

# Maximum Standardized Uptake Value of F-18 FDG-PET Inversely Correlated with Immunoexpression of p16<sup>INK4a</sup> in Patients with Non-Small Cell Lung Cancer

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**Abstract:** We have studied the possible relationship between the immunohistochemical expression of p16<sup>INK4a</sup> and the maximum standardized uptake value (SUV max) of F-18 fluorodeoxyglucose-positron emission tomography (F-18 FDG-PET) in 49 patients with non-small cell lung cancer (NSCLC). Immunohistochemical negativity of p16<sup>INK4a</sup> was verified in 71.4 % of the patients, higher in squamous cell carcinomas than in adenocarcinomas (p: 0.033). The SUV max correlates with negativity for p16<sup>INK4a</sup> (p: 0.042) and was higher (p: 0.024) in cases with p16<sup>INK4a</sup>-negative (17.3 ± 8.3) than in cases with p16<sup>INK4a</sup>-positive (12.7 ± 5.2). We can conclude that inactivation of the p16<sup>INK4a</sup> gene is very frequent in NSCLC, with higher values in squamous cell carcinomas. SUV max correlated positively and statistically with negativity for p16<sup>INK4a</sup>, which could explain why higher estimates of SUV max are found in squamous cell carcinomas when compared to adenocarcinomas. Only the clinical stage was found to be an independent prognostic value after multivariate analysis.

**Keywords:** F-18 fluorodeoxyglucose, PET, p16<sup>INK4a</sup>, non-small lung cancer, SUV max.

## INTRODUCTION

The protein p16<sup>INK4a</sup> is the product of the *CDKN2A/p16INK4* gene, which is very closely related to the cell cycle. It binds to and inhibits the complex cyclin D/CDK4, resulting in the inhibition of the cell cycle-dependent phosphorylation of the retinoblastoma protein. In oncology the interest lies in its function as a tumor suppressor gene, and its alterations (mainly promoter hypermethylation, gene deletion, missense mutation, etc.) are associated with a higher risk of developing tumors, lung cancer included. Alternatively its lack of expression can also be found in preneoplastic bronchial lesions [1] and in smokers [2]. It can also influence the sensitivity to cisplatin therapy [3].

In non-small cell lung cancer (NSCLC) the expression of p16<sup>INK4a</sup> does not correlate with age, gender, tumor site, histological subtype or clinical stage [4]. Some groups have confirmed its prognostic value [5-8]; others however, did not find it in tissue [9, 10] or in serum [11]. Some studies suggest a role for p16<sup>INK4a</sup> and cyclin D1 deregulation in progression of preinvasive bronchial lesions to invasive carcinoma [9]. Aberrant expression of p16<sup>INK4a</sup> and p53 were found to be significant and independent predictable prognostic factors for resected NSCLC, especially in early stages [4]. Methylation of the promoter region of the *p16* gene [12], as well as, cohypermethylation of *p16*<sup>INK4a</sup> and *fragile histidine triad* (FHIT) genes in patients with stage I NSCLC may be a

valuable biomarker for predicting the recurrence-associated prognosis of the disease [13].

Positron emission tomography (PET) with F-18 fluorodeoxyglucose (F-18 FDG) is an imaging technique widely used in patients with NSCLC [14, 15]. It has been shown to be of great value in staging/re-staging, early response to treatment and planning radiotherapy treatment. At the same time, the maximum standardized uptake value (SUV max) behaves, according to some groups, as an independent prognostic factor [16].

Some biological factors such as glucose transporters, cell proliferation (MIB-1), some enzymes involved in glycolysis, hypoxia-inducible factor 1 (HIF-1), etc., have recently been related with SUV max [17, 18]. SUV provides a semiquantitative value and is defined as the tissue concentration of tracer, as measured by PET, divided by the injected dose normalized to patient weight multiplied by a decay factor. In practice, the SUV is calculated by dividing the activity concentration in the region of the interest (ROI) drawn around the lesion by the injected dose divided by the body weight. Considering that a positive correlation has been found between SUV max and cellular proliferation and that p16 have been found to have the opposite effect, we have investigated the correlation between both parameters in patients with NSCLC.

## MATERIALS AND METHODOLOGY

The study group included 49 patients (45 males and 4 females, aged between 41 and 82 (63.7 ± 11.9) with NSCLC. The mean age of the patients with adenocarcinoma (14 males and 2 females) was 56.9 ± 12.5, and for the patients with

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squamous cell carcinoma (31 males and 2 females) was  $67.6 \pm 10.2$ . According to the clinical stage, the patients were classified as follows: stage IA: 3; stage IB: 8; stage IIA: 1; stage IIB: 8; stage IIIA: 10; stage IIIB: 9 and stage IV: 10 cases.

