The role of PET^{18} F-FDOPA in the evaluation of low grade gliomas.

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Key Points:

1) Low grade gliomas are in general relatively slow-growing brain tumor, but they have a very heterogeneous clinical and biomolecular behavior.

2) The optimal treatment of low grade glioma remains controversial, i.e “wait-and-see” versus treatment.

3) The MRI is considered the gold standard in the evaluation of low grade glioma, but has several limitations. Since the glioma growth does not obey an exponential evolution due to the diffusion of many newly produced tumor cells into the surrounding parenchyma. Therefore, the tumor density does not reach the minimal threshold required to appear on MRI.

4) The PET ^{18} F-FDOPA has good sensitivity and specificity in the evaluation of brain tumor recurrence, mainly in the evaluation of the recurrence of low grade or high grade malignant gliomas.

5) In our study, PET ^{18} F-FDOPA demonstrated not a potential diagnostic role, but also a prognostic value in predicting progression of disease.

LOW GRADE GLIOMA

According to the classification of World Health Organization (WHO) the term “low-grade glioma” (LGG) includes grade I and II gliomas. However, this group contains tumors that are clinically, histologically, and molecularly diverse. For example, the management of WHO grade I gliomas is very different from that of WHO grade II gliomas, the former being amenable to surgical cure, while the role of surgery for the latter is still being justified. According to WHO classification, the most important histological subtypes are astrocytomas, oligodendrogliomas, and oligo astrocytomas. Because of their initially indolent nature, the LGG may cause considerable morbidity, and inevitably lead to death [1].

The incidence of LGG in Europe is approximately 1.5/100.000 patients/year (astrocytic LGG, 1.2/100.000 patients/year; oligodendroglial LGG, 0.3/100.000 patients/year) [2], accounting for nearly 15% of all primary brain tumours. The peak incidence occurs in people between 35 and 44 years of age, and there is an increased prevalence among white people and male gender [2]. Despite a preponderance of astrocytomas, there has been an increased rate of diagnosis and/or incidence of pure oligodendrogliomas and mixed oligo-astrocytomas in recent years.

LGG have a predilection for eloquent areas, such as the supplementary motor area or the insula, especially astrocytomas. By contrast, oligodendrogliomas are seen most commonly along the convexity in subcortical areas, particularly in the frontal lobe [3].

Seizures represent the commonest presenting symptom, occurring in up to 80% of patients [4]. Seizures are more frequently associated with oligodendroglial tumors

and with cortically based tumors, especially in frontal, temporal and insular locations. Other symptoms include headache, lethargy, and personality changes. The survival rate at 5 years is approximately 40%-50% in astrocytomas, and 65%-80% in oligodendrogliomas [2].

The natural history of LGG results in transformation into high-grade tumors. This transformation is the main factor in determining a patient's survival. Also, is not predictable the timing of change to a high grade tumor.

It has been demonstrated that several factors may negatively affect survival: age older than 40 years, astrocytoma histology, maximum tumor diameter ≥ 6 cm, tumor crossing the corpus callosum, and the presence of a neurological deficit (except seizures) before surgery [4].

Molecular Markers

Recent reports have dramatically altered our conceptual framework of LGG molecular genetics, suggesting that isocitrate dehydrogenase (IDH) mutations are an early event in LGG pathogenesis. Previously, it was recognized that most LGGs harbored one of two mutually exclusive genetic changes: TP53 mutations in most low-grade astrocytomas, and deletions of chromosomes 1p and 19q in most pure low-grade oligodendrogliomas [5]. Various authors have demonstrated IDH1 mutations in 59% to 90% of grade II astrocytomas, 68% to 85% of grade II oligodendrogliomas, and 50% to 83% of grade II oligoastrocytomas [6-10]. Griffin et al observed that the IDH1 mutation is present in almost all secondary glioblastomas, but in only 7% of primary (de novo) glioblastomas [11]. Every IDH1 mutation occurs in codon 132, which normally codes for an arginine in the enzyme's active site [6,8,9]. IDH1 shares substantial homology with IDH2, a mitochondrial enzyme catalyzing the same reaction. Rare IDH2 mutations also were recently recognized in gliomas, especially oligodendrogliomas [7,9]. The mechanisms by which IDH mutations predispose to gliomagenesis remains unclear. Although the mutated protein is catalytically less active, consistently with a loss of function, these mutations are always heterozygous. Moreover, none of the mutations is a non-sense mutation, further suggesting the mutations may produce a dominant negative or gain of function [12]. Thus, IDH1 and IDH2 seem to function as tumor suppressor genes. Their inactivation has been shown to induce genes crucial to angiogenesis, although the relevance of this finding in LGGs lacking of angiogenesis remains unclair [12]. To what extent molecular markers such as 1p/19q codeletion status, IDH mutational status, and the presence or absence of O^6-methylguanine- DNA methyltransferase (MGMT). MGMT promotor hypermethylation (which is a prognostic factor in

glioblastoma outcome), predict responsiveness to specific therapies or overall prognosis remains uncertain. The presence of 1p/19q deletion seems to portend a predict better prognosis in LGG, although its favorable impact is weaker than in grade III gliomas. Kesari S et al. suggested that MGMT methylation status may predict improved overall survival [13], but the small size of this study, and a potential confounding correlation between MGMT methylation and 1p/19q codeletion, mandate support the need of larger studies. Similarly, in univariate analyses, IDH1 mutations conferred improved overall survival in LGG and anaplastic gliomas, as well as in glioblastomas, but it remains unclear whether mutational status retains prognostic significance when other prognostic markers are incorporated into multivariate models [8,10].

Treatment of low grade glioma

The optimal treatment of LGGs remains controversial. Nowadays, there are various possibility of treatment: surgery, radiotherapy and chemotherapy.

Surgical intervention

In patients with suspected LGG, the surgery has various aims: 1) histological confirmation of the nature of the lesion and evaluation of biomolecolar markers; 2) improvement of the neurological condition, particularly in the control of seizures [14]; and 3) prevention of malignant transformation [15].

Various studies supported the evidence that a resection is as extensive as safely possible once a surgery is planned.

Radiation therapy

The efficacy of radiotherapy in LGG has been demonstrated by a large randomized trial that found a longer time to progression after early radiotherapy in comparison to observation [16]. Also, it has been reported a better symptom control, especially epileptic seizures [17]. Moreover, it has been observed that radiotherapy has a relevant neurotoxicity with a reduction of quality of life [18,19].

