Prognostic Value of ^{18}F-FLT PET in Patients with Neuroendocrine Neoplasms: A Prospective Head-to-Head Comparison with ^{18}F-FDG PET and Ki-67 in 100 Patients

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Neuroendocrine neoplasms (NENs) constitute a heterogeneous group of tumors arising in various organs and with a large span of aggressiveness and survival rates. The Ki-67 proliferation index is presently used as the key marker of prognosis, and treatment guidelines are largely based on this index. 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) is a proliferation tracer for PET imaging valuable in the monitoring of disease progression and treatment response in various types of cancer. However, until now only data from 10 patients with NEN were available in the literature. The aim of the present study was to investigate ^{18}F-FLT PET as a prognostic marker for NENs in comparison with ^{18}F-FDG PET and Ki-67 index. **Methods:** One hundred patients were PET-scanned with both ^{18}F-FLT and ^{18}F-FDG within the same week, and the prognostic value of a positive scan was examined in terms of progression-free survival (PFS) and overall survival (OS). The correlation between the Ki-67 index and ^{18}F-FLT uptake was also investigated. **Results:** Thirty-seven percent of patients had a positive ^{18}F-FLT PET scan, and 49% had ^{18}F-FDG PET–positive foci. Patients with a high ^{18}F-FLT uptake had a significantly shorter OS and PFS than patients with low or no ^{18}F-FLT uptake. No correlation was found between Ki-67 index and ^{18}F-FLT uptake. In a multivariate analysis ^{18}F-FLT, ^{18}F-FDG, and Ki-67 all were significant prognostic markers of PFS. For OS, only ^{18}F-FDG and Ki-67 remained significant. **Conclusion:** ^{18}F-FLT PET has prognostic value in NEN patients but when ^{18}F-FDG PET and Ki-67 index are also available, a multivariate model revealed that ^{18}F-FLT PET only adds information regarding PFS but not OS, whereas ^{18}F-FDG PET remains predictive of both PFS and OS. However, a clinically robust algorithm including ^{18}F-FLT in addition to ^{18}F-FDG and Ki-67 could not be found. Accordingly, the exact role, if any, of ^{18}F-FLT PET in NENs remains to be established.

Key Words: ^{18}F-FLT PET; ^{18}F-FDG PET; neuroendocrine neoplasms; Ki67; prognosis

Neuroendocrine neoplasms (NENs) include a diversity of tumors derived from various organ systems, in particular the intestinal tract, pancreas, and lungs (*1–3*). Thus, the clinical course and prognosis of the disease can vary substantially. The treatment options are likewise multiple. Guidelines for diagnosis and therapy are based on tumor origin and histologic grading (*4–12*). At present, a key factor in the characterization of NEN is the Ki-67 proliferation index. The clinical course is associated with this index, and the treatment strategies are chosen accordingly (*13,14*). Determination of Ki-67 index requires tissue from the resected tumor or in nonresectable cases an invasive core biopsy.

Recently, ^{18}F-FDG PET demonstrated strong prognostic value in NENs, which may even exceed the value of the Ki-67 index and other traditional markers such as plasma chromogranin A or the presence of liver metastases (*15*).

The radiolabeled thymidine analog 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) is a tracer used for PET imaging of proliferation (*16,17*). ^{18}F-FLT is trapped inside the cells after phosphorylation by thymidine kinase-1. Thymidine kinase-1 is upregulated during the S-phase of cell proliferation as a key enzyme in the DNA salvage pathway, hence although ^{18}F-FLT is not incorporated into DNA, it represents an indirect measurement of proliferation (*16,17*). ^{18}F-FLT PET could be considered a noninvasive Ki-67 index (*18*) and might add valuable information about tumor aggressiveness and prognosis. The value of ^{18}F-FLT PET in the diagnosis and treatment of other cancer forms has been extensively evaluated, and in general the background uptake is high in the liver, bone marrow, and renal system, limiting the use in these organs (*19*). ^{18}F-FLT PET has been used in pulmonary lesions to differentiate malignant from benign disease (*20,21*) and to stage non–small cell lung cancer (*22,23*). ^{18}F-FLT PET has also been reported of diagnostic value in brain and breast cancer and in the evaluation of early response to chemotherapy in several types of cancer (*24–30*).

Until now, the prognostic value of ^{18}F-FLT PET has not been described in NEN. The aim of the present prospective study was therefore to investigate ^{18}F-FLT PET as a prognostic marker for NENs in comparison with Ki-67 index and ^{18}F-FDG PET.

MATERIALS AND METHODS

Patients

From 2011 to 2013, 103 consecutive patients were prospectively enrolled in the study at the European Neuroendocrine Tumor Society

(ENETS) European Center of Excellence for Treatment of Neuroendocrine Neoplasms in Copenhagen. Inclusion criteria were histologically verified NEN and presence of a primary tumor or metastatic disease at enrollment. All patients were scheduled for an ^{18}F-FLT PET and ^{18}F-FDG PET scan within the same week. One hundred patients completed both scans. Patient characteristics are shown in Table 1. Approximately 80% had tumors in the gastroenteropancreatic system, and 7% were lung NENs. The group described as others consisted of 3 patients with NENs derived from the stomach, 4 from the urogenital system, and 1 from the ovaries. In the group of patients with unknown primary tumor, the NEN diagnosis was based on biopsies from liver metastasis in 4 patients, lymph node metastasis in 3 patients, and bone metastasis in 1 patient.

