased uptake could not be attributed to a definite cause and the possibility of malignant lymphoma could not be excluded for certain, the scan was considered inconclusive. Inconclusive scans in particular indicated challenging situations in reporting a PET scan which, in most cases, would not correspond to recurrence but in which relapse could not be ruled out, thus requiring a careful and thorough clinical/diagnostic evaluation.

Semi-quantitative analysis results were obtained by calculating the maximum standard uptake value (SUV max) of the most avid lesion for each scan [circular regions of interest (ROI) > 70 pixels were drawn on trans-axial images), and by evaluating its target-to-background-ratio (TBR) using the target lesion SUV max relative to the liver SUV mean (measured within a 5-cm ROI on the healthy liver, in particular right lobe). Nonetheless, PET scans were not categorized on the basis of a threshold SUV max or TBR value, but by taking into account all available data, and in particular the site and degree of the radiotracer accumulation.

Clinical information and FDG PET results were available to FLT PET readers.

Histopathological results were considered the standard of reference, if sampling was clinically viable. When biopsy was not a clinically valid option due to practical or ethical reasons, clinical judgment and subsequent laboratory and imaging follow-up were considered to validate PET results (mean follow-up 10 months; range 6–41).

Re-staging was then performed in accordance with the Ann-Arbor staging system [26, 27].

Statistical analysis was performed employing the STATA® statistical software package. FLT PET/CT semi-quantitative data (SUVmax and TBR) were correlated to FDG data through a patient-based linear correlation. A non-parametric test (Wilcoxon–Mann–Whitney test) was employed to investigate the capability of both tracers to differentiate between benign lesions and malignancy. Furthermore, receiver operating characteristic (ROC) curves were defined in an attempt to identify an optimal SUV-based cut-off value. The performance of the two tracers (in terms of area under the curve — AUC) was also compared. When histopathological data (Ki-67, %) were available, patients were categorized into four groups (1: 0–25%; 2: 25–50%; 3: 50–75%; 4: 75–100%), and

a Spearman's rank correlation coefficient was calculated in order to investigate the correlation between FLT semi-quantitative data and the available proliferation index.

Results

Overall, from May 2010 to September 2016, 52 patients were enrolled: 32 males and 20 females, with a median age of 60 years (average: 55; range: 20–70). Of the above individuals, 40/52 (77%) had a clinical history of NHL, while 12/52 (23%) had a clinical history of HL. The former category included 15/40 follicular lymphomas (FL) (38%) and 17/40 DLBCL (42%), of which at least 3/17 (18%) transformed, 2/40 (5%) MALT, 4/40 (10%) mantle cell lymphoma (MCL), 2/40 (5%) T-cell lymphomas. As for the HL patient cohort, it included 1/12 classic HL (8%), 1/12 mixed-cells (8%), 2/12 lymphocyte-rich (LRCHL) (16%), and 8/12 not specified (68%). Regarding clinical indication for PET imaging, 7/52 patients (13%) were recruited at end-treatment, while 45/52 (87%) were recruited during follow-up. Patients characteristics are presented in Table 1.

Table 1 Patient characteristics

Patient characteristics	
Number of patients	52
Gender (female; male)	20; 32
Mean age (years)	55
Lymphoma type	No. of patients
Non-Hodgkin (NHL)	40/52 (77%)
Hodgkin (HL)	12/52 (23%)
Lymphoma sub-type (NHL)	No. of patients
FL	15/40 (38%)
DLBCL	17/40 (42%)
MALT	2/40 (5%)
MCL	4/40 (10%)
T Cell	2/40 (5%)
Lymphoma sub-type (HL)	No. of patients
Classic	1/12 (8%)
Mixed-cells	1/12 (8%)
LRCHL	2/12 (17%)
Not specified	8/12 (67%)
PET/CT indication	No. of patients
End-treatment	7/52 (13%)
Follow-up	45/52 (87%)

FL follicular lymphoma, *DLBCL* diffuse large B Cell lymphoma, *MALT* mucosa-associated lymphoid tissue, *MCL* mantle cell lymphoma, *LRCHL* lymphocyte-rich lymphoma

Recurrent or residual disease was excluded in 13/52 patients (25%), who were finally diagnosed as: 1/13 reactive follicular hyperplasia (8%), 5/13 reactive-inflammatory tissue (38%), 4/13 reactive nodes (31%), 2/13 nodal sarcoid-like disease (15%), 1/13 unspecified peri-caecal finding (8%). The validation of PET/CT results with histopathology was available only in 20/52 patients (38%), because biopsy sampling was not a possible option in 32/52 patients (62%).

