



FACULDADE DE MEDICINA

UNIVERSIDADE DE COIMBRA

**Clinical and Metabolic Characterization of Bronco-
Pulmonary Carcinoma in Surgical Stadium.**

José Alberto de Castro e Dias

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Pulmonary Carcinoma in Surgical Stadium.**

**Presented to the University of Coimbra in order to
complete the MSc degree in Medical School, under
the supervision of Professor Lina Carvalho and co-
supervised by Professor Manuela Grazina both from
Medical School of University of Coimbra**

José Alberto de Castro e Dias

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Medical School of University of Coimbra.

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ABBREVIATIONS

ADC – adenocarcinoma

ADSQC – adenosquamous carcinoma

BA – bronchioloalveolar

BPC – bronco pulmonary carcinoma

CBPG – complex bronchial-pulmonary group

CK7 – citokeratyn76

CK5.6 – citokeratyn5.6

CRGR – chromogranin A

FDG – 18F-Fluorodeoxyglucose

IASLC – International Association for the Study of Lung Cancer

IASLC/ATS/ERS – International Association for the Study of Lung Cancer /American Thoracic Society/and the European Respiratory Society

IHC – Immunohistochemistry

LCC – large cell carcinoma

LLL – left lower lobe

LUL – left upper lobe

mtDNA – mitochondrial DNA

Muc – mucinous

NSCLC – non small lung cell carcinoma

NE – neuroendocrine

PET – Positron Emission Tomography

PLEOC – pleomorphyc carcinoma

SCLC – small lung cell carcinoma

SQC – squamous cell carcinoma

SUV – standardized uptake value

TRU – terminal respiratory unit

TTF-1 – thyroid transcription factor-1

VIM – vimentin

WHO – World Health Organization

ABSTRACT

Bronchial-Pulmonary carcinomas (BPC) present in advanced stages in nearly 70% of cases. Clinical and pathological criteria have to be strict to fulfil personalized therapy and direct molecular pathology. The 18F-Fluorodeoxyglucose (FDG) uptake integrates clinical diagnosis and we hypothesized that FDG uptake together with CK7, CK5.6/p63, TTF1, CD56, Vimentin expression and mitochondrial DNA (mtDNA) content define criteria to stratify lung cancer in small biopsies.

A series of 40 surgical specimens whose patients had preoperative Positron Emission Tomography/Computed Tomography (PET/CT) was introduced in this study by recurring to archive tissue to submit to Immunohistochemistry (IHC) and mtDNA validation.

WHO 2004 and IASLC/ATS/ERS 2011 proposal classifications were applied to correlate with the previous parameters.

TTF1 Positive adenocarcinomas had lower 18F-FDG uptake ($p=0.006$) compared with TTF1 negative adenocarcinomas and these equal epidermoid carcinomas ($p=0.396$). Here called complex group of the other histological types have 18F-FDG uptake intermingled with the other types needed reliable IHC to sub classification; mtDNA correlated with 18F-FDG uptake ($p=0.0059$).

As an approach to sub classify BPC in small biopsies, 18F-FDG uptake together with IHC defined panel will allow stratification for personalized therapy and clinical follow up: epidermoid carcinoma, bronchial adenocarcinoma, TRU adenocarcinoma, adenosquamous -

RESUMO

Os carcinomas bronco-pulmonares (BPC) apresentam-se em estádios avançados em cerca de 70% dos casos. De forma a cumprir uma terapêutica personalizada são necessários critérios clínicos e patológicos e rigorosos. A captação de 18F-Fluordesoxiglucose (FDG) integra o diagnóstico clínico. Colocamos a hipótese que a captação de 18F-FDG aliada à expressão de CK7, CK5.6/p63, TTF1, CD56, Vimentina e ao conteúdo de DNA mitocondrial (mtDNA) definem critérios para a estratificação do carcinoma pulmonar em pequenas biopsias.

Foram incluídas neste estudo 40 peças cirúrgicas de doentes que previamente haviam feito uma Tomografia por Emissão de Positrões/Tomografia Computorizada (PET), com recurso a um banco de tumores para serem submetidos a imunohistoquímica (IHC) e à avaliação do número de cópias de mtDNA. Os parâmetros acima mencionados foram correlacionados com tipo histológico segundo as classificações WHO 2004 e IASLC/ATS/ERS 2011.

Os adenocarcinomas TTF1 positivos apresentam um menor captação de 18F-FDG ($p=0.006$) quando comparados com os adenocarcinomas TTF1 negativos, que por sua vez não se diferenciaram dos carcinomas epidermóides ($p=0.396$). Os restantes padrões histológicos dos tumores aqui chamados Grupo Complexo apresentaram uma captação 18F-FDG intermédia comparados os outros tipos tumorais tendo sido necessária IHC para a sua subclassificação; o mtDNA correlaciona-se com a captação de 18F-FDG ($p=0.0059$).

Como forma de subclassificar os BPC em pequenas biópsias, a captação 18F-FDG juntamente com o painel de IHC definido permitem uma estratificação para uma terapêutica personalizada e um seguimento clínico: carcinoma epidermóide, adenocarcinoma brônquico, adenocarcinoma da

unidade respiratória terminal (TRU), carcinoma adenoescamoso, carcinoma pleomórfico, carcinoma de grandes células.

