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Research Article

## In Non-Small Cell Lung Carcinomas, 18f-Fdg Suvmax Values are Independent of P63 Immunohistochemical Expression

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### Abstract

**Objective**: to study the possible relation between immunohistochemical expression of p63 and the maximum standardised uptake value (max SUV) of 18F-FDG PET in patients with non-small cell lung cancer (NSCLC)

**Material and Methods**: The study group included 49 patients (42 males), ranged between 41 to 82 years old with pre-treatment NSCLC (26 squamous, 15 adenocarcinomas and 8 large cell carcinomas) of whom the diagnosis was found in our centre. According to the clinical stage the classification was the following: 2 IA, 9 IB, 1 IIA, 5 IIB, 9 IIIA, 10 IIIB and 13 IV. Immunohistochemical expression of p63 was studied through the technique of tissue-matrix using Tissue Arrayer Device (Beecher Instruments, Sun Prairie, WI), using the Mab Clone 4A4 from Dako (Denmark).

**Results**: Positive p63 immunohistochemical expression was more frequent (p:0,0007) in squamous cell carcinomas (21/26; 80,7%) than in adenocarcinomas (2/15; 13,3 %), but not than in large cell carcinomas (4/8; 50%). There were not statistically significant differences on SuV max values in the patients classified according to p63 expression. The same findings were noted using different maxSuV qualitative cut-offs. When we considered exclusively the squamous cell carcinomas, we didn't observe statistically significant differences between the maxSuV values (qualitative and quantitative) of patients classified according to p63 expression also.

**Conclusions:** These results led us to consider that SuV max values are not correlated with immunohistochemical expression of p63 neither in patients with NSCLC considered as a whole nor in squamous cell carcinoma subtype, strong related to p63.

**Key words:** p63; Non-Small Cell Carcinomas; maxSuV-18F-FDG-PET

## Introduction

P63 is a member of p53 family [1] , mapping to 3q27 and it is deregulated in a broader range of tumors [2]. It is expressed in benign bronchial stem cells, in neoplastic cells with either squamous differentiation or squamous differentiating potential, as well as in a subpopulation of adenocarcinomas [3]. It is up-regulated in the early phase of epithelial abnormality in idiopathic pulmonary fibrosis [4] and also is involved in the control of maspin expression in non-small cell lung carcinomas (NSCLC), a member of the serpin family of protease inhibitors that inhibits tumor growth and suppresses metastasis in some malignancies, including lung cancer [5]. Massion et al. [6], analyzing p63 gene copy number, observed amplification in 88% of squamous carcinomas, in 42% of large cell carcinomas and in 11% of adenocarcinomas of the lung. Likewise, the predominant splice variant of p63 expressed was DeltaNp63alpha.

In clinical practice, it is a useful marker, with p40 and CK5/6, of NSCLC with squamous differentiation and squamous cell carcinoma [7-11]. Also, p63 is useful for distinction between EGFR/KRAS mutation positive and negative patients [12], and some studies have demonstrated that hypermethylation of RASSF1A (Ras-association domain family 1, isoform A) and negative expression of p63 was associated with poor recurrence free survival in stage I-II NSCLC [13]. Renouf et al. [14], after univariate analysis, described that bcl-2 and p63 were prognostic factors for improved disease-specific survival, but only bcl-2 was prognostic factor after multivariate analysis for improved overall survival and disease specific survival in patients with NSCLC. In this regard, Massion et al. [6] that 63 genomic amplification and protein staining intensity was associated with better survival. Similar findings have been described in other tumors [15-16]. P63 expression was of prognostic significance in neuroendocrine tumors and was associated with higher grade, but not in NSCLC [2].

Positron emission tomography (PET) is a non-invasive imaging modality that offers the possibility to visualize in vivo different metabolic cellular processes and tumor biology, and it is widely used in oncological patients, including patients with NSCLC [17-18]. It has been shown to be of a great value in staging/re-staging, early response assessment to treatment and planning radiotherapy treatment. The 18F-Fluorodeoxyglucose (18F-FDG) is the most commonly radiopharmaceutical used in PET studies, and it reflects the glucose metabolism. The standard uptake value (SUV) is a useful parameter in clinical practice. It is known that SUV maximum (SUVmax) is higher in squamous vs adenocarcinoma tumors [19-21] is associated with tumor size, node and metastasis stage, and SUV of most of metastatic lesions are greater (from half to double) than those of primaries [22]. In relation with survival, the results described in the literature are not conclusive. Yoo et al. [23] noted that higher SuVmax was associated with shorter disease free survival (DFS), whereas Lin et al. [24] did not observe the relation between both parameters. There is not a clear relationship between maxSuV and total volume of cancer, especially due to the tumor heterogeneity and the fact that 18F-FDG reflects the glucose uptake by the tumor cells. For this reason, other variations of this parameter as average SuV, metabolic tumor volume and total lesion glycolysis have been introduced recently in daily practice in order to obtain information with great clinical usefulness [25-26]. Hence SuVmax is an independent prognostic factor for some authors [27].