The radiotherapy is considered among the treatments, but there are some problems such as late toxicity of radiotherapic treatment in patients who has long estimated life [20-23]. Also, in young patients who have low increase tumors, and therefore long estimated life, cognitive deterioration and radionecrosis incidence has a big influence on quality of life [19, 24]. For this reason, today is not clear the optimal timing of this treatment, and therefore radiotherapy is delayed until recurrence and/or clinical-radiological progression [25].

For all these reasons, today, there is increasing attention on the potential efficacy of the chemotherapy on the evolution of LGG.

Chemotherapy

Shaw et al [26] demonstrated that the combination of procarbazine, lomustine and vincristine (PCV) after radiotherapy increase progression free survival, but not overall survival. Other studies reported higher efficacy of PCV and temozolomide (TMZ) chemotherapy with more frequent responses and longer duration of response in 1 p/19q co-deleted tumors [27-29].

Several studies demonstrated a chemotherapy activity in children and adults with newly diagnosed or recurrent LGG [30-32]. Also, various authors reported that the chemotherapy in LGG prolonged disease stabilization and leads to control of symptoms, including epilepsy [33].

Some authors has been hypothesized that the TMZ may represent an interesting alternative to radiotherapy after brain surgery [33-37]. It also exhibits an important clinical benefit with long disease stabilization, epileptic symptomatology control, and a good influence on quality of life [33]. Another study investigated the role of TMZ in LGG growth with standard schedule treatment. The authors demonstrated the positive effects of this kind of chemotherapy in LGG, although this beneficial effect was limited in time [38]. Kesari et al. [13] suggested that a protracted administration of daily TMZ is a well tolerated regimen and seems to produce an effective tumor control.

A Spanish study concluded that extended dose-dense schedule of TMZ appears to have activity, albeit modest, in patients refractory to previous standard TMZ

treatment, and that is associated with manageable toxicity [39]. These encouraging data come from a non homogeneous survey that included LGG in progression with radiological aspects, and sometimes histological check, of anaplastic evolution. LGG growth process is characterized by a slow expansion with a growth rate less than 1-3% [40,41], and this is considered to be a resistance factor for cytotossic radiotherapic and chemotherapic treatment. More recently has arisen the hypothesis that LGG biological features could make these tumors more sensitive to chronic chemotherapic drug exposition rather than fractioned elevated doses which are most useful in the treatment of tumors exhibiting elevated mitotic index [41]. Preliminary experiences with TMZ, taken at continuative schedule (low daily dose) in anaplastic gliomas have demonstrated good tolerability with hematologic toxicity lower than in standard schedule TMZ treatment (200 mg/mq for 5 consecutive days every 28 days) [42].

Various authors have demonstrated that heterozigosity of chromosomes 1 p and 19 q may be powerful predictors of chemosensitivity and survival in oligodendrogliomas [43,44]. It has been also suggested that genetic alterations may help to identify chemosensitivity in LGG [45].

At present, the treatment approach is the radical tumor resection, when possible and subsequent chemotherapy, especially in patients considered at “high-risk”.
However, evidence-based clinical data on treatment strategies to guide decision are still lacking.

The magnetic resonance imaging (MRI)

The fate of most LGGs is to progress to a higher grade of malignancy. Is not clear about their radiological history and growth rates during the “premalignant” phase. Today, the question on natural history of LGG is: do these tumors stabilize, grow irregularly, or grow continuously according to some common rules?

Actually, the MRI is the most important diagnostic tool for assessing LGG. It is useful for differential diagnosis, guiding biopsy or resection, planning radiotherapy and monitoring treatment response. The typical findings are of an infiltrative, non-enhancing mass lesion that arises in white matter and often extends into the cortex. These tumours are hypointense on T1-weighted sequences and hyperintense in T2-weighted sequences, with different degrees of white matter infiltration and oedema around the central part of the tumor.

Several studies demonstrated that the oligodendrogliomas exhibit calcifications in 20% of all cases [46].

Studies reported that in the majority of LGG the contrast enhancement is infrequent [47], and when present, it may indicate a focal area of high grade transformation.

The risk of anaplasia in non-enhancing lesions increases with patient age [48].

The MRI provide excellent anatomical information on the localization of brain lesions, but is inadequate in distinguishing between non-specific pathologies and tumor grades, exact delineation of tumor volumes, treatment-induced changes, and tumor recurrence [49].

Various studies reported low sensitivity and specificity for standard morphological MRI [49,50].

Furthemore, the glioma growth does not obey an exponential evolution because many newly produced tumor cells diffuse into the surrounding parenchyma, and their density does not reach the minimal threshold required to appear on MRI [40].

For this reason, the structural morphological information is supplemented by functional imaging. The use of advanced imaging techniques can increase the diagnostic accuracy. The radioactive tracer technologies like single-photon computed emission tomography (SPECT) and positron emission tomography (PET), along with MRI based methods (Functional MRI and spectroscopy MRI), can be used to obtain information on the molecular events (specific gene expressions), physiological processes (such as blood flow), and metabolic pathways (for example, glucose usage, protein synthesis, and DNA synthesis) characterizing tumor biology. The typical spectrum in the proton magnetic resonance spectroscopy (MRS) of LGG is a elevated choline, reflecting increased membrane turnover and decreased N-acetyl aspartate, reflecting neuronal loss. MRS is helpful in the diagnostic phase, but not in longitudinal monitoring [52].

Following treatment, the differentiation between treatment related changes and residual or recurrent tumor can be challenging. Given these limitations of anatomic imaging, metabolic imaging using PET with radiolabeled aminoacids may help to overcome some of these drawbacks. In the post treatment, PET has been advocated as a clinically useful imaging modality to differentiate treatment related changes from recurrent tumor.

Positron emission tomography (PET)

PET has been used for several neurologic, cardiac and oncological indications, and is currently revolutioning clinical management in oncology [53].

PET provides an in vivo metabolic and functional map of intact biologic systems. Anatomical, physiological or biochemical aspects of tumors, which distinguish neoplastic from normal brain tissue, can be visualized by means of PET.

The research field on PET is important for the development of tracers capable of measuring biologically relevant tumor cell mechanisms and the prediction of treatment efficacy on tumour cell metabolism [54]. In the literature, it is reported the use of various tracers and the PET can have various implications in clinical practice (see table 1).