The classification of PET/CT scan into positive, negative, or inconclusive, according to visual interpretation, is presented in Table 2. In particular, at standard imaging 43/52 patients (83%) resulted FDG-positive, of whom 37/43 (86%) later confirmed as true positives (TP), and 9/52 patients (17%) FDG-inconclusive, of whom only 2/9 (22%) with proven active disease. With regard to FLT, 33/52 showed FLT-positivity (63%) [of these, 31/33 were TP (94%)], 17/52 were FLT-inconclusive, with proven active disease in 8/17 of these cases (47%), and two patients were true FLT-negative.

Overall, the majority of patients (32/52 individuals, 62%) showed both FDG- and FLT-positivity, with 30/32 TP (94%). Nine/52 patients (17%) were FDG-positive but FLT-inconclusive (7/9 TP, 78%). Eight/52 patients (15%) were both FDG- and FLT-inconclusive (1/8 TP, 12%). Only one patient out of 52 (2%) was FDG-inconclusive but FLT-positive (1/1 TP, 100%), whereas 2/52 patients (4%) were FDG-positive but FLT negative (in 2/2 the disease was correctly excluded by FLT).

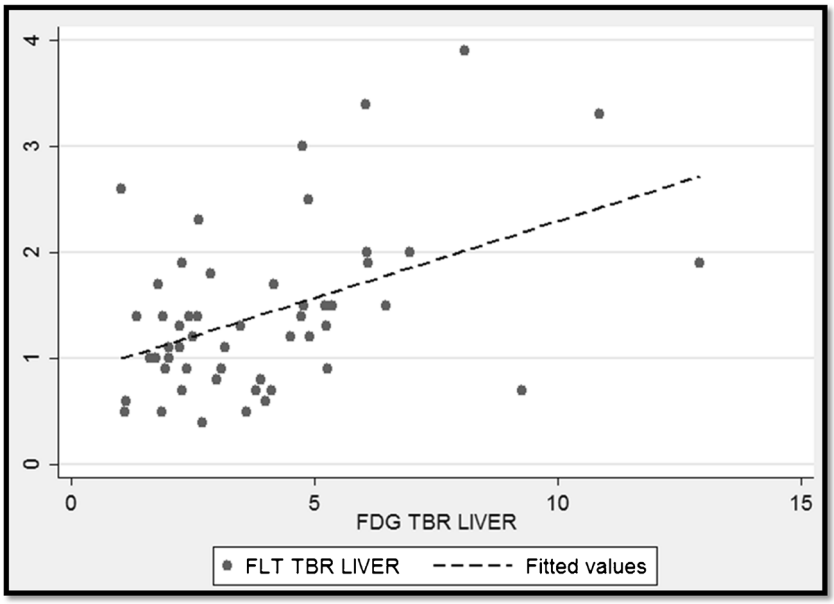
A statistically significant correlation was found when performing a linear correlation between FLT- and FDG target-to-background ratios (TBR) ($r = 0.46$; $p = 0.0007$) (Fig. 1).

Wilcoxon–Mann–Whitney test of our semi-quantitative parameters (Table 3) demonstrated significantly higher TBR and SUV max values in patients presenting with recurrent or residual lymphoma when compared to the disease-free group, for both tracers ($p = 0.0231$ and 0.0219 for FDG; $p = 0.0008$ and 0.0016 for FLT respectively).

Table 2 PET/CT results (visual analysis)

PET/CT results (visual analysis)		
Total patients:	52	True positive (TP)
FDG-positive:	43/52 (83%)	TP: 37/43(86%)
FDG-inconclusive:	9/52 (17%)	TP: 2/9 (22%)
FLT-positive:	33/52 (63%)	TP: 31/33 (94%)
FLT-inconclusive:	17/52 (33%)	TP: 8/17 (47%)
FLT-negative:	2/52 (4%)	TP: 0/2 (0%)
FDG- and FLT-positive	32/52 (62%)	TP: 30/32 (94%)
FDG-positive and FLT-inconclusive	9/52 (17%)	TP: 7/9 (78%)
FDG- and FLT-inconclusive	8/52 (15%)	TP: 1/8 (12%)
FDG-inconclusive and FLT-positive	1/52 (2%)	TP: 1/1 (100%)
FDG-positive and FLT-negative	2/52 (4%)	TP: 0/2 (0%)

Fig. 1 FDG-FLT TBR correlation



The performance of both the employed tracers, in terms of area under the curve (AUC), in discriminating between benign findings and malignancies (recurrent or residual disease) resulted in a slightly better performance of FLT-SUVmax (AUC = 0.8116), if compared to the other employed semi-quantitative parameters (FLT-TBR AUC = 0.7949; FDG-TBR AUC = 0.7140; FDG-SUV max AUC = 0.7120) (Table 4 and Fig. 2).