The results of the PET scans were masked to the patients and their clinicians, and during follow-up the patients were treated according to standard care and guidelines based on the routine CT and somatostatin receptor imaging performed (4–12,31). CT results from the routine follow-up, described by an expert in radiology, were used to determine progression according to RECIST (32). The diagnostic CT scan from the ^{18}F-FLT PET scan was used as baseline, and progression-free survival (PFS) was calculated as the time from the ^{18}F-FLT PET scan to the first CT scan hereafter showing progression or if the patient died. Patients without progression were censored at the time of their latest CT scan, whereas patients still alive in the overall survival (OS) assessment were censored at a cutoff date of June 27, 2015.

The regional scientific ethical committee approved the study (H-3-2011-092). A written informed consent form was obtained from all the participants.

^{18}F-FLT and ^{18}F-FDG PET

^{18}F-FLT was synthesized on a TRACERlab MX Synthesizer (GE Healthcare) using 3-N-Boc-5′-O-dimethoxytrityl-3′-O-nosylthymidine as a precursor. ^{18}F-FLT cassettes and reagents were purchased from ABX GmbH. The radiochemical purity of ^{18}F-FLT was 99.5% (97.7%–100%). ^{18}F-FDG was produced as a part of the routine production for clinical ^{18}F-FDG PET scans. The radiochemical purity of ^{18}F-FDG was 99.3% (98.4%–99.7). For ^{18}F-FLT, the patients received 5 MBq/kg and the mean injected dose was 328 MBq (206–473 MBq); for ^{18}F-FDG, patients received 4 MBq/kg and the mean injected dose was 303 MBq (179–444 MBq). Images were acquired at 1 h after injection after resting. Patients were positioned with arms above the head and scanned from forehead to mid thigh, 2–3 min per bed position depending on body mass index. The PET/CT scans were obtained on a Biograph 40 or 64 PET/CT scanner (Siemens Medical Systems). CT in combination with ^{18}F-FLT PET was performed as contrast-enhanced scans of diagnostic quality with intravenous and oral contrast medium. The CT scans of the ^{18}F-FDG PET/CTs were low-dose CTs to minimize the radiation burden. The CT data were used for attenuation correction of the PET images. PET, CT, and fused images were reconstructed in all 3 planes for analysis on the Siemens Leonardo workstation using TrueD software. Experts in nuclear medicine and radiology analyzed all images in consensus. Pathologic foci were reported, and tracer accumulation was quantified as the SUV_{max} in the tumor tissue. If no tracer accumulation was seen, the scan was reported as PET negative.

Ki-67 Immunohistochemical Determination

Ki-67 was detected with a monoclonal antibody MIB1 (DAKO) using the manufacturer's guidelines. The Ki-67 index was calculated as the mean percentage of cells with nuclear labeling in 20 hot spot areas and was measured as part of the clinical routine. Additional staining on existing paraffin-embedded histologic tissue was done if possible for patients without existing Ki-67 measurements. The median time between the Ki-67 measurements and the ^{18}F-FLT scan was 351 d (interquartile range, 104–754 d), and none of the biopsies for Ki-67 measurement were guided by ^{18}F-FLT/^{18}F-FDG PET images.

TABLE 1
Characteristics of Study Group of 100 NEN Patients Scanned with Both ^{18}F-FLT and ^{18}F-FDG PET

Characteristic	Data
Sex	
Male	60 (60%)
Female	40 (40%)
Mean age (y)	
At diagnosis	60 (range, 35–87)
At time of PET	64 (range, 40–87)
Type of tumor	
Ileal NEN	54 (54%)
Colonic NEN	8 (8%)
Pancreatico-duodenal NEN	15 (15%)
Lung NEN	7 (7%)
Other	8 (8%)
Unknown primary NEN	8 (8%)
Disease extension	
Primary only	5 (5%)
Primary and metastasis	23 (23%)
Liver metastasis	70 (70%)
Lymph node metastasis	41 (41%)
Bone metastasis	15 (15%)
Ki-67 proliferation index	
≤2%	18 (18%)
>2% to ≤20%	70 (70%)
>20%	9 (9%)
Missing Ki-67	3 (3%)
Ki-67 determined on	
Surgical specimen	50
Biopsy	47
Follow-up days	
From diagnosis	2,271 (range, 72–9,240)
From ^{18}F-FLT PET scan	918 (31–1,267)
Treatment during follow-up (n)	
Somatostatin analog	55 (55%)
α-interferon	34 (34%)
Surgery	9 (9%)
Liver embolization	6 (6%)
PRRT	18 (18%)
Everolimus	3 (3%)
Chemotherapy overall	30 (30%)
5-fluorouracil/streptozocin	17 (17%)
Carboplatin/etoposide	7 (7%)
Topotecan	3 (3%)
Temozolomide	9 (9%)

PRRT = peptide receptor radionuclide therapy.