The PET with 2-deoxy-2-[18 F] fluoro-D-glucose (FDG)

Malignant tumors are known to have higher rates of glucose utilization and glycolysis. Based on this knowledge, non invasive PET methods to image glucose uptake using 2-deoxy-2-[18 F] fluoro-D-glucose (FDG), a positron emitting analogue of glucose, have been developed. FDG is transported inside the cell by specific glucose transporters, and is phosphorylated by hexokinase but is not further metabolized to any significant extent. A recent study on 336 patients with primary brain tumor demonstrated that tumor grading according to FDG tumor uptake was more accurate than histopathology in prediction of survival [56]. Various studies have revealed several limitations in the applications of FDG-PET in patients with glioma : A) sensitivity in LGG detection by FDG-PET is low, as tumor to normal brain contrast is insufficient due to relatively small differences in the rates of glucose utilization

between normal brain and LGG [58]; B) increase in FDG uptake is non specific, as also observed in inflammatory lesions [57]. Even within tumors, about 24% of the FDG concentration in a tumor mass is actually in macrophages and other inflammatory cells [57]. For these reasons, FDG-PET demonstrates low sensitivity and specificity in distinguishing recurrent brain tumors from radionecrosis [58,59]; C) furthermore, FDG-PET has difficulties in precisely delineating tumour boundaries because of high uptake of FDG in the gray matter and difficulty in imaging the tumour infiltrative areas into the normal brain.

For these reasons, alternative PET tracers have been investigated for brain tumor imaging.

The PET with the L- [methyl-^{11} C]-methionine (^{11}C-MET)

The PET with the L- [methyl-^{11} C]-methionine (^{11}C-MET) is the most popular amino acid imaging modality for tumors, although its use is restricted to PET centers with an in-house cyclotron facility. Several studies have demonstrated that the^{11}C-MET is more efficient than FDG in delineating the tumor extent, especially in LGG and detecting tumor recurrences [60-62]. The overall sensitivity of ^{11}C-MET for gliomas, including high and low grade gliomas, has been estimated to be around 76-95% in various studies [63-66].

In the LGG, it has been reported that sensitivity lies between 65-85%, although its uptake may be lower in LGG than in high grade gliomas. [63, 66, 67]. By using the PET ^{11}C-MET it have been reported, as causes of false positives, demyelination, necrosis, subacute or chronic ischemia, brain abscess and hematoma. [65,66,68]

In a study of 194 patients, Narai et al. [67] demonstrated that while the ratio of standardized uptake value (SUV) normal hemispheric brain tumor (T/N) was significantly different in the low grade and high grade gliomas, there was no significant differences between grade I and II, and grade III and IV. A significant correlation was also found between ^{11}C-MET uptake and Ki-67 proliferation index. A higher T/N ratio of methionine uptake was found to be associated with short survival [67].

In conclusion, a lower semiquantitative ^{11}C-MET uptake has been associated with a better prognosis in patients with similar tumor grade and viceversa.

The role of PET ^{11}C-MET is important for the determination of tumor extent determination and in the differentiation of tumor recurrence versus radiotherapy injury. Various authors reported a significant reduction in ^{11}C-MET uptake and a semiquantitative index based on both MET uptake and MET defined volume in patients with LGG after chemotherapy with PCV [68].

In summary, the role of PET^{11}C-MET is very important at the initial diagnosis, at recurrence versus radiation injury, grading, prognostication, tumor extent, surgical resection biopsy planning, radiotherapy planning and therapy assessment. The use of ^{11}C-MET remains restricted to few PET centers possessing with available cyclotron unite onsite and could not be established in routine clinical practice despite convincing clinical results.

PET O-(2-[18F]-fluoroethyl)-L-tyrosine (^{18}F-FET)

Recent studies have investigated the use of PET O-(2-[18F]-fluoroethyl)-L-tyrosine (^{18}F-FET) in gliomas [69,70], demonstrating similar results between PET ^{11}C-MET and ^{18}F-FET [69,70].

Animal experiments have shown that ^{18}F-FET exhibits no uptake in inflammatory cells, and in inflammatory lymph nodes, promising a higher specificity for detection of tumor cells [71].

Various authors showed that, compared with morphological MRI, ^{18}F-FET PET adds valuable information to the data acquired in case of newly diagnosed cerebral lesions suspicious for LGGs. However, a histological biopsy-based evaluation of suspicious brain lesions remains necessary in most circumstances. Also, Floeth et al. [72] concluded that ^{18}F-FET PET should be considered as an additional tool in resection planning.

A prospective study in patients with LGG without contrast enhancement on MRI indicated that ^{18}F-FET PET in combination with MRI provides important prognostic information [73]. The authors observed that patients with LGG who showed normal or low 18F-FET uptake and circumscribed tumors on MRI had a good prognosis with lower risk for tumor progression and malignant transformation within the first 5 years after diagnosis. On the contrary, patients with LGG exhibiting increased ^{18}F-FET uptake on PET and diffuse tumors on MRI (T2- weighted/FLAIR images) had a worse prognosis with shorter life expectancy, rapid tumor progression, and malignant progression to high-grade tumors within only 2–3 years [73]. The duration of tracer uptake and image acquisition with any of these radiopharmaceuticals is about 30-50 minutes. The cost are comparable to those of ^{18}F-FDG PET.

The results of Pet ^{18}F-FET are promising, but at present there are only few clinical investigations.

PET with 3,4-dihydroxy-6-[^{18}F] fluoro-L-phenylalanine (^{18}F-FDOPA)

Another ^{18}F-labelled amino acid which has been used as a PET is 3,4-dihydroxy-6-[^{18}F] fluoro-L-phenylalanine (^{18}F-FDOPA) with a longer half-life (110 min) .

Its natural counterpart, DOPA, is the precursor of dopamine, which plays a crucial role in the cerebral co-ordination of motor function. Thus, ^{18}F-FDOPA has been extensively used for research in the field of movement disorders, in particular Parkinson's disease [74,75]. More recent studies have also demonstrated its usefulness in the imaging of neuroendocrine tumours [76-78].

^{18}F-FDOPA penetrates into the cells by a process mediated by amino acid transporters [79] . In the tumoral cells it has been observed an overexpression of these membrane transporters, in addition to the increase of vascular flow in tumoral lesions, resulting in an increased incorporation of amino acids at a cellular level. Also, the transportation of amino acids to the tumor cells is not related to the integrity of the blood-brain-barrier, although the uptake increases when this is injuried.

Several studies have demonstrated that ^{18}F-FDOPA may have a role in evaluation of gliomas [80-95] . In literature, it has been reported that ^{18}F-FDOPA has a greater sensitivity and specificity in the evaluation of tumor recurrence of brain disease compared with ^{18}F-FDG, mainly in the evaluation of the recurrence of low grade or high grade malignant gliomas [92].

It has also been reported that ^{18}F-FDOPA is superior to FDG and other radiopharmaceuticals in the evaluation of LGG at both onset and recurrence [90].