KEY WORDS: 18F-Fluorodeoxyglucose, small biopsies, bronchial-pulmonary carcinomas, mitochondrial DNA, immunohistochemistry.

INTRODUCTION

Pulmonary carcinoma is the most frequent cause of death worldwide (Boyle, 2008) Small cell lung cancer (SCLC) is a highly malignant tumor that accounts for 15% of lung cancer cases and Non-small cell lung cancer (NSCLC), accounts for the remaining 85% of cases, is divided into 3 major pathologic subtypes: adenocarcinoma(ADC), squamous cell carcinoma (SQC), and a group comprising adenosquamous carcinoma (ADSQC), large cell carcinoma(LCC) and pleomorphic carcinomas(PLEOC) (OMS2004) . ADC by itself accounts for 38.5% of all lung cancer cases, SQC 20% and the remaining types here called bronchial-pulmonary group (CBPG) for 2.9%. (Howlader et al. 2010 ; Herbst R.S et al. 2008) ADC presents clinical, radiological, pathological and molecular criteria that allow subtyping (Travis et al. 2004; Travis et al.1999). and the World Health Organisation (WHO) 2004 classification for surgical specimens and the International Association for the Study of Lung Cancer /American Thoracic Society/and the European Respiratory Society (IASLC/ATS/ERS 2011) suggested for biopsies (Travis et al. 2011) are similar, the latter needs simplification, to be complementary in order to serve personalized therapy.

In current WHO 2004 classification adenocarcinomas can be further categorized as acinar, papillary, bronchioloalveolar solid with mucin production, and mixed type, according to its histology. Mixed type is the commonest type and present patterns have to be discriminated decrescently.

Immunohistochemistry (IHC) is a simple, relatively unexpensive and reliable method (Jagirdar 2008; Beasley 2008) able to distinguish cell lineages in lung cancer even when facing limited

material from biopsies (Loo PS, et al. 2010; Nicolson AG, et al. 2010) and poorly differentiated carcinomas (Pardo J et al 2009; Rossi G et al. 2004)

Another hall mark of cancer is the altered metabolism (Deberardinis et al. 2008; Hsu et al.2008). The first to be discovered was the increase in glucose consumption (Warburg O. 1956.) and the development of F-18-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) was based in this basic principle, nowadays widely used in diagnosis and staging of lung cancer (Pauwels et al. 2000)

This higher glucose intake is related to the increased metabolic rate observed in tumors (Fuss, 2010), which have elevated energy demands for responding to the deregulated cell proliferation. It has also been documented that cancer cells have increased ROS production (Ralph et al., 2010), namely by the mitochondrial OXPHOS, that could affect mitochondrial genome (mtDNA).

As mitochondrial DNA (mtDNA) is highly susceptible to damage because its lack in protective histones, may have an important role in carcinogenesis. Decreased mtDNA content had been reported for renal (Selvanayagam et al.1996) gastric (Wu et al. 2005), breast (Mambo et al. 2005; Yu et al. 2007), previously- treated head and neck (Jiang et al. 2006), ovarian (Wang et al. 2006) and hepatic cancer (Lee et al. 2004; Yin et al. 2004; Morten et al. 2007), and increased mtDNA content was observed in prostate (Mizumachi et al. 2008), untreated head and neck (Jiang et al.2005) thyroid (Mambo et al. 2005), endometrial (Wang et al. 2005), and pancreatic cancer (Jones et al.2001). Our working group has recently observed that 67.6% of neoplastic tissue samples showed increased mitochondrial DNA copy number in lung cancer, by studying 37 paired cases of tumor/corresponding lung parenchyma (Bonifácio et al., 2012)

The aim of the present work is to investigate the correlation between FDG-PET, immunohistochemical expression and mtDNA copy number in bronchial-pulmonary carcinomas from surgical specimens of 40 patients.

Material and Methods

Patients, Histological Classification and Staging

The present study included patients related with 2010 and 2011 archive of surgical staged cases of the Service of Pathology of the University Hospital of Coimbra, comprising 30 males and 10 females, with ages ranging between 42 and 87 years old.

Bronchial-pulmonary carcinomas were classified according to WHO 2004 classification for lung cancer, and according with IASLC/ATS/ERS 2011, without any discrepancy.

Staging followed TNM classification (International Association for the Study of Lung Cancer, IASLC). Neoadjuvant chemotherapy was not prescribed to any patient.

Immunohistochemical Staining

Expression of citokeratin7 (CK7), thyroid transcription factor-1 (TTF1), vimentin (VIM), Citokeratin5.6 CK5.6, p63, Ki67, CD 56 , Chromogranin A was registered after Bond Polymer Refine Detection™ (DS9800; Leica Biosystems, Newcastle Ltd, United Kingdom) according to manufacturer's instructions on BOND-MAX™ (Leica Biosystems, Newcastle Ltd, United Kingdom). Primary antibodies against p63 (clone 7JUL; Novocastra Laboratories Ltd, Newcastle, United Kingdom) at a dilution of 1:50 for 60 minutes, CK5.6 (clone D5&16B4; Cell Marque, California, USA) at a dilution of 1:50 for 40 minutes, CD 56 (clone CD564;

Novocastra Laboratories Ltd, Newcastle, United Kingdom) at a dilution of 1:240 for 30 minutes, CK7 (clone OV-TL12/30; DakoCytomation, Glostrup, Denmark) at a dilution of 1:800 for 30 minutes, Chromogranin A (clone DAK-A3; DakoCytomation, Glostrup, Denmark) at a dilution of 1/3000 for 30 minutes, Ki67 (clone MIB-1; DakoCytomation, Glostrup, Denmark) at a dilution 1:150 for 30 minutes, TTF-1 (clone SPT24; Novocastra Laboratories Ltd, Newcastle, United Kingdom) at a dilution of 1:250 for 30 minutes and Vimentin (clone VIM 3B4; DakoCytomation, Glostrup, Denmark) at a dilution of 1:250 for 30 min, were applied to the sections and incubated at room temperature.