TABLE 2
Distribution of ^{18}F-FLT, ^{18}F-FDG, and Ki-67 in 100 NEN Patients

Tumor type/ Ki-67 index	^{18}F-FLT–positive		^{18}F-FDG–positive		Both positive (no.)	Both negative (no.)	G1 (no.)	G2 (no.)	G3 (no.)
	No.	Mean SUV$_{max}$ ± SEM	No.	Mean SUV$_{max}$ ± SEM					
NEN type									
Ileal NEN	20/54 (37%)	10.1 ± 1.7	22/54 (41%)	8.1 ± 1.5	17/54 (32%)	29/54 (54%)	14 (26%)	38 (70%)	
Colonic NEN	1/8 (13%)	27.0	3/8 (38%)	7.5 ± 0.7	1/8 (13%)	5/8 (63%)		7 (88%)	1 (13%)
Pancreatico- duodenal NEN	6/15 (40%)	11.5 ± 3.0	11/15 (73%)	14.2 ± 5.1	4/15 (27%)	2/15 (13%)	3 (20%)	12 (80%)	
Lung NEN	5/7 (71%)	8.1 ± 2.1	4/7 (57%)	14.4 ± 4.2	3/7 (43%)	1/7 (14%)		5 (71%)	2 (29%)
Other	2/8 (25%)	10.5 ± 0.2	3/8 (38%)	13.9 ± 4.0	1/8 (13%)	4/8 (50%)		4 (57%)	3 (43%)
Unknown primary NEN	3/8 (38%)	9.8 ± 0.3	6/8 (75%)	8.9 ± 2.0	3/8 (38%)	2/8 (25%)	1 (13%)	4 (50%)	3 (38%)
Total	37/100 (37%)	10.5 ± 1.2	49/100 (49%)	10.4 ± 1.4	29/100 (29%)	43/100 (43%)	18 (18%)	70 (70%)	9 (9%)
Ki-67									
≤2% (G1)	5/18 (28%)	7.3 ± 1.3	7/18 (39%)	9.8 ± 4.1	3/18 (17%)	9/18 (50%)			
3%–20% (G2)	27/70 (39%)	10.8 ± 1.4	35/70 (50%)	8.7 ± 1.1	21/70 (30%)	29/70 (41%)			
>20% (G3)	5/9 (56%)	12.1 ± 3.9	6/9 (67%)	14.1 ± 2.8	5/9 (56%)	3/9 (33%)			

For patients with more than one Ki-67 measurement, the one obtained closest to the ^{18}F-FLT scan was chosen and if more simultaneous Ki-67 indices existed the highest was chosen. Three patients without existing Ki-67 did not have tissue left for Ki-67 staining. The tumors were classified according to the classification from the World Health Organization (WHO) from 2010 for NEN of the gastrointestinal system into grade G1, Ki-67 ≤ 2%; G2, Ki-67 3%–20%; and G3, Ki-67 > 20% (33), including the lung NENs as suggested by Rindi et al. (34), although this is controversial.

Statistics

PFS and OS were chosen as endpoints to establish the prognostic value. The sample size was chosen on the basis of the observed event rates in a previous study of the prognostic value of ^{18}F-FDG PET in NEN patients. A 1-y follow-up among 100 patients in that study was sufficient to detect significant differences in PFS and OS among groups and perform multivariate analysis of PFS including 3–4 variables (15). ^{18}F-FLT and ^{18}F-FDG PET data were reported as negative or positive scans. Furthermore, the patients with high ^{18}F-FLT SUV$_{max}$ (above median) were compared with patients with lower SUV$_{max}$ (below median) or negative scans. Ki-67 index classified the patients into the WHO groups (G1, G2, and G3). Probabilities of PFS and OS were analyzed by the method of Kaplan and Meier, and differences between groups were tested by the log-rank test. Hazard ratios (HRs) were calculated by the Cox proportional hazards regression analysis. The multivariate analyses of independent prognostic factors were performed using stepwise backward elimination of variables with a P value above 0.05. The statistical analyses were performed using SPSS version 22 (IBM SPSS Statistics, IBM Corp.). Survival curves were created in Prism version 6 (Graph Pad Prism Inc.). The significance level was set at a P value of 0–05.

RESULTS

The results of the ^{18}F-FLT and ^{18}F-FDG PET scans in relation to tumor type and Ki-67 index are shown in Table 2.

Thirty-seven patients (37%) were ^{18}F-FLT PET–positive. Forty-nine patients (49%) were ^{18}F-FDG PET–positive. Twenty-nine patients (29%) were positive on both the ^{18}F-FLT and the ^{18}F-FDG PET scans, 8 were only ^{18}F-FLT–positive, and 20 were only ^{18}F-FDG–positive. Thus, 57 patients (57%) were positive on at least one of the scans and 43% were negative on both.

An example of a patient with an ^{18}F-FLT PET–positive focus in the cervical spine negative on the ^{18}F-FDG PET scan is shown in Figure 1. Figure 2 shows a patient with an intestinal focus positive on both the ^{18}F-FLT and the ^{18}F-FDG scan. In Figure 3, an intestinal focus is seen only by ^{18}F-FDG (and the diagnostic ^{68}Ga-DOTATOC scan) but not on ^{18}F-FLT.