Also, Becherer et al. [93] compared ^{18}F-FDOPA e ^{11}C-MET and concluded that the visualization of amino acid transport in brain tumours is feasible, even with compared with ^{18}F-FDOPA. Also the authors observed that the ^{18}F-FDOPA and ^{11}C-MET performed equally well in the imaging of brain tumours. The authors concluded that the ^{18}F-FDOPA might therefore be applied as a substitute for ^{11}C-MET in the diagnosis of residual or recurrent tumours, in particular in PET centres without a cyclotron [93].

Ledezma et al. [91] compared the results of cerebral PET with ^{18}F-FDOPA and MRI for the evaluation of residual disease. The metabolic alterations observed in PET preceded the morphological alterations detected by MRI, suggesting the use of ^{18}F-FDOPA PET in the absence of conclusive signs of residual tumor at MRI.

Also, it has been demonstrated that the kinetics of incorporation of ^{18}F-FDOPA varied depending on the grade of tumor malignancy [94].

The high grade gliomas presented a greater uptake of ^{18}F-FDOPA compared to those of low grade, showing that when the tumor uptake surpassed that of the striate nucleus, the sensitivity and specificity to predict tumor recurrence was of 92% and 95% respectively [95]. While the high grade tumors present a rapid decline in the curve after the maximum peak of incorporation, while those of LGGs have a slow descent. Furthemore, it is un-know whether tumor ^{18}F-FDOPA uptake correlates with tumor proliferative activity as measured by antibody staining of ki-67 antigen of tumor cells. Fueger et al. [89] demonstrated that in newly diagnosed tumors, an ^{18}F-FDOPA SUV max of 2.72 discriminated between low and high grade tumors, with a sensitivity and specificity of 85% and 89%, respectively.

Overall sensitivity of ^{18}F-FDOPA varies from 85% to 100%, and specificity varies from 89% to 100% [92,93].

Karunanithi et al. observed that PET ^{18}F-FDOPA and MRI were concordant in 74.3% and discordant in 17.1% of patients [83]. In a more recent study the authors observed that PET ^{18}F-FDOPA is an independent predictor of survival in patients with suspected recurrent gliomas, along size of recurrent tumor on MRI [80].

Metabolic imaging has been increasingly combined with anatomical imaging by MRI to improve the diagnosis, treatment, and follow-up of brain tumors

Aim of the study

The aim of the study was to evaluate the role of PET ^{18}F-FDOPA in the diagnosis, follow up and assessment of response of treatment of LGG.

Materials and Methods

Patients were enrolled at Department of Neurology of Regina Elena National Cancer Institute – Rome, in a prospective observational study between January 2013 and January 2014 after approvation from Institutional ethical committee.

Patients

We included all patients with a pathological diagnosis of LGG in according to WHO classification in all phase of disease: newly diagnosed, during the treatment with chemotherapy (TMZ or PC) and during observation phase. All patients aged ≥ 18 years, had a Karnofski Performance Scale (KPS) ≥60, and provided written informed consent. We excluded patients in pregnancy or breastfeeding. Clinical and demographic characteristic are collected at study enrolment. We considered age, sex, histological diagnosis, grade of tumour, disease duration, symptoms at onset, surgery or biopsy, radiotherapy, chemotherapy (type and duration).

All patients underwent ^{18}F-FDOPA PET and MRI examination in a span of 4 weeks.

Progression free survival and overall survival were considered as outcomes.

Image protocol MRI

The MRI has been carried out at Department of Neuroradiology of Regina Elena National Cancer Institute – Rome . MRI was performed with a 1.5 T superconductive system (OptimaTM MR450w, GE Medical System, Waukesha, WI, USA). We used a head-coil. All patients were imaged by the following: sequences fast spin echo (FSE) T2 on coronal plane , FSE T1, T2 and Fluid attenuated inversion recovery (FLAIR) in axial planes, with 3-mm slices thickness. After Gadolinium infusion (Gd-DTPA; 0.1mmol/kg) volumetric T1 fast-sequences (3D FSPGR) were acquired.

Both FLAIR and contrast-enhanced weighted sequences were considered for the assessment of response to treatment, according to Response assessment Neuro-Oncology criteria (RANO criteria) [96]. Gd-DTPA-enhancing lesions were considered positive for recurrence on MRI images.

Primary or residual tumors were manually defined by the agreement of two expert radiologists. The tumor boundary was identified, using morphological T2 FLAIR or T2 FSE weighted images as a guide to the tumor location. All sections containing tumor were outlined for each patient, excluding visually concomitant edema/gliosis and areas of malacia. Volumes were then quantified in cm^3 by means of 3D Slicer Software.

Image protocol PET^{18}F-FDOPA

The PET has been carried out at Department of Biomendicine and Prevention, University Tor Vergata, Rome. The PET/ computerized tomography (CT) was performed by tomograph PET/CT Discovery VCT (GE Medical Systems, TN, USA) USING A standard technique in 3D mode in 256x256 matrix. The images WERE reconstructed by alghoritm OSEM (4 iterations, 20 subsets). The system combined 20 rings (diameter 88.6 cm) for a total numbers of 13440 crystals (BGO), each with dimension of 4.7x6.3x30 mm; Axial field of view of 157 mm; Full width at half maximum 1 cm in axial;1 cm radius 5,6 mm in modality 3D mode. After fasting for at least 5 hours, patients were injected with 185 - 210 MBq/Kg di ^{18} F-FDOPA intravenously (iv); subsequently they were hydrated with 500 ml of saline solution iv. (NaCl) 0.9%.

The ^{18} F-FDOPA injection occurs in a dedicate room. The PET have a duration of 15 minutes. A CT scan of the head at a low amperage for attenuation correction (40 mA; 120kV) was executed before the acquisition of the PET images.

The PET CT images were interpreted as positive when the lesion definitely increased ^{18} F-FDOPA accumulation, taking into consideration the background and the controlateral site. The slices with a maximal ^{18} F-FDOPA uptake in the region of interest (ROI) were chosen for quantitative measurement of metabolic activity of the tracer [standardized uptake value (SUV)]. From these ROIs, the SUV was calculated using standard body weight method.

Statistical analysis

Descriptive statistics were used to summarize pertinent study information. Categorial variables were reported as frequencies and percentage values, while continuous variables were summarized through mean value and the relative standard deviation (SD), or median (interval), as appropriate.