We used a tissue arrayer device (Beecher Instruments, Sun Prairie, WI, USA) to produce tissue microarray (TMA) blocks, in accordance with conventional protocols [19]. All cases underwent histological review and the most representative areas were marked in the paraffin blocks. Two selected 1-mm-diameter cylinders from two different areas were included in each case from the carcinomas. All samples were obtained from the files of the Department of Pathology, Clinical University Hospital, Santiago de Compostela, Spain. Internal and external controls were included in each TMA. Immunohistochemical studies were performed on 4- $\mu$ m-thick paraffin sections using a peroxidase-conjugated dextran-labelled polymer (Dako EnVision Peroxidase/DAB; Dako, Glostrup, Denmark), in order to avoid misinterpreting endogenous biotin or biotin-like activity in cell cytoplasm or nuclei as positive staining. A commercially available monoclonal antibody was used for p16<sup>INK4a</sup> (Clone E6H4, CINtec Histology Kit, Heidelberg, Germany). Nuclear and cytoplasm immunoreactivity was considered positive for p16<sup>INK4a</sup>. Equivocal staining was considered to be negative. Immunohistochemical results were recorded as positive when >10% of neoplastic cells displayed immunoreactivity.

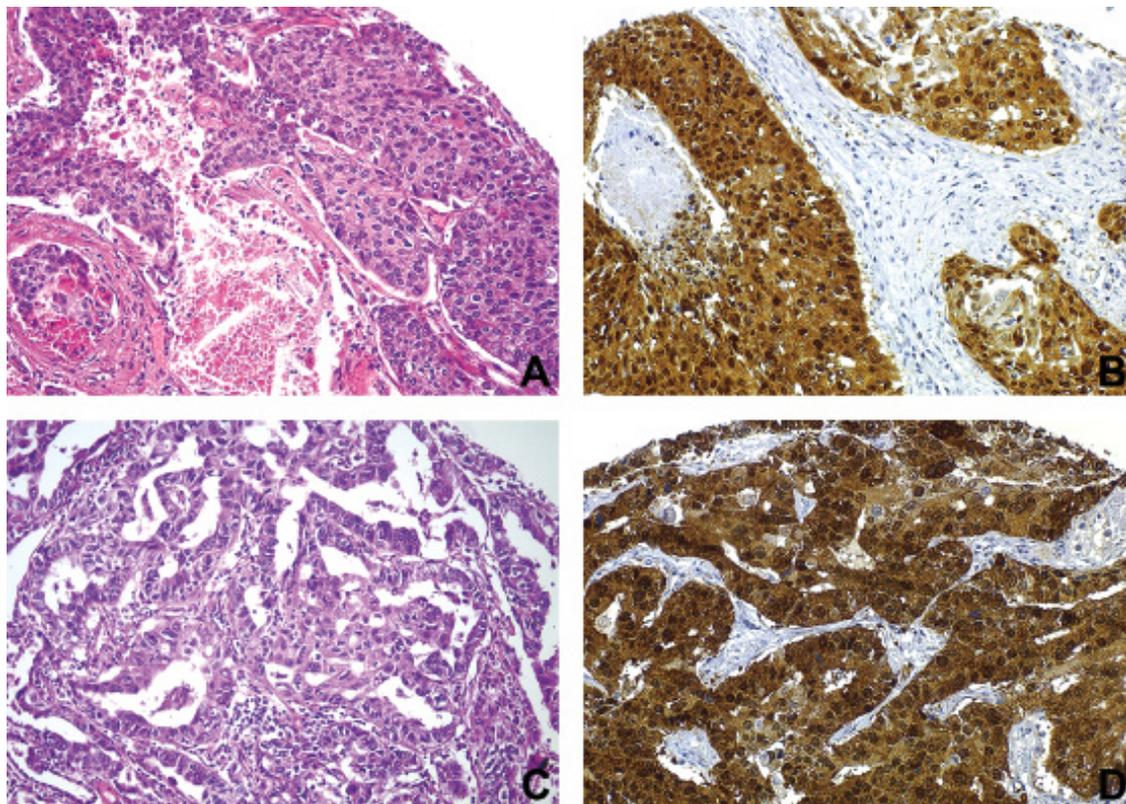
Glucose levels were measured (maximum level accepted 160-180 mg/dl) and a muscle relaxant was administered. 15-30 minutes later, F-18 FDG (350-518 MBq) was injected.