Somatostatin receptor scans were positive in 86 of the patients and negative in 14. No significant differences were seen in
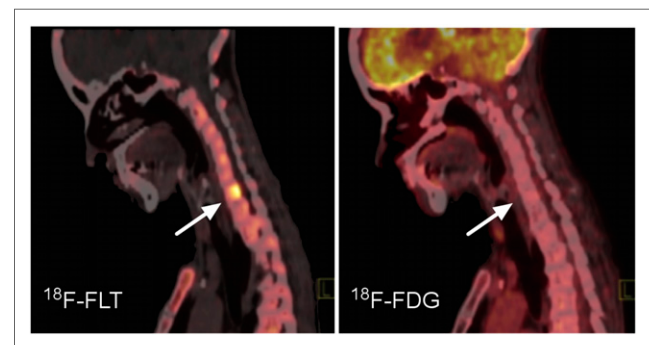

FIGURE 1. Sagittal image of head and neck in a patient with small bowel neuroendocrine tumor and metastases to lymph nodes and bone. On ^{18}F-FLT PET (left), a focus with high uptake is seen in cervical spine. ^{18}F-FDG PET (right) is without pathologic foci, and only high physiologic uptake in brain is seen.
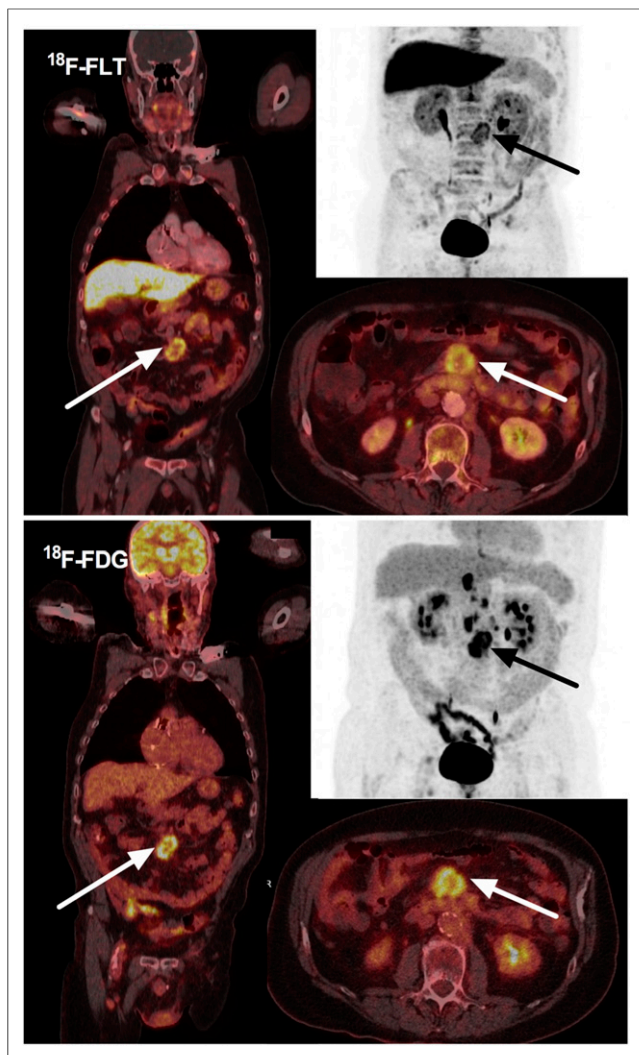
FIGURE 2. Whole-body fused ^{18}F-FLT or ^{18}F-FDG PET and CT as well as axial PET/CT images of intestinal tumor (arrow). Gray scale images: PET alone showing additional foci on ^{18}F-FDG PET (lower) compared with ^{18}F-FLT PET (upper).

found with increasing Ki-67 index. Pairwise comparison showed that G1 and G2 groups were significantly different in this cohort of patients only for PFS but not for OS. The highly proliferative G3 group, however, differed significantly from the G2 group both with regard to PFS and OS. HRs and *P* values are shown in the figures.

Looking exclusively at the G1 and G2 groups of patients (Fig. 5), both ^{18}F-FLT and ^{18}F-FDG stratified these patients into an FLT$_{low}$ and an FDG$_{neg}$ group with significantly longer PFS than the FLT$_{high}$ and the FDG$_{pos}$ counterparts (Figs. 5A and 5C). For OS (Figs. 5B and 5D), only a positive ^{18}F-FDG PET scan was significant.

No significant differentiation was gained by subdividing the ^{18}F-FDG groups further into FLT$_{high}$ or FLT$_{low}$ (data not shown).

Sixty events of progression and 26 events of death were observed in the 97 patients with available Ki-67 values. Thus, multivariate Cox proportional hazards regression analysis of 3 parameters was considered permissible.

In the multivariate analysis applied to PFS with ^{18}F-FLT and ^{18}F-FDG and WHO groups entered into the model, all 3 parameters remained significant predictors of PFS, with HRs of 2.0 (*P* = 0.028), 2.2 (*P* = 0.006), and 3.5 (*P* < 0.001), respectively. In the same analysis applied to OS (*n* = 97; 26 events), only ^{18}F-FDG and WHO groups remained significant predictors, with HRs of 4.2 (*P* = 0.002) and 3.9 (*P* = 0.001), respectively.

In G1 and G2 patients only, 51 events of progression and 19 deaths were observed. When multivariate analysis was applied to ^{18}F-FLT, ^{18}F-FDG, and WHO groups, only ^{18}F-FDG grouping remained significant, with HRs of 2.4 (*P* = 0.003) for PFS and 5.3 (*P* = 0.001) for OS.