The aim of this study was to study the possible correlation between immunohistochemical (IHC) expression of 63 and maxSuV 18F-FDG-PET in patients with non-small lung cell cancer considered as a whole, as well as classified according to the most relevant histological subtypes by WHO classification [28].

## Material and Methods

### Patients

The study group included 49 patients (42 males and 7 females), ranged between 41 to 82 years old (median 56) with pre-treatment NSCLC (26 squamous, 15 adenocarcinomas and 8 large cell carcinomas) of whom the diagnosis was found in our centre. According to the post-surgery clinical stage, the classification of them was the following: 2 IA, 9 IB, 1 IIA, 5 IIB, 9 IIIA, 10 IIIB and 13 IV.

### Samples

Lung carcinoma tissue samples were obtained at the time of surgery. Tissue slices had been fixed in 10% formalin and embedded in paraffin wax for histological and immunohistochemical studies. We used a Tissue Arrayer device (Beecher Instruments, Sun Prairie, WI) to construct two different TMA blocks, according to conventional protocols [29]. All cases were histologically reviewed 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 49 carcinomas. All cases were from the files of the Department of Pathology, Clinical University Hospital, Santiago de Compostela, Spain. Internal (basal cells of the bronchial mucosa) and external (p63-positive squamous cell carcinoma) controls were included in each TMA. TMA blocks were sectioned to produce 4-µm sections.

### IHC procedures

They were performed on tissue sections 4 µm thick using a p63 monoclonal antibody (Clone 4A4, ready-to-use, Dako, Denmark). The reaction was detected using a universal second antibody kit that utilized a peroxidase-conjugated dextran polymer (Dako EnVision Peroxidase/DAB; Dako, Glostrup, Denmark) in order to avoid misinterpreting endogenous biotin or biotin-like activity as positive staining. Scoring of the IHC results was performed according to the methods described by Ivan et al. [30], with minor modifications. At least 500 tumor cells were evaluated in the areas of highest positivity for p63 in each of the tumor samples. Only nuclear positivity for p63 was considered positive. In each case, a tumor has been considered positive diffusely (++) when >30%-100% p63-positive tumor cells

were found (Figure 1A), and heterogeneously positive (+) if there was between 10% -30% of p63-positive tumor cells (Figure 1B). Any tumor was considered negative for p63 staining if equivocal (weak) stain and/or less than 10% of p63-positive tumor cell were detected.

### PET Procedures

Patients were fasted at least six hours prior administration. Glucose levels were measured (maximum level accepted 160-180 mg/dl) and a muscle relaxant was administrated 15-30 minutes before the intravenously injection of 350-518 MBq of 18F-FDG. The image was acquired 60 minutes after the 18F-FDG administration in a PET Advanced System (General Electric Medical Systems). A semiquantitative analysis was performed by the determination of maximum SUV indexes for each observed lesion, considering the SUV (according with the following formula: *SUV= tissue radioactivity concentration (Bq/mL)/ [injected dose (Bq)/patient weight (g))* as the tracer uptake in the region of interest (ROI) in relation to the injected dose and the body weight. Sequential studies were not performed and it was only considered the uptake observed in the investigation.

## Statistical Analysis

Data obtained were evaluated using the SPSS 15.0 software for Windows (SPSS, Chicago, IL. USA). With the maxSUV that did not follow a normal distribution, values were presented as range, and median. We used the Chi square test with Yates correction, if necessary, for qualitative variables comparison and the Mann Whitney test for continuous ones. A p-value ≤ 0.05 was considered as statistically significant.

## Results

Positive p63 immunohistochemical expression was more frequent (p:0,0007) in squamous cell carcinomas (21/26; 80,7%) (Figure 1) than in adenocarcinomas (2/15; 13,3 %), but not than in large cell carcinomas (4/8; 50%).