We also searched for optimal FLT-SUV max cut-offs. The receiver operating characteristic (ROC) curves showed 95% sensitivity, 81% accuracy, but only 39% specificity for an FLT-SUV max cut-off value ≥ 3. If considering a higher cut-off value ≥ 7, our specificity reached 100% but the relative sensitivity was 41% only, with an accuracy of 56% (Fig. 3).

The proliferation index, as measured by Ki-67, was reported in 14 out of the 20 histopathological analyses and classified in 4 groups (0–25%; 25–50%; 50–75%; 75–100%). No statistically significant correlation was observed between the two FLT indices (SUV max and TBR) and Ki-67 groups (Fig. 4).

Finally, based on FDG and FLT PET/CT results, a disease re-staging was performed, according to the Ann-Arbor system, observing a concordance between the employed tracers in 34/52 of cases (65%), while in the remaining 18/52 cases (35%) relevant discrepancies in interpretation were recorded.

Figures 5 and 6 show a TN FLT case and a FP FLT case respectively.

Table 3 Disease vs disease-free: two-sample Wilcoxon rank-sum (Mann–Whitney) test

Disease vs disease-free: two-sample Wilcoxon rank-sum (Mann–Whitney) test			
FLT (TBR liver)			
	Range (TBR)	Mean (TBR)	
Disease	0.4–3.9	1.6	
Disease-free	0.5–1.8	0.9	
			$p = 0.0016$
FLT (SUVmax)			
	Range (SUVmax)	Mean (SUVmax)	
Disease	1.7–16.8	7.0	
Disease-free	1.2–6.8	3.9	
			$p = 0.0008$
FDG (TBR liver)			
	Range (TBR)	Mean (TBR)	
Disease	1–12.9	4.4	
Disease-free	1.1–4	2.7	
			$p = 0.0219$
FDG (SUVmax)			
	Range (SUVmax)	Mean (SUVmax)	
Disease	3–31	10	
Disease-free	2.3 - 8.8	5.9	
			$p = 0.0231$

Table 4 Area under the curves (AUC) per SUVmax and TBR

	^{18}F-FDG	^{18}F-FLT
AUC per SUVmax measurement		
AUC	0.712	0.812
95% confidence interval for AUC	0.57–0.86	0.69–0.93
AUC per TBR (liver) measurement		
AUC	0.714	0.795
95% confidence interval for AUC	0.57–0.86	0.65–0.94