With only 9 patients with G3 tumors included in our study, isolated evaluations within this group are speculative. However, FLT$_{high}$ scans detected the 3 patients with the shortest time to progression (*P* = 0.002).
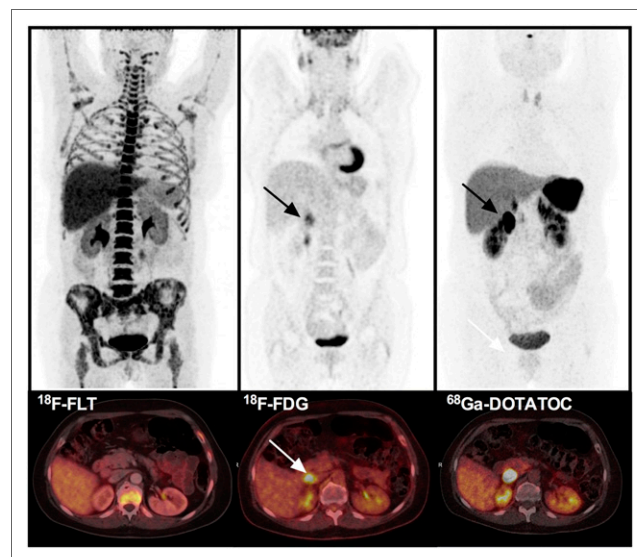
^{18}F-FLT or ^{18}F-FDG positivity in these groups. Thus, 38% (33/86) and 28% (4/14) were ^{18}F-FLT–positive, and 48% (41/86) and 42% (6/14) were ^{18}F-FDG–positive.

Ninety-seven patients could be categorized according to Ki-67 index, and a slightly higher percentage of the G3 patients were positive in both the ^{18}F-FLT and the ^{18}F-FDG PET scans. Ki-67 index did not correlate to ^{18}F-FLT (data not shown). A comparison of only Ki-67 indices obtained within 1 y from ^{18}F-FLT scans also did not reveal any correlation.

^{18}F-FLT PET–positive versus –negative scans showed no relation to prognosis. However, both PFS and OS were significantly shorter in patients with an ^{18}F-FLT SUV$_{max}$ above the median value (FLT$_{high}$) than in patients with values below median or negative scans (FLT$_{low}$) (Figs. 4A and 4B).

Patients with a positive ^{18}F-FDG PET scan (FDG$_{pos}$) also had a significantly worse prognosis than patients with a negative ^{18}F-FDG PET (FDG$_{neg}$) scan (Figs. 4C and 4D).

PFS and OS for patients in the 3 Ki-67 index–based groups are shown in Figures 4E and 4F. A significantly worse prognosis was

FIGURE 3. Transversal PET/CT fused images of 3 different PET scans in the same patient. ^{18}F-FLT PET (left) does not show intestinal focus (arrows) found on ^{18}F-FDG PET (middle) and ^{68}Ga-DOTATOC (right). Whole-body PET images on top are ^{18}F-FDG and in the middle maximum-intensity projections for ^{18}F-FLT scan (left) and ^{68}Ga-DOTA-TOC scan with physiologic uptake in pituitary gland (right).
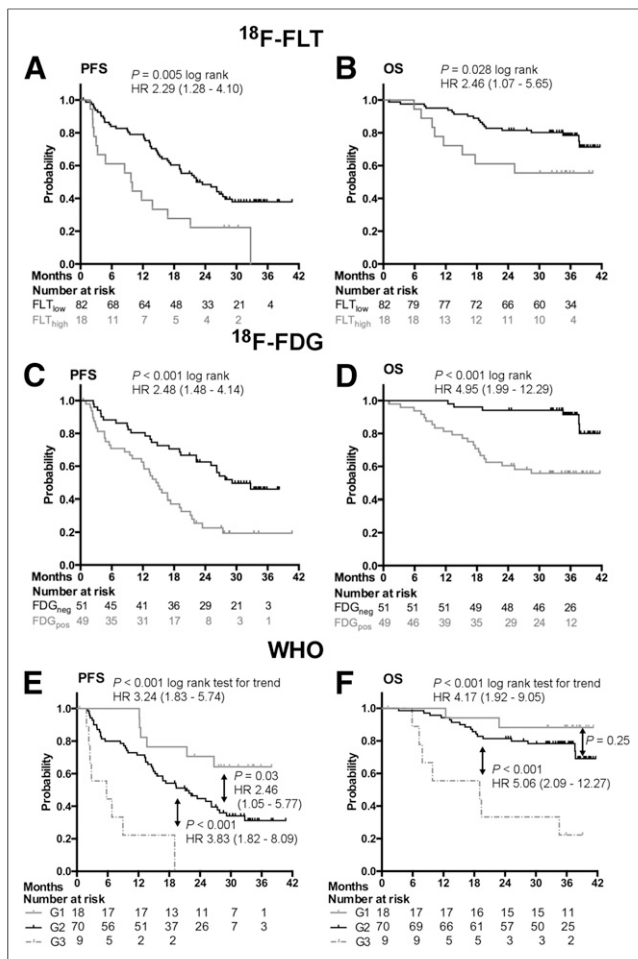
FIGURE 4. PFS and OS according to results of ^{18}F-FLT PET (A and B; $n = 100$); ^{18}F-FDG PET (C and D; $n = 100$); and WHO groups G1, G2, and G3 (E and F; $n = 97$).