The agreement between MRI and PET ^{18}F-FDOPA was estimated by using the Cohen's Kappa statistic, and was interpreted on the basis of the Landis and Koch classification criteria [97]

The SUV-max ratio cut-off value was obtained with a minimum C1 error from the Receiver operating characteristics (ROC) analyses. In the calculation C1 was equal to the following: 1-(sensibility+specificity)/2.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were then obtained in order to evaluate the diagnostic performance of the contrast enhancement.

We also conducted progression free and overall survival analyses by using Cox univariate models and adopting the most suitable prognostic category as referent group. A multivariate Cox proportional hazards model also was developed using stepwise regression (forward selection), with predictive variables that were significant in the univariate analyses. The enter limit and the remove limit were $P = .10$ and $P = .15$, respectively.

P-values < 0.05 denote statistically significant associations. Statistical analyses were carried out using SPSS software (SPSS version 21.0, SPSS Inc., Chicago, Ill., USA).

Results

Patients and tumor characteristics

We enrolled 56 (35 males and 21 females) patients affected by LGG. In Table 2 we reported demographic and clinical data of the whole sample. The mean (SD) age at diagnosis was 44.5 (14.5) years; the median disease duration was 4 (interval from 1 to 30). Out of the 56 patients recruited for this study, 13 (23%) were a newly diagnosed, 25 (45%) in chemotherapy treatment, and 18 (32%) were untreated but had a regular follow-up. The majority of patients (n=27, 48%) were affected by astrocytoma, while the remaining patients were affected by oligodendrogliomas (n=17, 31%), and gliomatosis (n=12, 21%).

MRI features

At enrolment, MRI features were as follows (see also Table 3): 10 (18%) patients had a negative scan; 33 (59%) patients had a stable tumor with residual; 7 (12%) had a partial response; 6 (11%) had a progression disease. Contrast-enhancement was observed only in 18 (39%) patients, while in 28 (61%) patients the contrast-enhancement was absent. The median size of lesion was 27 cm^3 (interval from 2 to 506).

PET features

^{18}F-FDOPA were positive in 41 (73%) patients, and negative in the remaining 15 (27%), based on interpretation criteria mentioned in method section. The mean SUV max was 1.6 (0.6) (see table 4 a).

Follow-up data

All patients had 12 months of regular follow-up after the study entry. At the end of follow-up, 12 (21%) patients showed a progression of disease after a mean time of 223 (40) days; of them, 3 patients died after a mean time of 338 (20) days.
The remaining 44 (79%) were progression-free survivals.

Concordance between MRI and PET

The Table 4 b summarize the agreement between MRI and PET.
The Kappa statistic for the whole sample (n=56) was equal to 0.301 (95%CIs from 0.21 to 0.40) showing a fair concordance between the two tests (value between 0.21 and 0.40 according to the scale of Landis e Koch) in terms of diagnostic capability.
The concordance analysis was repeated at the end of follow-up to test whether the agreement between the two diagnostic tools was increased after splitting patients according to disease progression (n=12) or not (n=44).
When we considered only progression-free patients (n=44), the Kappa statistic was equal to 0.250 (95%CIs from -0.050 to 0.401) showing a fair concordance between the two tests (value between 0.21 and 0.40 according to the scale of Landis e Koch) in terms of diagnostic capability.
When we considered the patients who had a disease progression (n=12), the Kappa statistic was equal to 0.435 (95%CIs from -0.014 to 0.883) showing a moderate concordance between the two tests (value between 0.41 and 0.60 according to the scale of Landis e Koch) in terms of diagnostic capability.
We found a good correlations between residual volume and SUV max (r=0.435, p=0.002 by Spearman Rank Correlation Coefficient), indicating that the greater the

residual volume, the higher the SUV max was (see Figure 1/a). This finding was confirmed even after removing 8 patients with very high residual volume, i.e. more than 5 folds the median value of the whole sample, showing that the correlation between residual volume and SUV max still reached the statistical significance (r=0.335, p=0.03 by Spearman Rank Correlation Coefficient, see Figure 1/b).
The area under the ROC curve for the SUV max predicting the presence of contrast-enhancement at baseline (i.e. at study entry) was 0.789. According to the ROC analysis, the best possible prediction value of SUV max was 1.65 (see Figure 2). The diagnostic values, expressed as sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy was reported in Table 5.

Role of PET in predicting progression and overall survival

In table 6 and 7 are shown the findings of two Cox regression models in which disease progression and death were the dependent variables, and age, disease duration, KPS, residual volume, SUV max, qualitative ^{18}F-FDOPA uptake were the independent variables.
Regarding the progression-free survival, the univariate analysis shows that the variables useful in predicting disease progression were: the disease duration (HR=0.68, 95% CIs from 0.47 to 0.97, p=0.03) and the SUV max, not only considering its absolute value (HR=2.80, 95% CIs from 1.28 to 6.13, p=0.01), but also according to the cut-off value of 1.65 found by means of the ROC analysis (HR=5.27, 95% CIs from 1.15 to 24.10, p=0.032). Moreover, the residual volume reached a marginal significant predictive value (HR=1.00, 95% CIs from 1.00 to 1.01, p=0.054). The Figure 3 shows the Kaplan-Meier curve depicting survival time

stratified by SUV max higher than 1.65 (n=26, continuous line) and lower than 1.65 (n=30, dotted line) (p=0.01 by the Log-Rank test).

After the stepwise procedure, the multivariate analysis showed that a SUV max greater than 1.65 was the only independent predictor of disease progression (HR=4.59, 95% CIs from 0.99 to 21.31, p=0.054). This implies that a patient with a SUV max higher than 1.65 had an almost 5-fold increased risk of disease progression, regardless of its clinical and MRI characteristics.

Regarding the overall survival, there was no significant predictor of death, mainly due to the small number of patients who reached the outcome (n=3).

Discussion

We prospectively investigated the diagnostic and prognostic role of PET ^{18}F-FDOPA in imaging of low grade glioma.

The low grade glioma are a group of tumors with distinct clinical, histological and molecular characteristics. The natural history of LGG is progression to a higher grade of malignancy. The indolent nature of LGG may cause considerable morbidity and lead to death [1].

The MRI is useful for differential diagnosis, guiding biopsy or resection, planning and monitoring treatment response, but in evaluation of LGG history presents several limitations. Very little is known about their radiological history and growh rates during the “premalignant phase”. Mandonnet et al [40]indicate that the growth of tumor diameter is apparently linear. Swanson et al [41] found that the glioma growth does not obey an exponential evolution because many newly produced tumor cells diffuse into surrounding parenchyma and their density does not reach the minimal threshold required to appear on MRI. Thus, these authors concluded that the measurement of the volumetric doubling time is not appropriate to describe the growth curves [40,43].