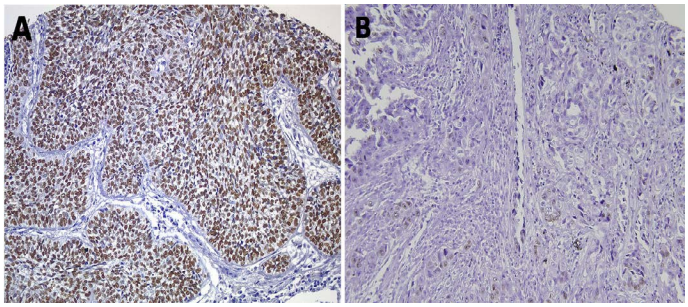


**Figure 1.** A. Squamous cell carcinoma showing strong and diffuse nuclear posititivity for p63 Original magnification, 200X). B. Squamous cell carcinoma with heterogeneously positive for p63 (200X).

Each PET study was presented using axial, coronal, sagital tomographic images and trough the volumetric projection (see Figure 2). Only one reader was required. In this regard, maxSuV values obtained in the patients with NSCLC, considered as a whole and classified according to p63 immunohistochemical expression, are depicted in Table 1. There were not statistically significant differences between the three subgroups of patients. The same findings were noted using different maxSuV cut-offs (See Table 2).

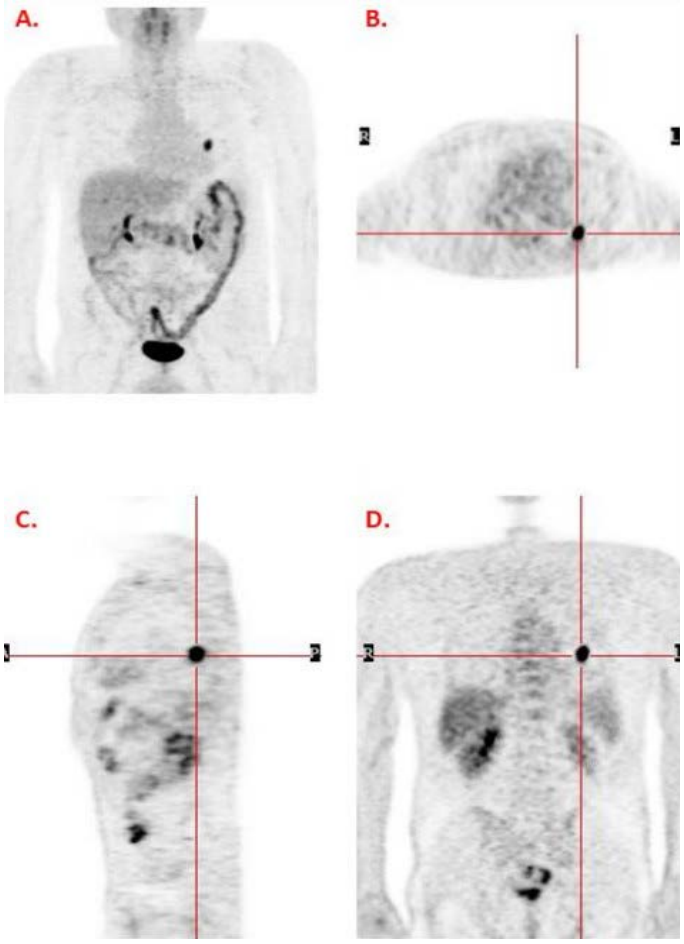


**Figure 2.** 18F-FDG PET in a patient with NSCLC (squamous cell lung carcinoma).

**Table 1.** maxSuV values observed in NSCLC considered as a whole and classified according to p63 immunohistochemical expression.

| P63 EXPRESSION | Nº | RANGE | MEDIAN | 25pt | 75pt |
|---|---|---|---|---|---|
| NEGATIVE | 22 | 3-47 | 15 | 10 | 20 |
| + POSITIVE | 16 | 9.1-24,9 | 15.5 | 12.4 | 17.5 |
| ++ POSITIVE | 11 | 4-24 | 12.4 | 10.0 | 16 |

Not statistically significant differences between the groups.