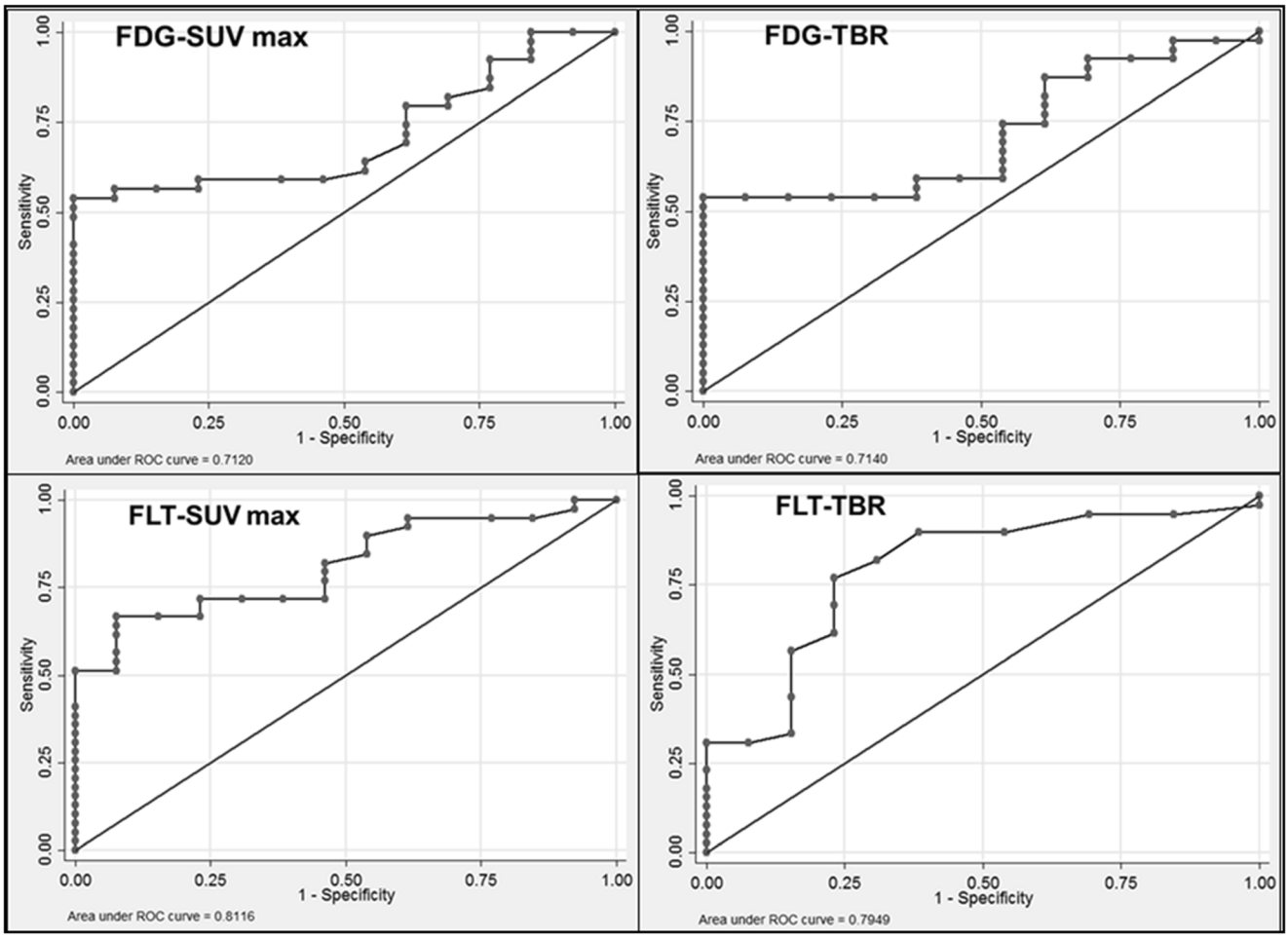


Fig. 2 ROC analyses

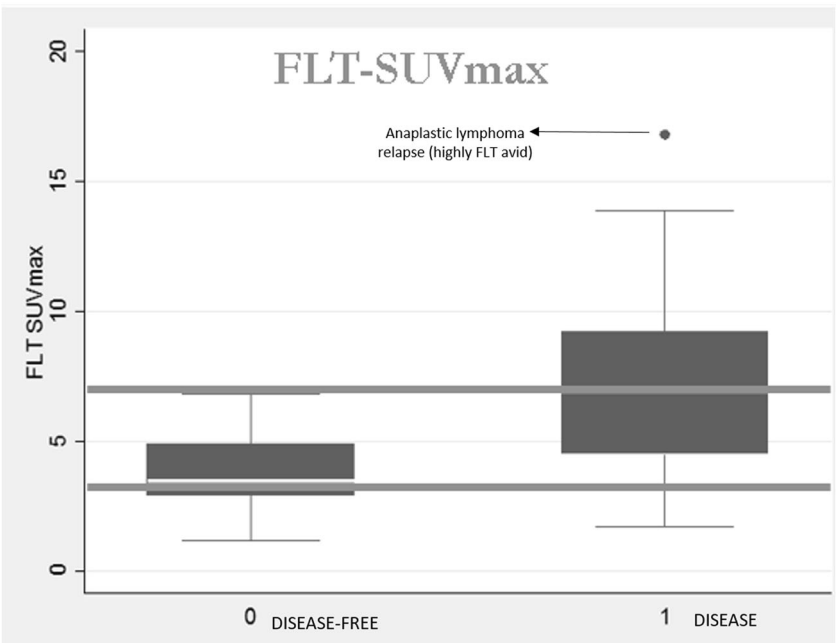


Fig. 3 Optimal cut-off values of FLT-SUVmax

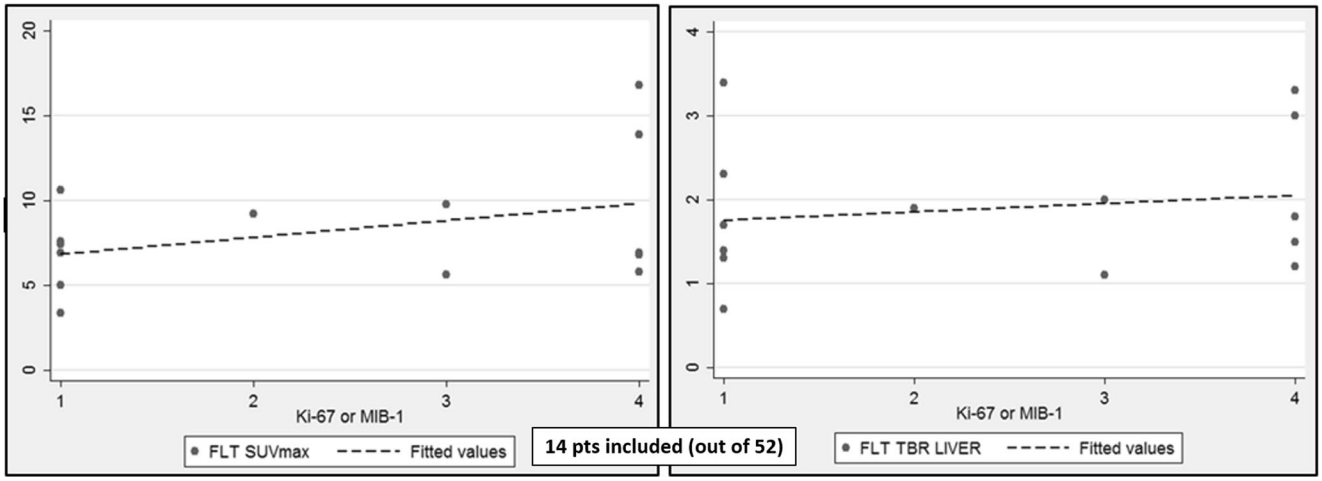


Fig. 4 Correlation of FLT parameters to proliferation index

Discussion

Ideally, FLT could differentiate malignant involvement from inflammatory lymph nodes, which are common findings in lymphoma patients and turn out falsely positive at FDG PET/CT scanning. In this sense, it would allow for a non-invasive confirmation of malignant disease, therefore optimizing lymphoma patient management by eventually reducing the number of potentially risky surgical procedures required to justify an extra line of chemotherapy. Furthermore, during the planning phase of our experimental design, F-18-FLT had never been prospectively employed as an experimental PET/CT tracer, to our knowledge, in a clinical setting of suspected residual disease or recurrence of lymphoma. At the same time, its role in assessing lymph node proliferation, through a direct comparison with histopathological and/or clinical findings, was still not clear.

However, the initial enthusiasm about the higher specificity of FLT has been tempered by the observations that FLT uptake also occurs in granulomatous inflammatory lesions such as tuberculosis and in reactive lymph nodes, related to the high proliferation rate of macrophages and B-lymphocytes respectively [28]. Other authors also questioned FLT effectiveness in differentiating between reactive lymph nodes and nodal disease in a setting of head-and-neck cancer [29]. Nevertheless, a recent review of the available literature for several tumour species including lymphoma confirmed the role of FLT PET/CT as a good predictor of early response to therapy,