In the management of LGG, the identification of early changes may drive different clinical and terapeutical approach. Given these limitations of anatomic imaging, in clinical practice the PET has been advocated for evaluation of glioma.

Several studies showed that the PET ^{18}F-FDOPA has higher sensitivity and specificity for glioma compared with other tracers [85,88,90,92]. ^{18}F-FDOPA has half-life of 110 minutes and thus can be send without an onsite cyclotron. Ledezema et al [91] observed that the metabolic alteration PET preceded the morphological alteration detected by MRI. A more recent study on glioma recurrence reported that

result of MRI and PET ^{18}F-FDOPA were concordant in 74.3% and discordant in 17.1% [83]. Also, the authors concluded that this tool is more specific than MRI for high and low grade glioma [83].

Our results confirm that PET ^{18}F-FDOPA has high sensitivity and specificity for low grade glioma. Our data show a fair concordance between MRI and ^{18}F-FDOPA. These results are different than those Karunanithi et al [83], because we considered only low grade glioma. When we considered the patients with progressed glioma we observed a better concordance between the two examinations according to the scale of Landis e Koch [97]. In the majority of LGG, there is not contrast-enhancement at MRI scan. Our findings are consistent with this latter observation, since only 38% of patients exhibited contrast-enhancement. The ^{18}F-FDOPA PET provide a linear quantification of tumor activity, the SUV, that can provide useful information. In our cohort, a SUV of 1.65 exhibited a sufficient sensitivity (60%) and excellent specificity (90%) in identifying contrast-enhancement. Moreover, we also found a good correlation between SUV and lesion size, even after removing patients with very high residual. All these findings might suggest that SUV max can be more useful than MRI in early detecting LGG at risk of imminent anaplastic evolution.

In literature, several prognostic factors are reported to influence survival such as KPS, age, tumor grade and histology, extent at surgery, biomolecular markers and tumor size [4]. The univariate analysis showed that the duration of disease and a SUV cut off>1.65 were predictors of progression in LGG, whilst lesion size on MRI showed a statistically significant trend but not confirmed in a multivariate analysis. Few studies evaluated the potential role of PET as predictor of survival in recurrence glioma [56,67,73, 98-100]. PET with FDG uptake is a strong predictor of survival in

glioma patients [98]. Nyazi et al [99] found that uptake kinetics of [18F]FET-PET is an idependent determinant of overall and progression free survival. ^{11}C-MET/PET has been used not only for diagnosis, but also for prognostication recurrent glioma [100] . Recently, it has been demonstrated that T/N ratio on ^{18}F-FDOPA PET is an independent predictor of survival in patients with suspected recurrent glioma. Also a T/N ratio>1.5 or size >2.5 cm of recurrent tumor on contrast enhancement sequences were predictor of poor survival [80]. Walter et al. found that patients with a positive ^{18}F-FDOPA/PET had significantly shorter survivals than those with negative ^{18}F-FDOPA imaging [101]. In a retrospective analysis on patients treated with Bevacizumab, the authors concluded that the voxel-wise changes in ^{18}F-FDOPA may be a predictor for PFS and OS in recurrent malignant gliomas [88]. Our study reinforces the role of ^{18}F-FDOPA/PET as a tool useful in predicting progression. In the multivariate analysis COX model the presence of a SUV max>1.65 was the only independent predictor of progression free survival, irrespective for other clinical and MRI characteristics considered. We failed to identify any features (clinical, MRI or PET) associated with overall survival, probably due to the small sample size of patients reaching the outcome.

This is the primary, prospective and observational study aimed at evaluating diagnostic and prognostic impact of ^{18}F-FDOPA in low grade glioma.

However, our findings need to be replicated in larger samples. Moreover, a longer follow up is warranted to explore whether ^{18}F-FDOPA PET could predict the survival. Another interesting issue could be the investigation of the potential of ^{18}F-FDOPA PET in monitoring chemotherapy response.

Conclusions

In conclusion, to lead to a correct management of LGG, the neuro-oncologist should rely not only on clinical, biomolecular and MRI radiological features, but also on ^{18}F-FDOPA PET.

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Table1 Summary of the role of PET in clinical management of cerebral gliomas

Clinical managment	**State of the art**
Initial diagnosis	Particularly useful for low grade gliomas
Recurrence versus radiotherapy injury	Correlation between uptake and recurrence
Grading	Correlation between Ki67 and uptake
Prognostication	Higher semi-quantitative uptake

	associated with poorer prognosis
Tumor extent	Better delineation
Surgical resection biopsy planning	Radiotherapy planning
Therapy assessment	Prognosis associated with degree and extent of uptake after therapy

Table 2 Demographic and clinical features of patients

Patients characteristics		
Characteristics	**N**	**%**
Total sample	**56**	
Sex		
Male	35	63
Female	21	27
Age (yrs)		
Mean (SD)	44,5 (14,5)	
Median (range)	41	(19-77)
<50 years	40	71
>50 years	16	29
KPS score (%)		
Median (range)	100 (70-100)	
100	41	73

<100	15	27
Diagnosis		
astrocytomas	27	48
Gliomatosis	12	21
oligodendrogliomas	17	31
disease duration(yrs)		
Median (interval)	4 (1-30)	
Radiotherapy		
Yes	26	46
No	30	54
Chemiotherapy		
Pre	13	23
On going	25	45
Follow-up	18	32

Table 3 MRI features

Parameter	**N**	**%**
RM		
Negative	10	18
Stable with residual	33	59
Partial response	7	12
Progression	6	11
contrast-enhancement		
Yes	18	39
No	28	61
Median size of residual,cc		
	27	(interval 2-506)

Table 4/a PET ^{18}F-FDOPA features

Parameter	N	%
PET ^{18}F-FDOPA		
Negative	15	27
Positive	41	73
SUV MAX mean±SD	1.06 (0.6)	

Table 4/b Concordance between MRI and PET ^{18}F-FDOPA

	^{18}F-FDOPA results			
	Negative		Positive	
RM	**N**	**%**	**N**	**%**
Negative	7	47	3	7
Stable with residual	7	47	26	63
Partial response	1	6	6	15
Progression	0		6	15

Table 5 ROC analysis between contrast-enhancement

contrast-enhancement/Suv Max	Value	Var	SE	95%CI
Area under Curve	0,789			
Sensibility	0,58	0,0094	0,0969	(0,328;0,794)
Specificity	0,9	0,003	0,0548	(0,668;0,980)
Accuracy	0,75	0,0034	0,0579	(0,576;0,871)
positive predictive value	0,833	0,0077	0,0878	(0,511;0,967)
negative predictive value	0,711	0,0054	0,0736	(0,527;0,894)