25pt: percentile 25

75pt: percentile 75

When we considered exclusively the squamous cell carcinomas, the most frequent p63 positive tumor subtype, we didn't observe statistically significant differences between the maxSuV values of patients classified according to p63 expression (See Table 3). The same findings were also ob-

served using different quantitative cut-offs [15, 20 , 25] (Data not shown). Clinical stage (I-II: r: 11,2-32,1; 17.1+/-6,4; median 15,2 vs III-IV: r: 4-24,9; 14,1+/-5,2; median 13,9; p: ns) and histological grade (HG2: r: 4-24,9; 15,0+/-5,0; median 14,05 vs HG3: range 9,1-.32,1; 16,3+/-8,1; median 13,9; p: ns) didn´t also influence on maxSuV values in + positive and ++ positive squamous cell carcinomas.

| p63 IHC EXPRESSION | | | | |
|---|---|---|---|---|
| CUT-OFF | NEGATIVE | + POSITIVE | ++ POSITIVE | POSITIVE |
| >15 | 10/22 | 8/16 | 5/11 | 13/27 |
| >18 | 5/22 | 3/16 | 2/11 | 5/27 |
| >20 | 4/22 | 3/16 | 2/11 | 5/27 |
| >24 | 2/22 | 1/16 | 0/11 | 1/27 |

Not statistically significant differences between the groups.

**Table 2.** maxSuV values observed in NSCLC considered as a whole and classified according to p63 immunohistochemical expression (IHC) as well as different qualitative cut-offs.

| P63 EXPRESSION | Nº | RANGE | MEDIAN | 25pt | 75pt |
|---|---|---|---|---|---|
| NEGATIVE | 5 | 7,9-32,1 | 16.6 | 15 | 22,5 |
| + POSITIVE | 12 | 9,1-24,9 | 12,8 | 11,9 | 16,9 |
| ++ POSITIVE | 9 | 4-24 | 15,2 | 12,4 | 16,0 |

Not statistically significant differences between the groups.

25pt: percentile 25

75pt: percentile 75

**Table 3.** maxSuV values observed in squamous cell carcinomas classified according to p63 immunohistochemical expression.

## Discussion

Several studies aiming to find the prognostic and predictive value have shown numerous biological factors in patients with non-small lung cell cancer, heterogeneous group of malignant diseases with a poor prognosis. One of them is p63, gene located in chromosome 3q27-29, containing 15 exons and at least 6 protein isoforms. Six different isotypes have been described, suggesting that the proteins coded from them have biological functions differing from one to another [31]. P63 has been implicated in promotion of squamous differentiation in various tissues. In clinical practice, it is a useful marker, alone or with CK5/6, of NSCLC with squamous differentiation and squamous cell carcinoma [32]; likewise, several antibodies have been generated against p63 [33] and its genomic amplification and protein staining intensity was associated with better survival [6]. Also p63 is a useful marker in distinguishing poorly differentiated squamous cell carcinoma from poorly differentiated adenocarcinoma and large cell neuroendocrine carcinoma, but it is not useful in distinguishing the metastasis from primary lung carcinoma from other squamous cell tumors [31].

We observed positive p63 immunohistochemical expression in 80,7% of squamous cell carcinomas, higher than those noted in in adenocarcinomas, but not than in large cell carcinomas. Our results are similar to those described by other authors so much in squamous carcinomas [5-7], as in adenocarcinomas and large cell [2, 11,31] . The most important feature is the higher positive p63 expression in squamous carcinoma type, that it was neither associated with neither prognosis nor survival [14; 31]. In adenocarcinomas, different percentages of positives results with immunohistochemical techniques are described, ranged between 10-30%, and it is interesting to stand out that there is slight delta-N p63 positivity and TA p63 negativity [31]. Another group [34] observed that cytoplasmatic expression of p63 is an adverse prognostic factor in patients with adenocarcinoma of the lung. We have not observed any association between p63 expression and differentiation grade, whereas Bir et al. [31] have described that in well-differentiated tumors, staining is strong in peripheries of the tumor mass and is weakened in the center.

Up to our knowledge, there is not a paper concerning the possible relation between p6 maxSuV values obtained in the patients with NSCLC. We observed that SuVmax values (quantitative and qualitative) were similar in the subgroups of patients with NSCLC classified in relation to negativity and positivity (+ and ++) of p63 immunohistochemical expression. The same results were obtained when only squamous cell carcimomas were considered. In positive p63 expression tumors, SuVmax values were neither related with clinical stage not differentiation grade. The lack of association between 18F-FDGmax values and other biological parameters as platelet derived endothelial growth factor has been reported in the literature [35].

These results, preliminary due to reduced number of tumors included in our study, led us to consider that max SuV values are not associated with immunohistochemical expression of p63, neither in patients with NSCLC considered as a whole nor in squamous cell carcinoma subtype, strong associated with p63.

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