DISCUSSION

To our knowledge, this is the first study to describe ^{18}F-FLT PET in a large group of patients with NEN. In a previous study using ^{18}F-FLT PET in 10 patients with well-differentiated gastroenteropancreatic NENs, all were negative (*35*). It was suggested that this might be due to the low proliferation rate of the tumors. However, in our study ^{18}F-FLT PET was positive in more than one fourth of the patients with Ki-67 $\leq$ 2 and in 37% of all the patients. The simultaneous ^{18}F-FDG PET scan was positive in 49% of the patients, and the proportions both positive on ^{18}F-FLT PET and ^{18}F-FDG PET increased with increasing proliferation index. For NENs, Ki-67 index is a key factor in the treatment decision algorithms as outlined in the ENETS guidelines (*5–12*). This is based on the fact that Ki-67 index correlates well with both PFS and OS in NEN patients, which we confirmed in the present study. However, a noninvasive way to assess tumor proliferation and predict prognosis would be preferable especially when repeated examinations are needed to monitor disease. Also a noninvasive method for whole-body evaluation would elegantly circumvent sampling error as seen with biopsies. Surprisingly, we found no correlation between ^{18}F-FLT PET SUV$_{max}$ and Ki-67 index in our study. Conflicting results have been seen in studies investigating the correlation between Ki-67 and ^{18}F-FLT uptake.

In a meta-analysis by Chalkidou et al. (*18*), it was concluded that a significant correlation between ^{18}F-FLT uptake and Ki-67 was more often found in studies comparing SUV$_{max}$ with a mean or average value of Ki-67 instead of Ki-67 max (hotspot). Furthermore, best correlations were seen when surgical samples were examined for Ki-67 rather than biopsies (*18*). Our study was not designed to obtain the optimal conditions for correlations between ^{18}F-FLT uptake and Ki-67 index. Neuroendocrine tumor centers determine the Ki-67 index in NENs by counting in hotspot areas and often only from small biopsies. In contrast, the ^{18}F-FLT uptake is calculated from the whole tumor and because of tumor heterogeneity the Ki-67 index might not represent and characterize the whole tumor well enough (*36*). Also, if hotspot-determined Ki-67 should correspond with SUV$_{max}$ of ^{18}F-FLT PET, the latter would need to have a much higher resolution. A prerequisite to expect good correlation between the Ki-67 index and ^{18}F-FLT uptake is that biopsies or surgical specimens used for Ki-67 should be from the same site from which ^{18}F-FLT uptake is measured. None of the tissue samples for Ki-67 determination were taken guided by the ^{18}F-FLT PET images, and in only 4 of the 37 ^{18}F-FLT PET–positive patients the ^{18}F-FLT SUV$_{max}$ focus was at the site of Ki-67 indexing. We believe this lack of concordance is the main reason that we found no correlation between Ki-67 and ^{18}F-FLT uptake. We used Ki-67 data from tissue samples taken before initiation of the present study, for some patients several years. However, if we restricted correlation analysis to Ki-67 samples taken within 1 y of the ^{18}F-FLT scan, we did still not find any correlation pointing to the topographic explanation being most important.

We found that a high level of ^{18}F-FLT uptake was a negative prognostic marker for both PFS and OS. A positive ^{18}F-FDG PET scan likewise predicted a worse outcome. HR for OS was higher for ^{18}F-FDG than for ^{18}F-FLT. The prognostic value of ^{18}F-FDG has earlier been shown (*15*) and is now confirmed in the present
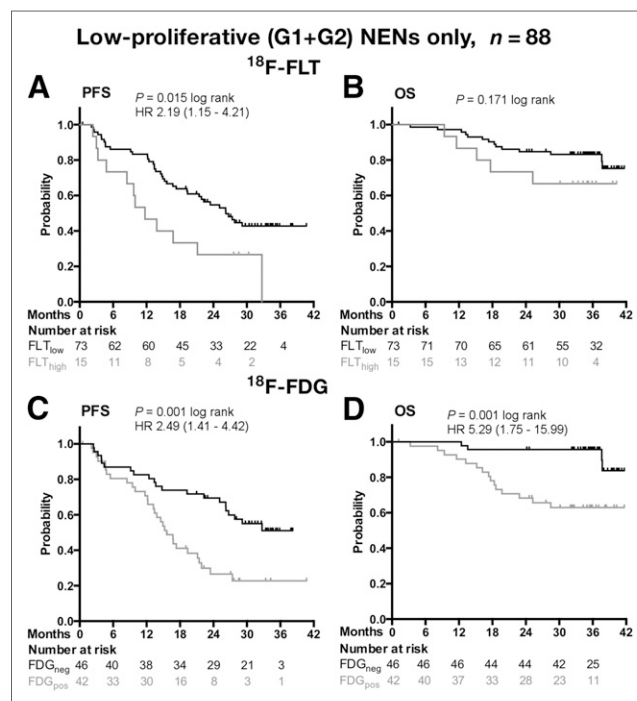

FIGURE 5. PFS and OS according to results of ^{18}F-FLT PET (A and B) and ^{18}F-FDG PET (C and D) in 88 patients with G1 and G2 NENs.

series of NEN patients. The WHO 3-tier system based on Ki-67 index as a prognostic factor was also confirmed although no significant difference was found for OS between the G1 and G2 groups. It has been discussed whether the G3 and maybe also the low-proliferative patients could be further stratified to individualize the treatment more. We included only 9 patients with G3 tumors in our study, which made further evaluation of this group difficult and speculative. Nevertheless, FLT_{high} scans significantly detected the 3 patients with the shortest time to progression, and OS was also significantly different for the FLT_{high} group. No such correlation was found for ^{18}F-FDG in the G3 group, possibly indicating that ^{18}F-FLT is superior to ^{18}F-FDG in the stratification of G3 patients.