Patients had to fast for at least six hours prior to administration. The image was acquired 60 minutes after the administration of the radioisotope in a PET Advanced System (General Electric Medical Systems, Milwaukee, WI, USA). Semiquantitative analysis was performed after determination of SUV max indexes for each observed lesion, considering the SUV max to be the uptake of the region of interest in relation to the injected dose and the body weight. Axial, coronal, and sagittal tomographic images along with the volumetric projection were visualized. Sequential studies were not performed and only the uptake observed was considered in the investigation.

The SPSS programme for Windows was employed for the statistical analysis. Continuous variables were expressed as the mean  $\pm$  SD. To verify any statistically significant difference, Chi-square distribution, with Yates correlation, was used for qualitative variables, while Student's t-distribution was used for the continuous variables, as well as for the analysis of variance after adjustment of the clinical stage. Spearman's rank correlation coefficient was used to measure the variables. Survival curves were analyzed by means of the Kaplan-Meier method and their difference by the log-rank test. Multivariate analysis for survival was performed using the Cox proportional hazards models. The criterion for significance was considered  $p < 0.05$ .

## RESULTS

Immunostaining for p16<sup>INK4a</sup> was negative in 71.4 % of the cases, with higher values ( $p: 0.033$ ) in squamous cell carcinomas (71.8%) than in adenocarcinomas (50%) (Fig. 1). SUV max in the study group ranged between 1.6 and 47,



**Fig. (1).** p16<sup>INK4a</sup> in non-small cell lung cancer. Squamous cell carcinoma (A) (HE) showing immunoreactivity for p16<sup>INK4a</sup> (B). p16<sup>INK4a</sup> immunostain (D) was also appreciate in this adenocarcinoma (HE) (C). (A, B, C and D x200). HE indicates hematoxylin and eosin.

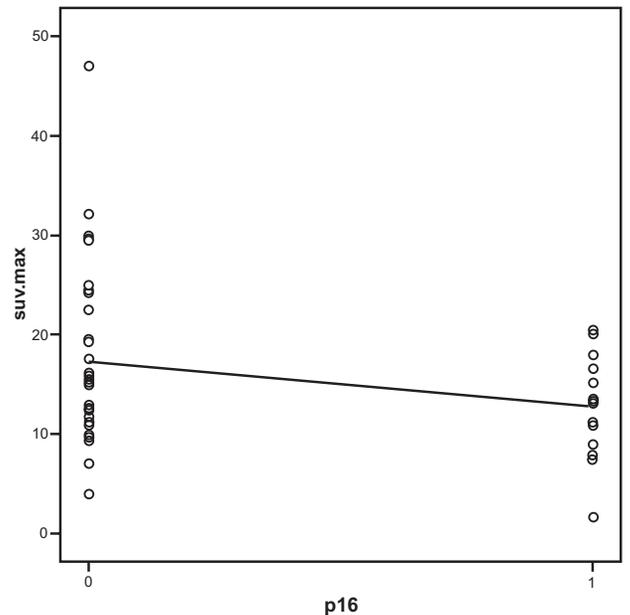
with a mean of  $16.0 \pm 7.8$  and a median of 15.1. SUV max for adenocarcinomas ranged between 1.6 and 47 ( $15.1 \pm 10.6$ ; median 13.2) and these results did not differ significantly from the values found in squamous cell carcinomas (range: 4.5-32.1;  $16.4 \pm 6.3$ ; median 15.6).

In the study group considered as a whole, SUV max were correlated statistically and negatively with p16 expression ( $r: -0.29$ ;  $p: 0.042$ ) (Fig. 2). SUV max were also higher ( $p: 0.024$ ) in those cases with p16<sup>INK4a</sup>-negative ( $17.3 \pm 8.3$ ) than in p16<sup>INK4a</sup>-positive ( $12.7 \pm 5.2$ ). SUV max distribution in relation with the clinical stage and p16<sup>INK4a</sup> expression is shown in Table 1. We found that in those cases with p16<sup>INK4a</sup>-positive SUV max tended to be statistically higher ( $p: 0.07$ ) in stages III-IV than in I-II (Fig. 3), although this did not occur in those with p16<sup>INK4a</sup>-negative. On the other hand, in stages I-II SUV max was higher in the patients with p16<sup>INK4a</sup>-negative than in those with p16<sup>INK4a</sup>-positive ( $p: 0.007$ ). There was no difference in stages III-IV.