In parallel, known positive and negative controls were used.

Antibodies were graded quantitatively in a four point scale concerning the percentage of cytoplasmic nuclei positivity: (0; 1 [$<10\%$]; 2 [$10\%-50\%$], 3 [$>50\%$]).

F-FDG PET/CT

Before PET/CT scan all patients fasted for at least 6 hours and their blood glucose levels were required to be less than 130mg/dl.

Fifty minutes after intravenous injection of 370 MBq of ^{18}F -FDG, the PET/CT imaging was obtained from head to the upper portion of thigh on the following integrated PET/CT scanner (General Eletronics Discovery ST4)

A low dose CT scan was performed for attenuation correction with 4 slice multidetector CT of the hybrid scanners General Eletronics Discovery ST4 ®, followed by 3D-mode acquisition. The reconstruction algorithm (iteration 2 and subset 30) created a raw data of PET imaging into a 128 x 128 matrix.

General Eletronics Advanced Workstation was used to determine max Standardized Uptake Value (SUVmax), using 1 cm diameter region of interest (ROI) within the lesion on the slice with the highest uptake.

mtDNA content

Either neoplastic tissue and respective redundant preserved pulmonary parenchyma were submitted to DNA extraction using the *Maxwell[®] 16 FFPE Tissue LEV DNA Purification Kit*, with the *Maxwell[®] 16 Instrument Kit* (Promega, Madison, USA) according to manufacturer's instructions.

Concentration and purity of extracted DNA were determined by optical density spectrophotometer, with the instrument *NanoDrop[®] ND-1000* (ThermoScientific, Wilmington, USA)

mtDNA copy number has been determined by real-time PCR assay (all these methods are extensively described in Bonifácio et al (2012)).

Statistical Analysis

Because the measurement of SUVmax is semiquantitative and the distribution was not normal, non-parametric test were used to analyse the significance of difference between groups. Mann Whitney test was used to access the correlation between variables and groups. Linear regression was used to compare SUVmax and mtDNA copy number.

Statistical analysis was carried out by using IBM[®] SPSS[®] Statistics 20.0 software

RESULTS:

Patients and Bronchial-Pulmonary Carcinomas

In this group of 30 males and 10 females, 21 were smokers and 19 non-smokers. The mean age was 65.53 ± 10.19 years (ranging from 42 to 87 years). Tumor mean size measured in surgical specimens was $3.65\text{cm} \pm 2.11\text{cm}$, ranging between 1.2 to 10.5cm and comprised 18 ADC, 12 SQC, 3 neuroendocrine carcinomas (NE), 4 pleomorphic carcinomas (PLEOC), 2 ADSQC, 1 SCLC, 1 large cell lung carcinoma.

All 18 adenocarcinomas expressed CK7 and 14 were TTF1 positive and 3 were negative, while 1 had low nuclear expression with this immunomarker and were included in our study as a negative TTF1 case. Squamous cell carcinoma had consistently negative expression of CK7 and TTF1 and positivity of CK 5.6/P63, with heterogeneous expression of vimentin in the complex group no specific immunohistochemical pattern was defined (Fig. I).