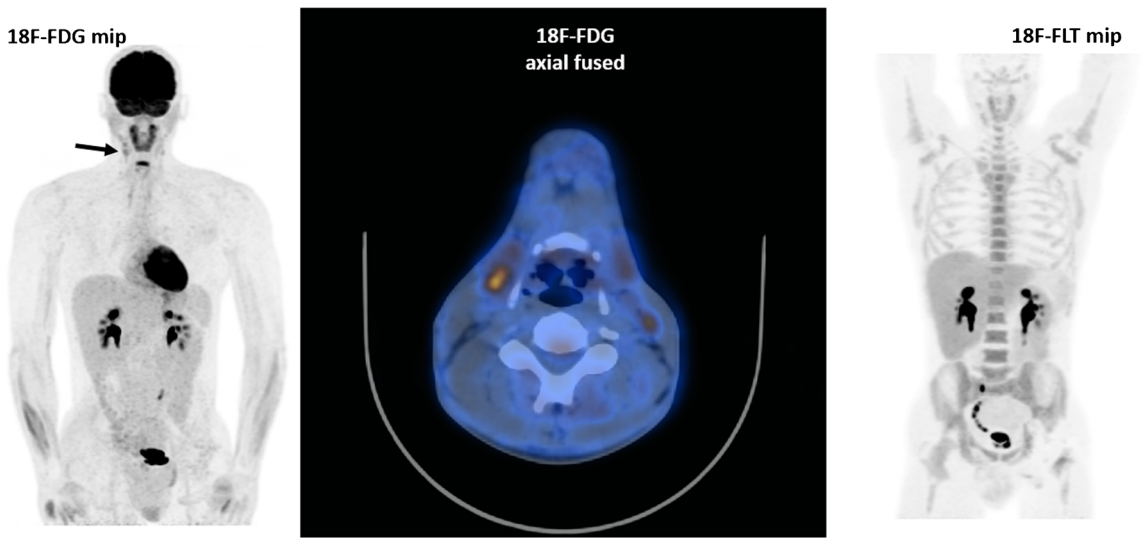


Fig. 5 Suspected para-pharingeal and laterocervical HL relapse: false-positive FDG and true-negative FLT in dental inflammation. 18F-FDG was suspicious for HL relapse at the level of the left para-pharingeal space (SUVmax = 7; TBR = 3.9, original site of disease at diagnosis) and bilateral cervical nodes (*black arrow* in *mip*, SUVmax = 4.1 right; SUVmax = 2.3 left; *FDG axial fused*), not confirmed by 18F-FLT which showed only faint, diffuse, homogeneous uptake (*FLT-mip*, SUVmax = 2.8; TBR = 0.8) in particular at the level of the Waldeyer ring, most likely inflammatory; final clinical evaluation identified an ongoing dental inflammation and excluded recurrent lymphoma.