After univariate analysis SUV max did not behave as a prognostic value, while p16<sup>INK4a</sup> expression did ( $p: 0.069$ ; HR 2.49, CI: 0.93-6.09). The p16<sup>INK4a</sup> protein lost this property, however, after multivariate analysis, with the clinical stage ( $p: 0.004$ ) remaining as the only prognostic value (Table 2).

**DISCUSSION**

The p16<sup>INK4a</sup> gene is involved in controlling phase G1 of the cell cycle. The frequent deletion or mutation of CDKN2A(p16<sup>INK4a</sup>) in tumor cells suggests that p16<sup>INK4a</sup> acts as a tumor suppressor. Lukas, *et al.* [20] showed that wild-type p16<sup>INK4a</sup> arrests normal diploid cells in late G1, whereas a tumor-associated mutant of p16<sup>INK4a</sup> does not. Significantly, the ability of p16<sup>INK4a</sup> to induce cell cycle arrest was lost in cells lacking functional retinoblastoma protein. Thus,

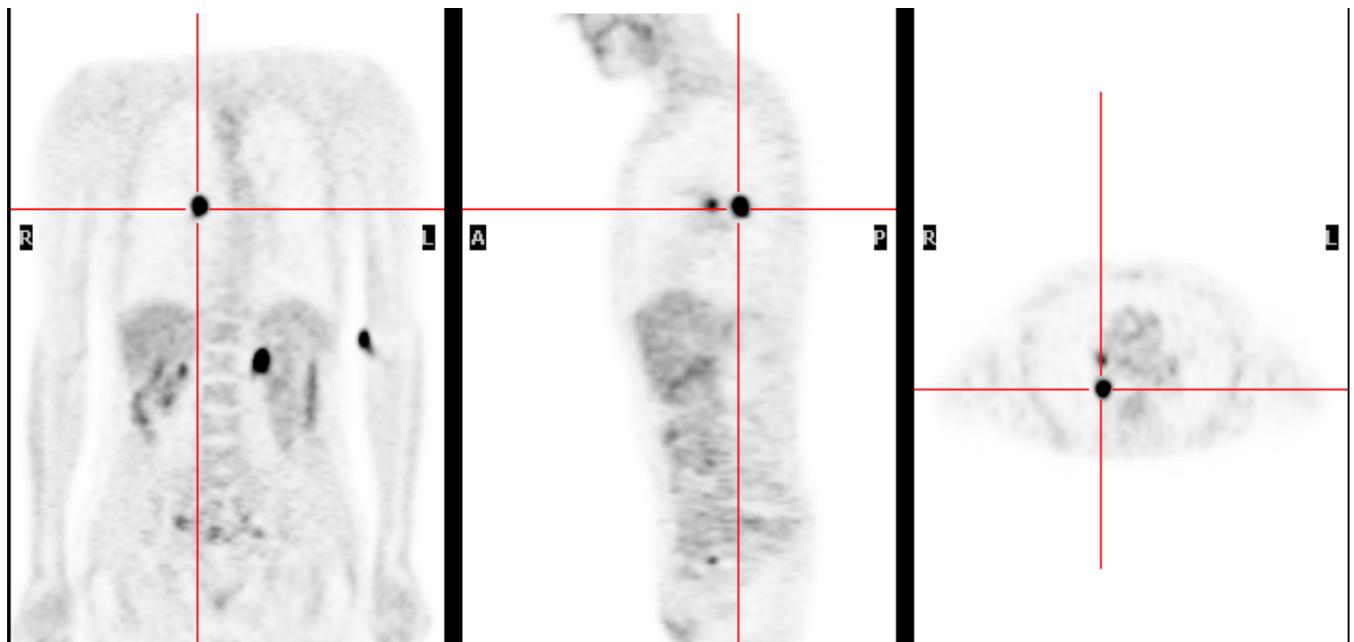


**Fig. (2).** Inverse correlation between maximum standardized uptake value (SUV max) and p16<sup>INK4a</sup> expression. 0: p16<sup>INK4a</sup>-negative expression; 1: p16<sup>INK4a</sup>-positive expression.

**Table 1.** Distribution of SUV Max Values According to Clinical Stage and p16<sup>INK4a</sup> Expression

Clinical Stage	p16 <sup>INK4a</sup> -Positive	p16 <sup>INK4a</sup> -Negative	p
I-II	(5) $9.3 \pm 4.7$	(15) $19.5 \pm 9.7$	0.007
	$p = 0.07$	ns	
III-IV	(9) $14.5 \pm 4.7$	(20) $15.7 \pm 7.0$	ns

Note: ( ), number of cases; ns, not significant.