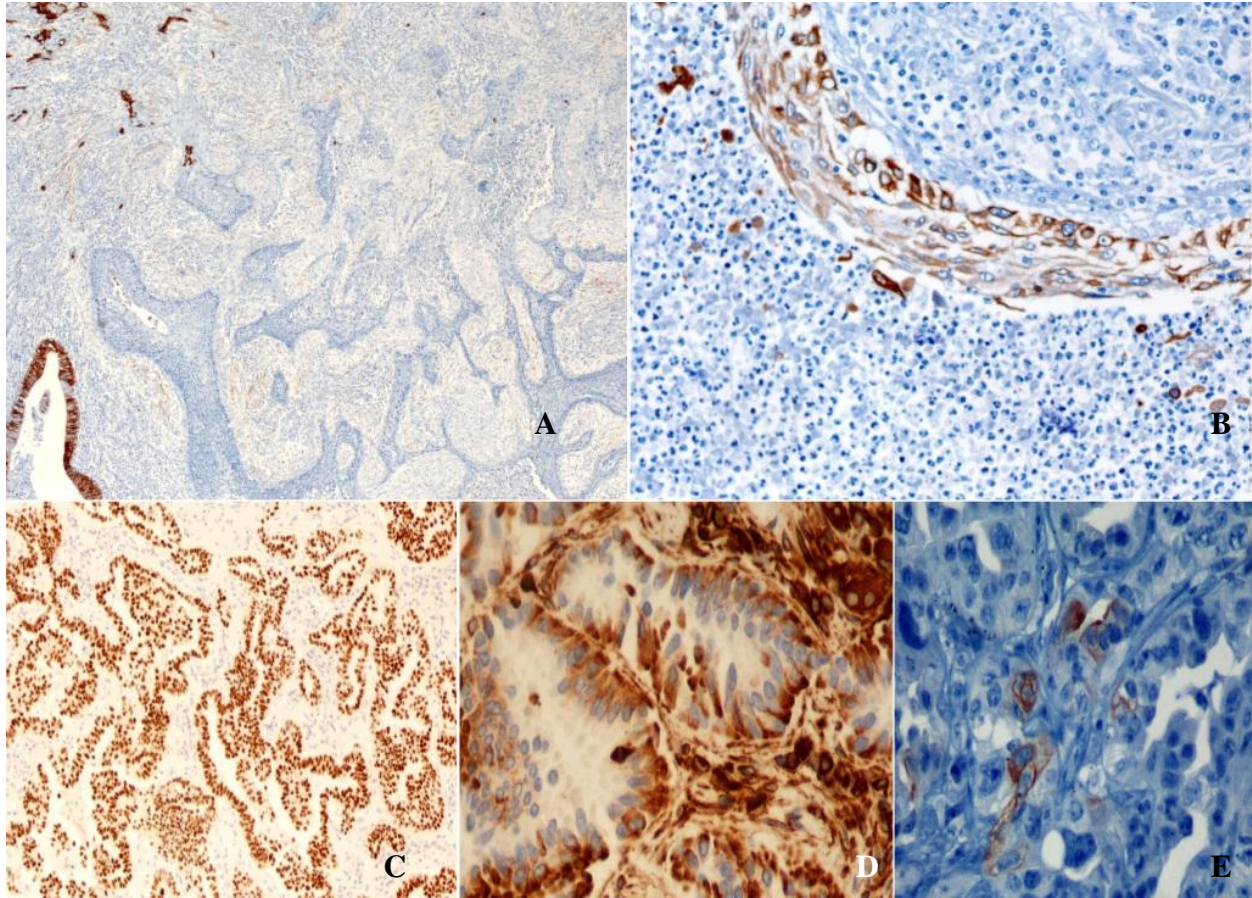


Figure I. **A-** Tumor n° 5, SQC not expressing CK7 or VIM, 40X. **B-** Tumor n°8, SQC expressing CK7, 200X. **C-** Tumor n° 20, ADC expressing TTF1, 100X. **D-** Tumor n°21, ADC expressing VIM 400X. **E-** Tumor n° 34 NE carcinoma combined with ADC expressing CRGR, 400X.

F-FDG PET/CT was performed before surgery by a median of 48.3 days ranging 27 to 68 days, with median SUVmax of 16.2 ranging between 2.1 – 97.9. Tables I, II, III and IV register histological classification, subtyping, staging, IHC, as well as SUVmax and patients cohort.

Table I – Epidermoid Carcinomas Group

	Histology	Age	Gender	Tobaco	pT	Size	pN	CK7	TTF1	CK5.6/p63	CD56	CRGR	Vim	Ki67	SUVmax
1	SCQ- endobronchial	61	2	0	2a	5	0	0	0	2	0	0	0	2	16.4
2	SQC	63	1	0	2a	4	0	0	0	3	0	0	0	2	17.2
3	SQC- papillary endobronchial	62	1	1	2a	4.5	0	0	0	3	0	0	0	3	41.8
4	SQC- papillary and basaloide	58	1	0	2a	4.5	1	0	0	3	0	0	0	2	7.4
5	SQC	46	1	1	1b	3	1	0	0	3	0	0	0	2	9.36
6	SQC	60	1	1	2a	1.8	0	0	0	3	0	0	0	2	11.49
7	SQC papillary	59	1	1	1a	2.5	0	0	0	3	0	0	0	2	23.1
8	SQC	63	1	1		6.8	0	0	0	3	0	0	0	2	31.2
9	SQC- with necrosis	79	1	0	3	7	0	0	0	3	0	0	1	2	17
10	SQC- clear cells	87	1	0	3	5.3		2	0	3	0	0	1	3	31.8
11	SQC	84	1	0	2a	1.2	0	1	0	3	0	0	1	2	3.8
12	SQC-clear cells	61	1	1	2a	4.3	0	1	0	2	0	0	2	2	17.6