Table 6 Cox-Regression analyses Progression Free Survival: Univariate and multivariate analyses

	Univariate analyses			Multivariate analyses		
Parameter	**HR**	**95%CI**	p-value	**HR**	**95%CI**	p-value
KPS basale value	0,95	0,90;1,00	0,064			
KPS basale value						
<100	1,00					
100	0,32	0,10;1,06	0,063			
Age	0,97	0,93;1,02	0,280			
Age						
<50 yrs	1,00					
>50 yrs	0,74	0,16;3,41	0,698			
Residual (cc)	1,00	1,00;1,01	0,054			
Suv Max	2,80	1,28;6,13	0,010			
Suv Max						
<1.65	1,00			1,00		
>1.65	5,27	1,15;24,1	0,032	4,59	0,99;21,31	0,054

Disease duration (yrs)	0,68	0,47;0,97	0,030	0,69	0,49;0,99	0,410
^{18}F-FDOPA						
positive	1,00					
negative	0,24	0,03;1,83	0,168			

Table 7 Cox-Regression analyses Overall Survival: Univariate and multivariate analyses

	Univariate analyses			Multivariate analyses		
Parameter	**HR**	**95%CI**	p-value	**HR**	**95%CI**	p-value
KPS basale value	1,02	0,86;1,20	0,852			
KPS basale value						
<100	1,00					
100	0,77	0,07;8,69	0,836			
Age	1,05	0,97;1,13	0,210			
Age						
<50 yrs	1,00					
>50 yrs	7,42	0,67:82,8	0,104			
Residual (cc)	1,00	0,99;1,02	0,781			
Suv Max	3,81	0,79;18,4	0,095			
Suv Max						
<1.65	1,00					
>1.65	61,60	0,005;691740,1	0,386			
Disease duration (yrs)	0,12	0,012;1,23	0,074			
^{18}F-FDOPA						

positive	1,00		
negative	0,03	0,00;1950,0	0,536

Figure 1a Spearman Rank Correlation Coefficient between SUV max and residual volume

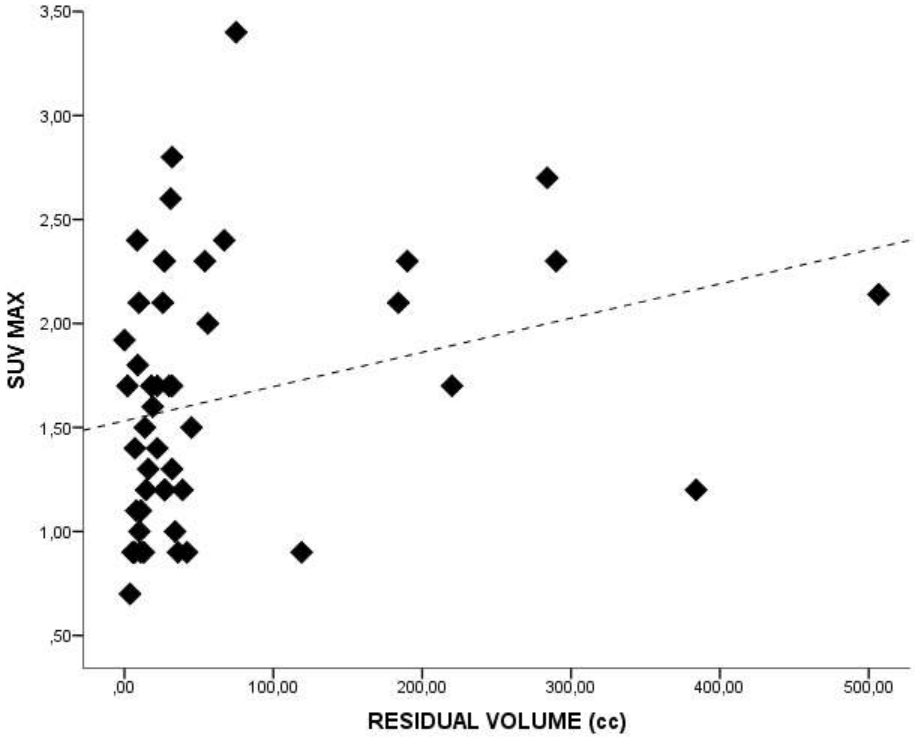


Figure 1/b Spearman Rank Correlation Coefficient between SUV max and residual volume after removing 8 patients with very high residual volume (outliers)