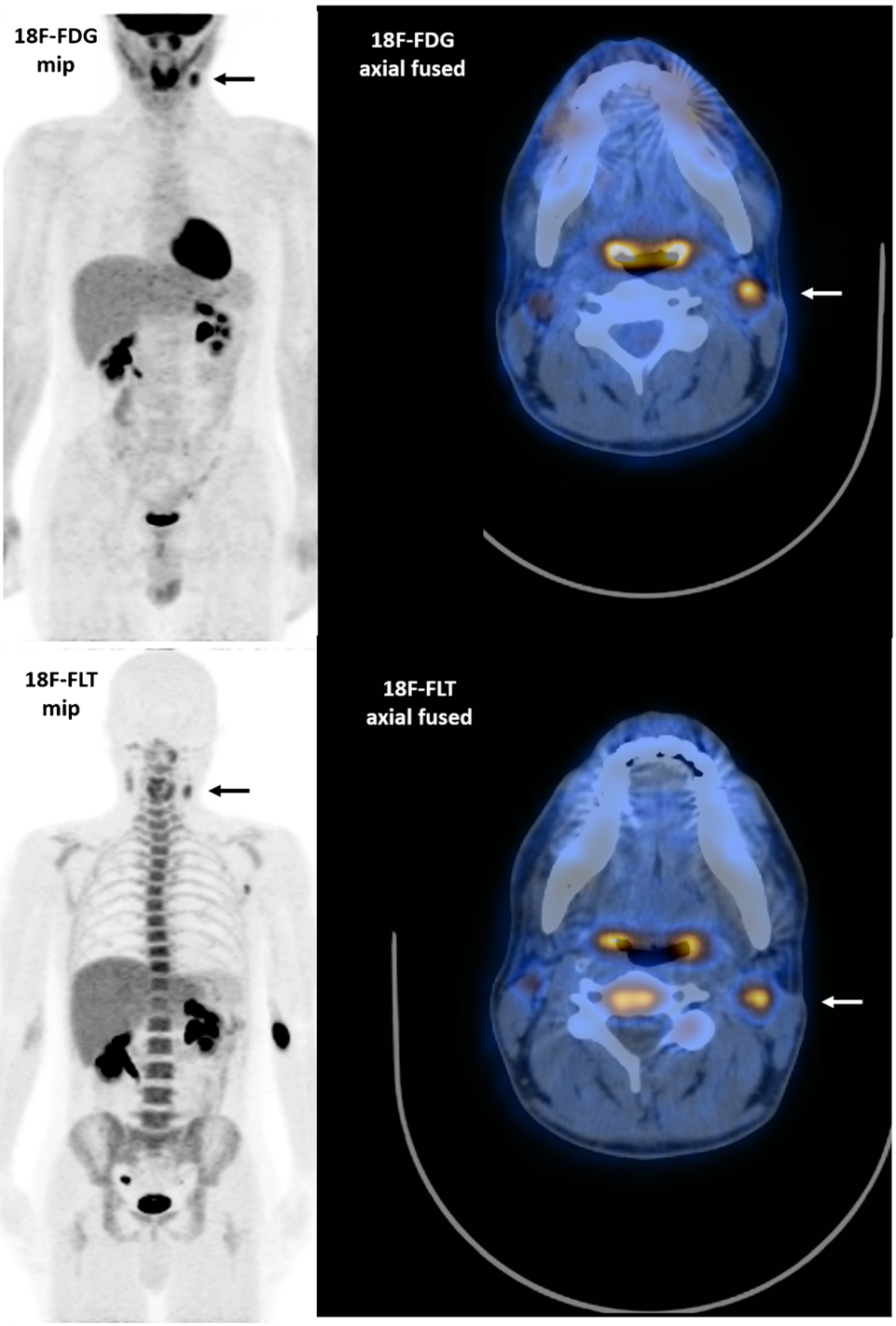


Fig. 6 False-positive FDG and FLT due to reactive follicular hyperplasia. FDG- and FLT-positive left cervical lymphadenopathy (*arrows*, SUV max = 6.9 and 6.8; TBR = 2.9 and 1.8 respectively) appeared during follow-up evaluation compatible with HL relapse. However the histological examination demonstrated reactive follicular hyperplasia with elevated Ki-67 in germinal centers

and potentially a useful tool to tailor different treatment strategies to each patient [30]. A few studies investigating early interim FLT-PET imaging showed its prognostic role as significant predictor of PFS and OS [31], its potential to identify patients with delayed response and non-favorable prognosis despite clinical complete response [32], and more recently a significantly higher PPV than standardized FDG PET/CT-based interpretation in prospective multicentre series of 46 DLBCL [33]. However, early response assessment was not the setting of our study. Concerning the evaluation of response at therapy completion, Kasper and collaborators, who investigated post-radiochemotherapy residual masses in 48 patients, in 2007 concluded that there is no advantage of combined FDG/FLT studies over FDG alone with respect to the prediction of survival [20]. In our series, the sub-group of suspected residual active disease after treatment counts only seven out of 52 patients (13%); therefore, a direct comparison with previously published results on larger populations [20, 22] cannot

be performed. However, we confirmed that FLT-SUVmax was significantly higher in tumours than in benign lesions, as previously documented [22]; furthermore, correlation with PFS and OS were not endpoints of our study.

Although FDG was already proven to detect more lesions than FLT [20], the additional biological characterization of tumour tissue by FLT was still considered to be potentially useful for the identification of recurrence and reasonable to be further explored.

As the prognosis of relapsed lymphoma patients significantly improves if recurrences are detected early, especially in the case of aggressive NHL and HL, in which a PET-based follow-up, at least in the first 1 or 2 years after remission, can be justified, and several chemotherapy lines are available to treat it, the importance of differentiating between inflammatory and malignant lesions could not be overstated. Whether the FLT role could be complementary to that of FDG PET/CT, or the former be performed alone in a different system of clinical guidelines for recurring/relapsing lymphoma patients, was also open to investigation. Another advantage that this new tracer could potentially offer lay in its ease-of-use within an established imaging protocol, as it does not require substantial modifications to an FDG-PET/CT scanning protocol. Concerning dosimetry, organ dose estimates for F-18-FLT are comparable to those associated with other commonly performed nuclear medicine examinations [34]. Furthermore, recently the European Pharmacopeia (9.0) of alovudine (F-18) injection (FLT) was published (01/2014:2460), thus moving FLT tracer from bench to bedside.