SQC – Squamous cell carcinoma

Table II – Adenocarcinomas Expressing TTF1

	Histology	Age	Gender	Tobaco	pT	Size	pN	CK7	TTF1	CK5.6 /p63	CD56	CRGR	Vim	KI67	SUVmax
13	ADC solid and microacinar	72	1	1	2a	1.5	0	3	2	0	0	0	1	1	11.5
14	ADC BA non Muc	76	1	0	2a	1.2	0	3	2	1	0	0	1	1	4.1
15	ADC acinar and solid	74	1	1	2a	1.5	0	3	2	0	0	0	0	1	5.8
16	ADC acinar and solid	44	1	1	2b	5	1	3	2	0	0	0	1	1	11.6
17	ADC micropapillary and solid	64	1	1	1a	2		3	2	0	0	0	0	1	3
18	ADC acinar and solid	59	2	1	2a	5	0	3	2	0	0	0	0	1	12.89
19	ADC BA Muc	71	2	0	1a	2.3	0	3	2	0	0	0	0	1	2.9
20	ADC acinar	60	1	1	3	2.5	2	3	3	0	0	0	0	1	18.4
21	ADC acinar	83	2	0	2b	1,6	0	3	3	0	0	0	2	1	8.6
22	ADC Acinar, papillar and micropapillary	73	1	0	1a	2	1	3	3	0	0	0	1	1	3.6
23	ADC acinar and BA Muc	68	2	0	2a	1.9	0	3	3	0	0	0	0	2	5
24	ADC acinar and BA Muc	62	2	0	2a	1.6	0	3	3	0	0	0	1	1	4.6
25	ADC Acinar and BA	75	1	1	1b	4.2		3	3	0	0	0	0	2	9.36
26a	ADC acinar - LLL	61	2	1	4	3	0	3	3	0	0	0	2	1	7.4

ADC – adenocarcinoma; BA – bronchioloalveolar; Muc – mucinous ; LLL-left lower lobe

Table III – TTF1 Negative Adenocarcinomas

	Histology	Age	Gender	Tobaco	pT	Size	pN	CK7	TTF1	CK5.6 /p63	CD56	CRGR	Vim	KI67	SUVmax
27	ADC acinar non Muc and micropapillary	63	2	0	2a	3.2	0	3	1	0	0	0	1	1	21.8
28	ADC acinar e papillary	51	1	1	3	9	1	3	0	0	0	0	0	1	34.7
29	ADC Acinar e papillary Muc	54	2	1	1b	3	0	3	0	0	0	0	0	2	21
30	ADC solid, BA non Muc and micropapillary	79	2	0	2a	2	0	3	0	0	0	0	2	1	12.37

ADC- adenocarcinoma; Muc – Mucinous; BA- bronchioloalveolar

Table IV – Bronchial-Pulmonary Complex Tumors

	Histology	Age	Gender	Tobaco	pT	Size	pN	CK7	TTF1	CK5.6/ p63	CD56	CRGR	Vim	KI67	SUVmax
26b	PLEOC ADC, solid giant cell pattern, LUL	61	2	1	4	3.2	0	3	1	0	0	0	2	1	3.4
31	PLEOC ADC acinar	63	1	0	1b	1.2	2	3	0	2	0	0	1	1	5
32	PLEOC- ADC and giant cells and fusiform cells	57	1	1	1b	2.5	0	3	1	1	0	0	1	2	7.41
33	PLEOC ADC solid Muc	66	1	0	3	5.5	2	3	0	1	0	0	2	2	97.9
34	NE combined withADC	53	1	0	2a	6	2	3	0	0	1	1	0	2	12
35	NE combined with SQC	68	1	1	2a	4.8	0	3	0	2	2	0	0	3	18
36	NE combined with SQC	68	1	0	2a	3.5	0	3	0	2	2	1	0	3	40.2
37	SCLC combined with ADC	70	1	1	1a	1.5	0	0	0	0	2	2	1	3	15.7
38	ADSQC	70	1	1	2a	4	0	0	1	2	0	0	0	3	8
39	ADSQC	60	1	1	2a	3	1	3	0	2	0	0	1	2	28
40	Giant Cell Carcinoma	42	1	1	4	10.5	0	3	0	0	0	0	0	3	2.1

PLEOC - pleomorphic carcinoma; ADC – adenocarcinoma; SQC – squamous carcinoma; ADSQC – adenosquamous carcinoma; NE – neuroendocrine carcinoma; Muc – mucinous; LUL – left upper lobe

Median mtDNA copy number was 531.48 ranging between 30.00 and 1098, presenting standard deviation of 250.73. The correlation between SUVmax and mtDNA copy number was accessed by Linear regression ($p= 0.0059$) - Table V and Fig. VI.

Table V – SUVmax and mtDNA copy number

	Histology	SUVmax	mtDNA copy number
1	SCQ- endobronchial	16.4	447
2	SQC	17.2	546
3	SQC- papillary endobronchial	41.8	346
4	SQC- papillary and basaloide	7.4	407
5	SQC	9.36	513
6	SQC	11.49	349
7	SQC papillary	23.1	635
8	SQC	31.8	936
16	ADC acinar and solid	11.6	340
17	ADC micropapillary and solid	3.0	286
21	ADC acinar	8.6	354
24	ADC acinar and BA Muc	4.6	569
25	ADC Acinar and BA	9.36	346
31	PLEOC ADC acinar	5.0	730
33	PLEOC ADC solid Muc	97.9	581
34	NE combined with ADC	12.0	30
35	NE combined with SQC	18.0	343
36	NE combined with SQC	40.2	787
31	PLEOC ADC acinar	15.7	448
33	PLEOC ADC solid Muc	8.0	1098
34	NE combined with ADC	28.0	860

SQC – squamous cell carcinoma; ADC – adenocarcinoma; BA- bronchioloalveolar;

Muc-mucinous ; PLEOC – pleomorphic carcinoma; NE - neuroendocrine

Using WHO 2004 lung cancer histological classification and IASLC/ATS/ERS 2011, a significant difference in SUVmax was found between ADC and SQC groups with lower SUVmax in ADC group ($p=0.047$) (Fig II).