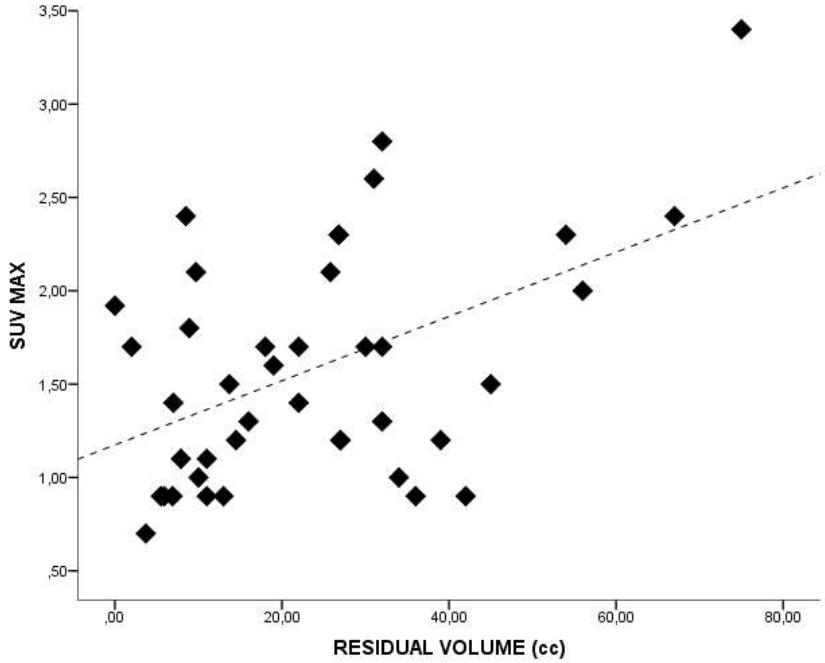


Figure 2 ROC analysis

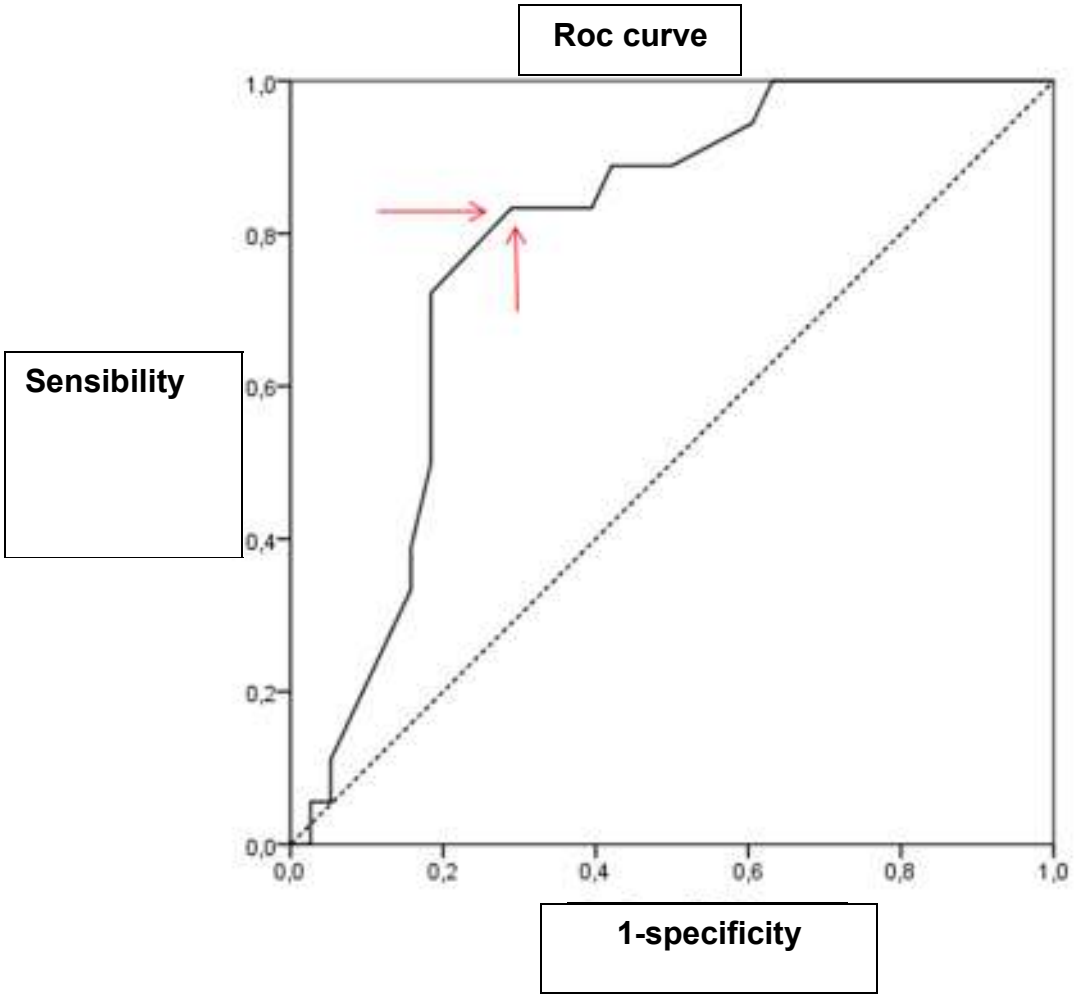
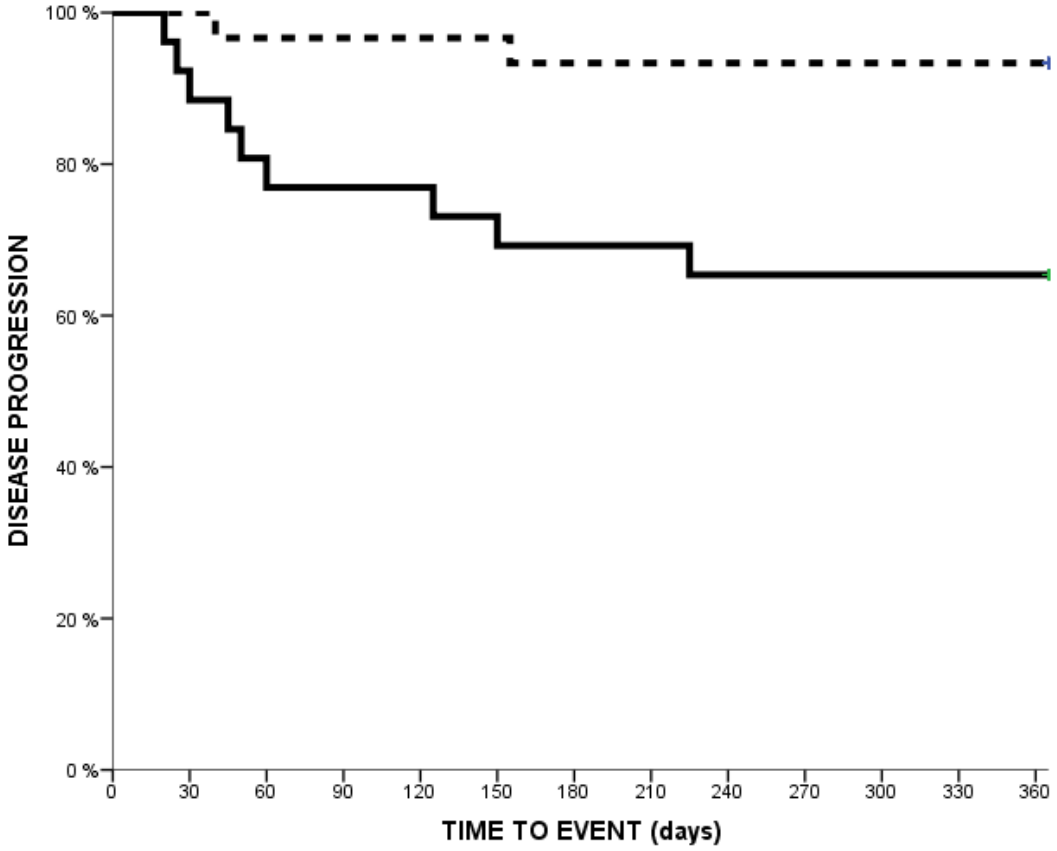

Roc curve
Sensibility
1,0
0,8
0,6
0,4
0,2
0,0
0,0
0,2
0,4
0,6
0,8
1,0
1-specificity

Figure 3 Kaplan-Meier curve depicting survival time stratified by SUV max higher than 1.65 (n=26, continuous line) and lower than 1.65 (n=30, dotted line) (p=0.01 by the Log-Rank test).



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