In the 88 G1 and G2 patients, WHO groups, ^{18}F-FLT, and ^{18}F-FDG all significantly identified patients with longer PFS whereas only ^{18}F-FDG–positive scans could predict a group with significantly shorter OS. Likewise, only ^{18}F-FDG remained significant in the multivariate analysis including WHO groups, ^{18}F-FDG, and ^{18}F-FLT for both PFS and OS. In this large low-proliferative group, ^{18}F-FDG is a stronger predictor than the existing WHO groups and ^{18}F-FLT cannot challenge ^{18}F-FDG in this.

In the multivariate analysis of all patients, including the G3, ^{18}F-FLT PET remained an independent predictor of PFS also when WHO groups and ^{18}F-FDG were included in the model, indicating that ^{18}F-FLT adds additional information about prognosis on top of that from the WHO group and ^{18}F-FDG. However, a simple clinical algorithm using all 3 parameters could not be found. In the multivariate analysis of OS, only WHO group and ^{18}F-FDG remained significant predictors. However, it is crucial to emphasize that especially in NEN patients, who have a long life expectancy despite disseminated disease, PFS is an important parameter for monitoring disease control.

As only 37% of the patients had a positive ^{18}F-FLT PET and additional foci were often seen on other scans (Figs. 2 and 3), it is not justified to use ^{18}F-FLT PET as a primary diagnostic tool in NEN patients. The high background uptake of ^{18}F-FLT in the liver challenges the imaging of liver metastases, and PET-based somatostatin receptor ligand tracers are far superior in that respect (*37,38*). A rather high percentage (71%) of lung NENs had positive ^{18}F-FLT PET scans. A preclinical study showed that an early ^{18}F-FLT PET scan could predict the effect of everolimus treatment in human xenograft NENs of lung origin in mice (*29*). Further studies are needed to show whether ^{18}F-FLT might have particular value in NENs from specific sites or for monitoring of treatment response in ^{18}F-FLT–positive NENs.

CONCLUSION

^{18}F-FLT PET has prognostic value in NEN patients and predicts both OS and PFS. However, if ^{18}F-FDG PET and Ki-67 index are also available, ^{18}F-FLT PET added independent information regarding PFS but not regarding OS, whereas ^{18}F-FDG PET had independent information regarding both PFS and OS. However, a clinically robust algorithm including ^{18}F-FLT in addition to ^{18}F-FDG and Ki-67 could not be found. Accordingly, the exact role, if any, of ^{18}F-FLT PET in NENs remains to be established.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance

REFERENCES

1. Modlin IM, Lye KD, Kidd M. A 5-decade analysis of 13,715 carcinoid tumors. *Cancer.* 2003;97:934–959.
2. Kaltsas GA, Besser GM, Grossman AB. The diagnosis and medical management of advanced neuroendocrine tumors. *Endocr Rev.* 2004;25:458–511.
3. Fraenkel M, Kim MK, Faggiano A, de Herder WW, Valk GD. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer.* 2014;21:R153–R163.
4. Janson ET, Sørbye H, Welin S, et al. Nordic guidelines 2014 for diagnosis and treatment of gastroenteropancreatic neuroendocrine neoplasms. *Acta Oncol.* 2014;53:1284–1297.
5. Caplin ME, Baudin E, Ferolla P, et al. Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Ann Oncol.* 2015;26:1604–1620.
6. Delle Fave G, O'Toole D, Sundin A, et al. ENETS consensus guidelines update for gastroduodenal neuroendocrine neoplasms. *Neuroendocrinology.* 2016;103: 119–124.
7. Niederle B, Pape UF, Costa F, et al. ENETS consensus guidelines update for neuroendocrine neoplasm of the jejunum and ileum. *Neuroendocrinology.* 2016;103: in press.
8. Ramage J, de Herder WW, Delle Fave GF, et al. Consensus guidelines update for colorectal neuroendocrine neoplasms (NEN). *Neuroendocrinology.* 2016;103: 125–138.
9. Pape UF, Niederle B, Costa F, et al. Consensus guidelines for neuroendocrine neoplasms of the appendix (excluding goblet cell carcinomas). *Neuroendocrinology.* 2016;103:144–152.
10. Falconi M, Eriksson B, Kaltsas G, et al. Consensus guidelines update for the management of functional p-NETs (F-p-NETs) and non-functional p-NETs (NF-p-NETs). *Neuroendocrinology.* 2016;103:153–171.
11. Pavel M, O'Toole D, Costa F, et al. Consensus guidelines update for the management of distant metastatic disease of intestinal, pancreatic, bronchial neuroendocrine neoplasms (NEN) and NEN of unknown primary site. *Neuroendocrinology.* 2016;103:172–185.
12. Garcia-Carbonero R, Sorbye H, Baudin E, et al. Consensus guidelines for high grade gastroenteropancreatic (GEP) neuroendocrine tumours and neuroendocrine carcinomas (NEC). *Neuroendocrinology.* 2016;103:186–194.
13. Yao JC, Hassan M, Phan A, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol.* 2008;26:3063–3072.
14. Rindi G, Petrone G, Inzani F. The 2010 WHO classification of digestive neuroendocrine neoplasms: a critical appraisal four years after its introduction. *Endocr Pathol.* 2014;25:186–192.
15. Binderup T, Knigge U, Loft A, Federspiel B, Kjær A. ^{18}F-fluorodeoxyglucose positron emission tomography predicts survival of patients with neuroendocrine tumors. *Clin Cancer Res.* 2010;16:978–985.
16. Shields AF, Grierson JR, Dohmen BM, et al. Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat Med.* 1998;4:1334–1336.
17. Rasey JS, Grierson JR, Wiens LW, Kolb PD, Schwartz JL. Validation of FLT uptake as a measure of thymidine kinase-1 activity in A549 carcinoma cells. *J Nucl Med.* 2002;43:1210–1217.