**Fig. (3).** F-18 fluorodeoxyglucose-PET for staging a 60 year-old-male with non-small cell lung cancer. There is high focal uptake in the right lung (triangled in coronal, sagittal and transaxial view), coincident with the lesion previously detected in the CT scan. There is also a focus, less than 10 mm in diameter, in the right hilar area, suggesting lymph node involvement, later confirmed (biopsy) as an adenocarcinoma.

loss of p16<sup>INK4a</sup>, overexpression of D-cyclins, and loss of retinoblastoma have similar effects on G1 progression, and may represent a common pathway to tumorigenesis. Inactivation of p16<sup>INK4a</sup> has been observed in numerous tumors including lung cancer [21-23]. Along with p53, p16<sup>INK4a</sup> is considered the most important gene in oncology. Within lung cancer it is very relevant in carcinogenesis and its functional alteration is associated with the smoking habit, premalignant lesions, and genesis of different tumor subtypes, sensitivity to specific therapies and even with prognosis at an early stage [12]. Inactivation of p16<sup>INK4a</sup> can be associated with other biological parameters [24, 25].

**Table 2. Cox's Proportional Hazard Model. Statistical Values of Clinical Stage as Prognostic Factor After Multivariate Analysis**

Variable		p	HR	95% CI
Clinical stage				
	II	0.107	2.158	0.847-5.501
	III	0.009	2.643	1.281-5.454
	IV	0.000	4.759	2.022-11.196
p16 <sup>INK4a</sup>		0.413	1.293	0.699-2.389

Note: HR, Hazard ratio; CI confidence interval.

We have observed negative immunoeexpression of p16<sup>INK4a</sup> protein in 71.4% of our cases; this is superior to that observed by other groups with values between 27-54%, but similar to Pankiewicz *et al.* [26] and Guzman *et al.* [27]. Regardless of the antibody used in the tissue evaluation, this discrepancy could be due to the different histological subtypes included in the studies. In fact, we found that negativity was considerably higher in squamous cell carcinomas (71.8%) than in adenocarcinomas (50%), with statistically significant differences (p: 0.033) in both subtypes. This has also been confirmed by other studies [26].

We have not found differences in p16<sup>INK4a</sup>-negative tumors with regards to the clinical stage (I-II: 15/20 vs III-IV: 20/29) in accordance with previous reports [4, 28].

The most relevant finding of our study was the negative statistical correlation of SUV max with p16<sup>INK4a</sup> expression (r: -0.29; p: 0.042) with p16<sup>INK4a</sup>-negative tumors reaching higher statistical significance than those that were p16<sup>INK4a</sup>-positive. It is possible that the higher SUV max in squamous cell carcinomas in comparison with adenocarcinomas reported in the literature might be the consequence of their association with negativity for p16<sup>INK4a</sup>. In tumors with immunonegative p16<sup>INK4a</sup> SUV max did not differ in clinical stages I-II vs III-IV, although in those with p16<sup>INK4a</sup>-positive, SUV max was higher in clinical stages III-IV vs I-II, and very close to being statistically significant.

The univariate analysis proved that p16<sup>INK4a</sup> expression is a prognostic factor, although this was not found with SUV max. Regarding p16<sup>INK4a</sup> there is considerable discrepancy in the literature; it seems that its value is confirmed only in early clinical stages and, in general, along with other biological parameters. SUV max has been accepted as a prognostic factor [16, 18]; however not all studies have proved

this [29]. After multivariate analysis we found that the prognosis value of p16<sup>INK4a</sup> had disappeared, with the clinical stage remaining as the only indicator of later tumor behaviour.

## CONCLUSION

We can conclude that inactivation of the p16<sup>INK4a</sup> gene is very frequent in NSCLC, with higher values in squamous cell carcinomas than in adenocarcinomas. SUV max correlated positively and statistically with negative immunoeexpression of p16<sup>INK4a</sup>, which could explain why higher estimates of SUV max are found in squamous cell carcinomas when compared to adenocarcinomas. Only the clinical stage was found to be an independent prognostic value after multivariate analysis.

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## ABBREVIATIONS

- F-18 FDG-PET = F-18 fluorodeoxyglucose-positron emission tomography  
 FHIT = Fragile histidine triad  
 HIF-1 = Hypoxia-inducible factor 1  
 MIB-1 = Mindbomb homolog-1  
 NSCLC = Non-small cell lung cancer  
 PET = Positron emission tomography  
 SUV max = Maximum standardized uptake value  
 TMA = Tissue microarray

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