We also found that negative TTF1 adenocarcinomas had higher uptake of 18F-FDG than TTF1 positive adenocarcinomas ($p=0.06$) (Fig III).

There were no differences in between TTF1 negative lung adenocarcinomas and squamous cell lung carcinomas (Fig IV) concerning SUVmax ($p=0.396$).

The lowest SUVmax was observed in ADC TTF1+, ADC TTF1- had the highest values significantly different from ADC TTF1 + But not from SQC. There are no significant SUVmax differences between the complex group of bronchial-pulmonary carcinomas and ADC TTF1+ ($p=0.147$) ADC TTF1- ($p=0.240$) SQC ($p=0.424$). (Fig. V).

mtDNA copy number and SUVmax (Fig VI).were compared using linear regression and a statistical significant direct proportionality was found ($p=0.0059$)

Because tumor size has influence on SUV max, we conducted further analysis to determine a possible association between tumor size and histological classification. We found that tumor size did not differ significantly among the 3 groups mentioned in this study.

Figure II. SUVmax in adenocarcinomas and squamous cell carcinomas. Adenocarcinomas has significantly lower SUVmax than the squamous cell carcinoma. Mann-Whitney $p= 0.047$.

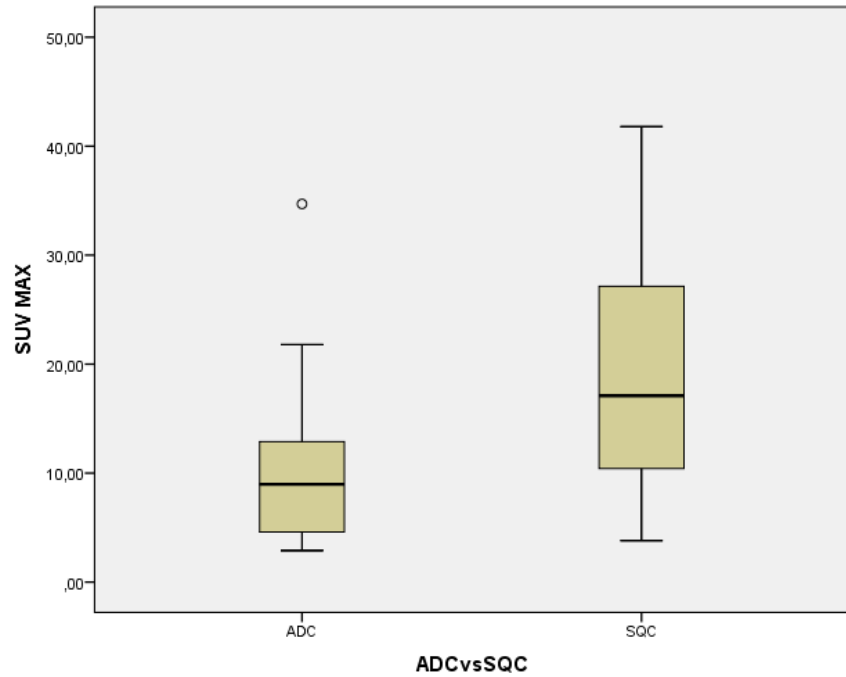


Figure III. SUVmax in adenocarcinomas TTF1- and adenocarcinomas TTF1+. Adenocarcinomas TTF1+ has significantly lower SUVmax than the adenocarcinomas TTF1-. Mann-Whitney $p= 0.006$

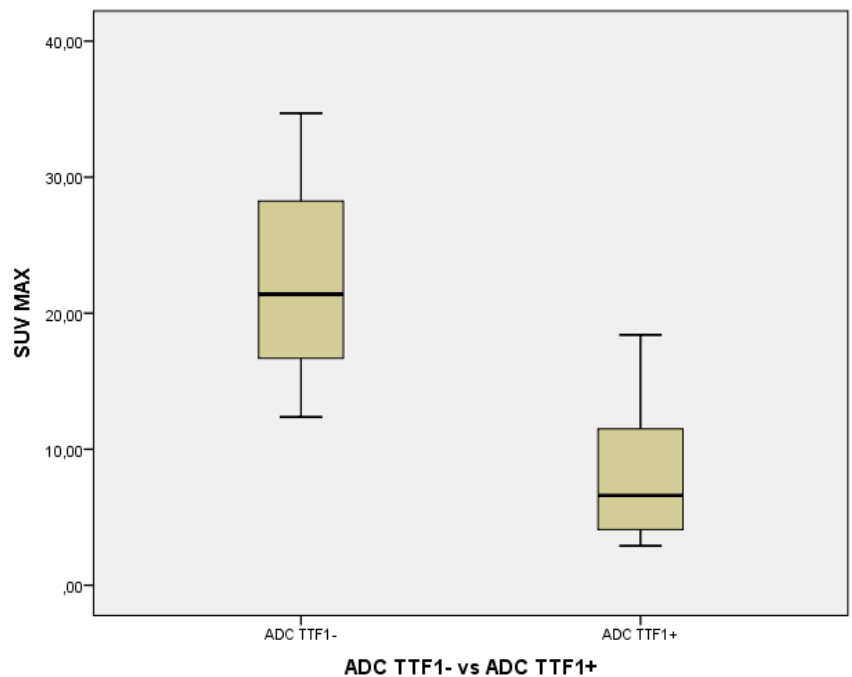


Figure IV. SUVmax in adenocarcinomas TTF1 - and squamous cell carcinomas.