18. Chalkidou A, Landau DB, Odell EW, Cornelius VR, O'Doherty MJ, Marsden PK. Correlation between Ki-67 immunohistochemistry and 18F-fluorothymidine uptake in patients with cancer: a systematic review and meta-analysis. *Eur J Cancer.* 2012;48:3499–3513.

19. Tehrani OS, Shields AF. PET imaging of proliferation with pyrimidines. *J Nucl Med.* 2013;54:903–912.

20. Tian J, Yang X, Yu L, et al. A multicenter clinical trial on the diagnostic value of dual-tracer PET/CT in pulmonary lesions using 3′-deoxy-3-′18F-fluorothymidine and 18F-FDG. *J Nucl Med.* 2008;49:186–194.

21. Li X-F, Dai D, Song X-Y, Liu J-J, Zhu Y-J, Xu W-G. Comparison of the diagnostic performance of 18F-fluorothymidine versus 18F-fluorodeoxyglucose positron emission tomography on pulmonary lesions: a meta analysis. *Mol Clin Oncol.* 2015;3:101–108.

22. Yamamoto Y, Nishiyama Y, Kimura N, et al. Comparison of 18F-FLT PET and 18F-FDG PET for preoperative staging in non-small cell lung cancer. *Eur J Nucl Med Mol Imaging.* 2008;35:236–245.

23. Yang W, Zhang Y, Fu Z, et al. Imaging of proliferation with 18F-FLT PET/CT versus 18F-FDG PET/CT in non-small-cell lung cancer. *Eur J Nucl Med Mol Imaging.* 2010;37:1291–1299.

24. Li Z, Yu Y, Zhang H, Xu G, Chen L. A meta-analysis comparing 18F-FLT PET with 18F-FDG PET for assessment of brain tumor recurrence. *Nucl Med Commun.* 2015;36:695–701.

25. Wang J, Kuo W-H, Shih TT-F, Yen R-F. Using 18F-FLT PET to distinguish between malignant and benign breast lesions with suspicious findings in mammography and breast ultrasound. *Ann Nucl Med.* 2014;28:941–949.

26. Jensen MM, Erichsen KD, Björkling F, et al. Early detection of response to experimental chemotherapeutic Top216 with [18F]FLT and [18F]FDG PET in human ovary cancer xenografts in mice. *PLoS One.* 2010;5:e12965.

27. Jensen MM, Erichsen KD, Johnbeck CB, et al. [18F]FLT and [18F]FDG PET for non-invasive treatment monitoring of the nicotinamide phosphoribosyltransferase inhibitor APO866 in human xenografts. *PLoS One.* 2013;8:e53410.

28. Jensen MM, Erichsen KD, Johnbeck CB, et al. [18F]FDG and [18F]FLT positron emission tomography imaging following treatment with belinostat in human ovary cancer xenografts in mice. *BMC Cancer.* 2013;13:168.

29. Johnbeck CB, Munk Jensen M, Haagen Nielsen C, Fisker Hag AM, Knigge U, Kjær A. 18F-FDG and 18F-FLT-PET imaging for monitoring everolimus effect on tumor-growth in neuroendocrine tumors: studies in human tumor xenografts in mice. *PLoS One.* 2014;9:e91387.

30. Sanghera B, Wong WL, Sonoda LI, et al. FLT PET-CT in evaluation of treatment response. *Indian J Nucl Med.* 2014;29:65–73.

31. Öberg K, Knigge U, Kwekkeboom D, Perren A, ESMO Guidelines Working Group. Neuroendocrine gastro-entero-pancreatic tumors: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2012;23(suppl 7): vii124–vii130.

32. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45: 228–247.

33. Bosman FT, Carneiro F, Hruban RH, Theise ND. *WHO Classification of Tumours of the Digestive System.* Lyon, France: International Agency for Research on Cancer; 2010.

34. Rindi G, Klersy C, Inzani F, et al. Grading the neuroendocrine tumors of the lung: an evidence-based proposal. *Endocr Relat Cancer.* 2013;21:1–16.

35. Giammarile F, Billotey C, Lombard-Bohas C, et al. 18F-FLT and 18F-FDG positron emission tomography for the imaging of advanced well-differentiated gastro-entero-pancreatic endocrine tumours. *Nucl Med Commun.* 2011;32:91–97.

36. Yang Z, Tang LH, Klimstra DS. Effect of tumor heterogeneity on the assessment of Ki67 labeling index in well-differentiated neuroendocrine tumors metastatic to the liver: implications for prognostic stratification. *Am J Surg Pathol.* 2011;35:853–860.

37. Johnbeck CB, Knigge U, Kjær A. PET tracers for somatostatin receptor imaging of neuroendocrine tumors: current status and review of the literature. *Future Oncol.* 2014;10:2259–2277.

38. Kjær A, Knigge U. Use of radioactive substances in diagnosis and treatment of neuroendocrine tumors. *Scand J Gastroenterol.* 2015;50:740–747.