Our study investigated the performance of this proliferation-targeting tracer, in comparison to the commonly employed FDG for PET/CT imaging of suspected recurrent lymphomatous disease, and to the available histopathological, or clinical, evidence. We observed a slightly better performance of FLT, semi-quantitatively evaluated by calculating the SUV max of visually positive findings, in discriminating benign from residual or recurrent disease when compared with the other investigated semi-quantitative parameters. Those included—for both F-18-FDG and F-18-FLT—SUV max for the most avid lesion as well as TBR, relative to the liver SUV mean. The use of "reference tissue" SUVmax values and normalization of lesion/target SUV measures to those of selected reference background is an alternative approach which has been suggested to assist reduction in variability of SUV measurements, and which allows comparison of different radiotracers. A number of tissues have been advocated in literature as reference background, including blood pool and liver; however, liver conventionally represents a good comparator for positivity criteria in many diseases, showing the best inter-patient reproducibility and the least variance. In our series, there was a wide overlap between TBR and SUVmax ranges (Table 3), which suggests no additional discriminatory value using FLT. Furthermore, some authors recently reported on a better performance of FLT-TBR, in terms of AUCs, when employing mediastinal blood-pool instead of liver SUV as background [18]. We could not determine a valid optimal cut-off value for FLT-SUVmax. To that extent, if aiming for a higher sensitivity, a cut-off value of 3 would offer the better performance, although with a very limited specificity (95% sensitivity, 39% specificity, 81% accuracy). On the other hand, in our clinical setting, in which FLT represents and additional complementary procedure, a higher level of specificity and a lower number of false-positive findings represented a more relevant target, as we were already able to rely on a very sensitive standard radio-tracer, namely F-18-FDG. Thus, we found that a cut-off of FLT-SUVmax of 7 might represent the best option (41% sensitivity, 100% specificity, 56% accuracy); nevertheless, accuracy remains inadequate in both cases.

With regard to the recorded 35% relevant discrepancies (18/52 cases) in interpretation between FDG and FLT, in terms of Ann-Arbor disease re-staging, in almost all cases (17/18, 94%) downstaging was detected with FLT compared with the standard tracer FDG; it should be noted that in 11/17 patients (65%), the final stage was correctly reduced. On the contrary, in 6/17 patients (35%), FLT downstaging was considered false by clinicians and attributed to its well-known lower sensitivity. In the remaining single case (1/18, 5%), FLT erroneously upstaged the disease at the level of the skeleton, whose assessment is hampered by physiological FLT uptake throughout the proliferating bone marrow.

Whether FLT is really able to correlate with the proliferation activity is still under debate, and although strong evidence supports the presence of a significant correlation between FLT uptake and Ki-67 expression in solid cancers such as brain, lung, and breast (Chalkidou et al.) [35], we could not successfully establish a clear correlation to our FLT semi-quantitative parameters. Furthermore, our histopathological investigation was limited by either practical or ethical reasons, and was characterized by a reduced availability of proliferation data (an absolute value of Ki-67 was only occasionally documented within the routine report), and by the forced, inevitable subgrouping into four percentage classes.

Our study did suffer from a few further limitations. These included a limited availability of F-18-FLT, as our commercial provider discontinued production in June 2013, thus causing a delay in tracer availability between then and January 2014, when our Radio-pharmacy Unit was able to initialize stable, regular synthesis. Furthermore, during patient enrolment, all those subjects presenting either very small lesions (non-viable sampling due to procedural difficulties), or whose lymph nodal lesions could be clearly linked to co-existing non-lymphomatous conditions (additional FLT PET imaging not indicated due to ethical concerns), could not be included in the study. Remarkably, that excluded from our cohorts those patients who could potentially most benefit from a tracer able to

differentiate between inflammation and proliferation, in cases of equivocal FDG findings. Another limitation is the heterogeneity of the population (both HL and NHL) because all histological types of lymphomas could be included, taking into account neither grading nor aggressiveness: in particular, almost half of the population was affected by non-aggressive lymphomas (21/52 patients, 40%). Finally, even patients who were judged as negative for residual or recurrent disease with the experimental tracer FLT, considering its low sensitivity, still required further validation, implying a longer clinical and diagnostic follow-up monitoring.

Conclusion

According to our results in a clinical setting of recurrent or residual lymphoma, FLT is not significantly superior to FDG and it is unlikely that it will be employed independently. FLT may be restricted to a few specific cases, as complementary to standard FDG imaging, to confirm a diagnosis or to define a better target to biopsy. However, due to FLT suboptimal performance, many findings would remain inconclusive, requiring further diagnostic procedures and reducing the effectiveness of performing an additional FLT scan.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Local Ethical Committee code: 77/2009/O/Sper.

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Informed consent Written informed consent was obtained from all individual participants included in the study.

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