Adenocarcinomas TTF1- has significant difference in SUVmax than the squamous cell carcinoma. Mann-Whitney: $p= 0.396$

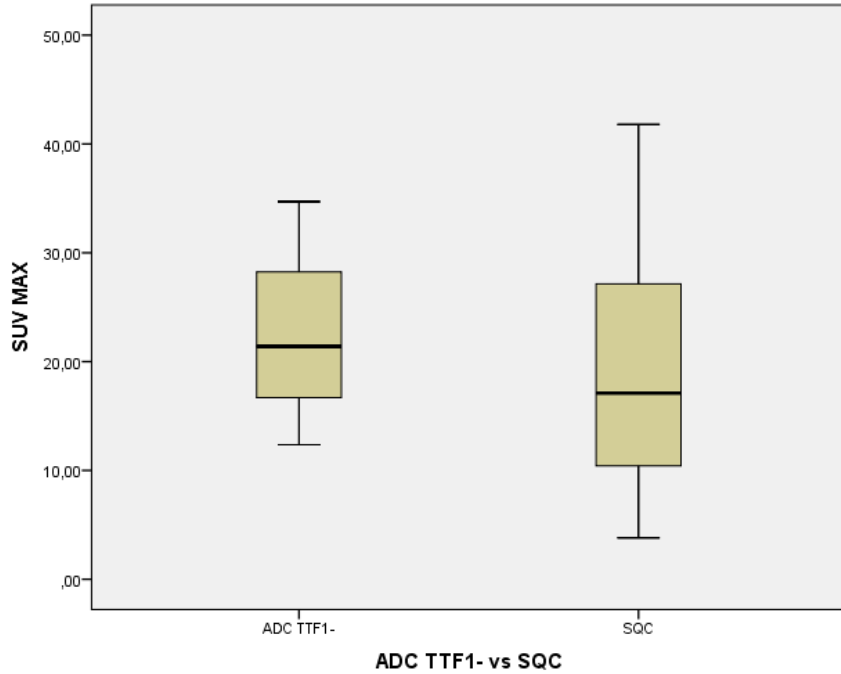


Figure V. SUVmax all four groups, ADC TTF1+ ; ADC TTF1-; SQC; CBPG.

CBPG presented high variance and its values are not significantly different compared to ADC TTF1+ ($p= 0.147$) ADC TTF1- ($p=0.240$) SQC ($p=0.424$)

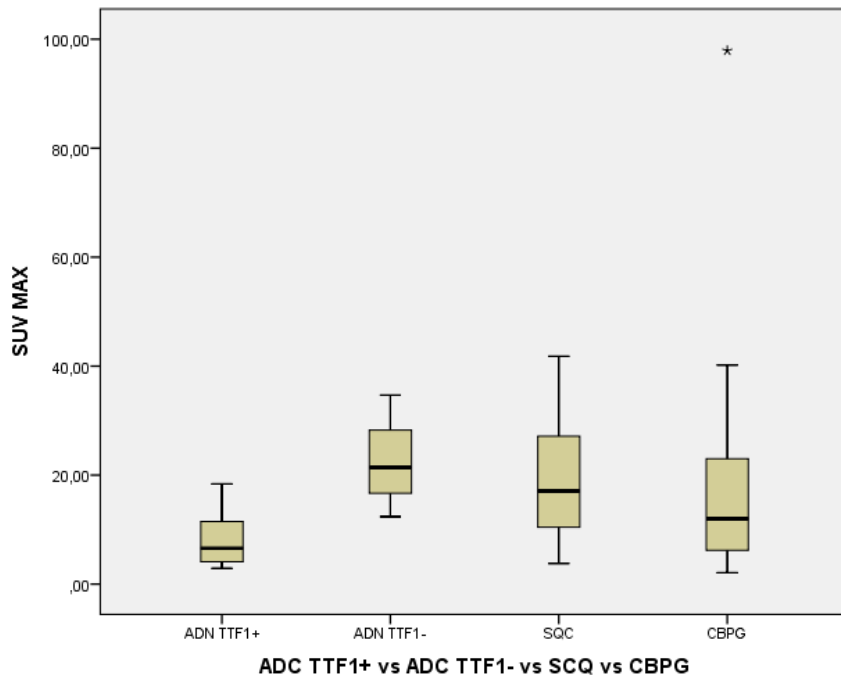
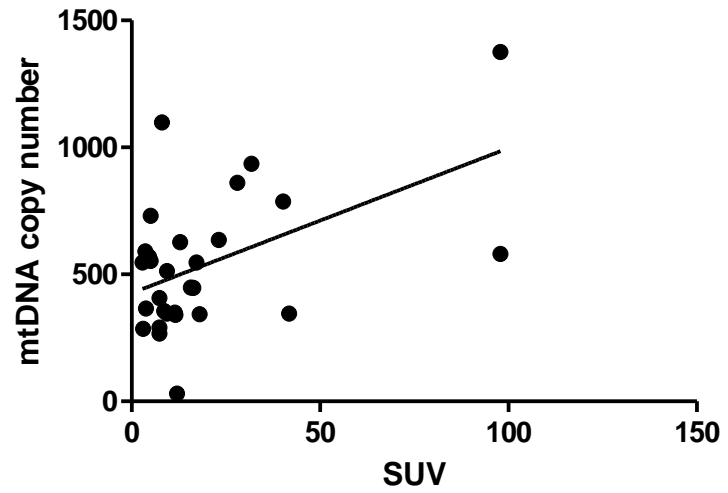


Figure VI. Comparison of SUVmax values and mtDNA copy number.

There is a significant association between SUVmax and mtDNA copy number. Linear regression ($p=0.0059$)



Discussion

Lung Cancer division in two main categories, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), is not useful for patients and personalized therapy. (Howlader et al.2010; Herbst et al. 2008)

Adenocarcinoma and squamous cell carcinoma are the two major types of lung cancer, and sub classification is not yet used clinically. Pathologic evidence indicates that lung adenocarcinoma is a heterogeneous group of diseases with different prognosis. (Motoi et al. 2008; Sica et al. 2010) and from epidermoid carcinomas, a group of basal cell carcinoma is emerging, either in pathology observation and clinically in advanced stages.

In this research the WHO 2004 classification of the lung cancer and the adapted IASLC/ATS/ERS showed no discrepancy for surgical specimens and the applied IHC panel was able to define all the different histological subtypes, making itself of most use in biopsies where tissue sample may lack quality or quantity, but is enough to follow up the patients and understand the here called complex group of bronchial-pulmonary carcinomas (Carvalho 2009; Pelosi et al. 2012; Pelosi et al. 2011)

In this study we support that IHC patterns relate to histological morphology of the lung cancer and correlate with 18F-FDG glucose uptake. Together these two biomarkers can have biological implications in the classification of bronchial-pulmonary carcinomas. Adenocarcinomas expressing TTF1 differ from those that do not express TTF1 by showing a lower SUVmax forming the group of Pulmonary Adenocarcinomas. TTF1 negative adenocarcinomas do not differ from SQC and together they form the bronchial carcinomas group. The particular group of

SQC expressing CK7 need further clinical follow up to understand a bronchial basal cell carcinoma.

Differences on F-FDG uptake (SUVmax) indicate that the hallmarks of deregulated glucose metabolism are dissimilar in the tumor groups(Aquino et al. 2007; Geus-Oei et al. 2007; Suzawa et al. 2011) We achieved that different SUVmax values relate to different histological types of lung cancer allowing a cell lineage classification together with IHC. SUVmax is after all a clinical parameter that takes part of the first diagnostic opinion by the oncologist.

Japanese researchers have proposed histological classification of adenocarcinoma according to morphological features - Terminal Respiratory Unit (TRU) type and Non TRU. TRU type is more likely to contain TTF1 expression (Yatabe et al.2002; Yatabe et al. 2005). Other studies based on morphology showed that TRU Adenocarcinomas had significant lower glucose up take compared to non TRU adenocarcinomas (Chiu et al 2011)

Previous studies showed higher glucose uptake in SQC compared to ADC. In our study TTF1+ cases support TRU adenocarcinomas and have lower uptake of 18F-FDG while TTF1 negative cases are similar to SQC as already stressed, so in this work we verified a relation between TTF1 expression and lower SUVmax.

Research works applied this same approach and showed that solid predominant growth pattern presented higher F-FDG max uptake (Chao-Hua Chiu et al 2011) but in our study this conclusion was not reached. Furthermore there are evidence that tumors with high SUV demonstrate aggressive behaviour (Nguyen, et al. 2007; Van Baardwijk et al. 2007)

In the same basis we tried to explore the direct correlation of IHC and Max FDG uptake as to further pathological sub-classification to interpret the Complex Group of tumors in a primary SUVmax. This complex group may be foreseen in small biopsies and FDG uptake will be the first clinical approach.

There is evidence that higher mtDNA content in tumors is a compensatory increase due to the reduction of aerobic glycolysis (Barrientos et al. 1997). Regulation of glycolysis depends on many factors, including oxidative phosphorylation, which is partially encoded by mitochondrial genome. mtDNA copy number in tumor tissue was studied to understand its role related with glucose uptake. We have found out that mtDNA significantly correlated positively with SUVmax in 18F-FDG-PET Tumor cells have high intake of glucose, and GLUT-1 transporters, responsible for this cellular intake are usually highly expressed in tumor cells (Chiu et al. 2011). According to these findings it's probable that tumors with high SUVmax and high mtDNA copy number use the anaerobic glycolytic pathway to produce the desired energy for tumor growth, and deserve special attention for a personalised therapy. However more studies are needed in order to understand the mechanism involved in mtDNA replication, depending on glucose intracellular levels.

There were some limitations in this work that could influence the results. It is worth to mention that maximum effort was made to achieve a panel of IHC with the minimum number of antibodies to be applied after correct histological criteria to spare tissue needed for molecular pathology in small biopsies.

In conclusion, SUVmax may be then associated with IHC panel on small biopsies able to give more information about clinical prognostic parameters as together they were able to correlate to a

specific subtype of bronchial-pulmonary carcinoma. Our results indicate that TTF1 negative adenocarcinomas form a particular entity in terms of SUVmax, which has biological and clinical implications. We are aware that our results need to be replicated in a larger sample, but we believe that the present paper is an important contribution to the understanding of metabolic and molecular changes in lung cancer.

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