

Aim of the study: To explore the correlation of the expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) with maximum standardised uptake value (SUV_{max}) of ^{18}F FDG-PET-CT in non-small cell lung cancer (NSCLC).

Material and methods: Thirty patients received PET-CT imaging protocol first, and then all the patients underwent needle biopsy through CT guidance. The tumour tissue of each patient was taken from two biopsy areas: one area was $SUV_{max} = 2.5-5$, and the other was $SUV_{max} > 5$. Expression of VEGF and EGFR were detected and analysed by immunohistochemical staining.

Results: The expressions of both VEGF and EGFR had no statistically significant correlation with SUV_{max} in age, gender, pathology, or differentiation ($p > 0.05$). The expressions of both VEGF and EGFR had statistically significant correlation with SUV_{max} in tumour size and clinical stage ($p < 0.05$). SUV_{max} correlated positively with VEGF and EGFR expressions ($r = 0.879$, $p < 0.05$).

Conclusions: SUV_{max} correlated positively with expressions of VEGF and EGFR ($r = 0.879$, $p = 0.000$; $r = 0.839$, and $p = 0.000$, respectively). This study may provide guidance for radiotherapy of NSCLC.

Key words: vascular endothelial growth factor, non-small cell lung cancer.

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The correlation of expression of VEGF and EGFR with SUV_{max} of ^{18}F FDG-PET-CT in non-small cell lung cancer

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Introduction

Lung cancer is currently one of the most common malignant tumours, and non-small cell lung cancer (NSCLC) accounts for about 75–80% of lung cancer [1, 2]. Due to lack of early diagnostic methods 75% of patients are diagnosed too late and lose the opportunity for surgery [3, 4]. Non-small cell lung cancer is prone to distant metastasis, and currently there is no effective treatment to control it. So far, in NSCLC, the 5-year survival rate after treatment is only 10% [5]. So how to further improve the efficacy of NSCLC is still a major issue of clinical research. For stage I, II, and III of some selective patients, surgical resection is the preferred method of treatment, but unfortunately only 25–30% patients choose radical surgery [6, 7]. For unresectable NSCLC, comprehensive treatment with radiotherapy and chemotherapy provides a possible chance of cure. In NSCLC, radiotherapy is an important local treatment. It shows that about (64.3 ± 4.7%) of patients at different stages in their treatment need to receive radiation therapy [8, 9].

Radiotherapy has developed from ordinary radiotherapy to conformal radiotherapy, intensity-modulated radiation therapy (IMRT), and image guided radiotherapy (IGRT). The GTV (tumour volume) is determined to be dependent on imaging, the so-called image target area. There are still low rates of local control in many tumours given equal doses because of radiation-related cancer genes [such as vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR)] heterogeneity expression in tumours [10–12]. In theory, tumours should be given different doses based on the heterogeneity expression of radiation-related genes to develop radiotherapy into the biological target era.

This study aims to detect the correlation between the expressions of VEGF and EGFR in different parts of tumour tissue by immunohistochemistry and SUV_{max} (standardised uptake value) values in PET-CT (positron emission tomography-computed tomography) in NSCLC, to provide guidance in determining radiation doses.

Material and methods

Patients and specimens

We selected 30 patients with NSCLC who were treated at the Affiliated Hospital of Qingdao University Medical College between June 2009 and December 2010. The study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of the Affiliated Hospital of Qingdao University Medical College. Written informed consent was obtained from all participants. The clinical and pathological characteristics of the 30 patients with NSCLC are as follows: 18 (60%) were male and

12 were female. Eleven (36.7%) were diagnosed as adenocarcinoma, and 19 were diagnosed as other types of carcinoma (squamous carcinoma, 17; large cell carcinoma, 1; adenosquamous carcinoma, 1). Two (6.7%) were stage I, 9 (30%) were stage II, 13 (43.3%) were stage III, and 6 (20%) were stage IV according to the NSCLC staging system. All patients were primary lung cancer without chemotherapy and radiotherapy.

All patients underwent PET-CT within two weeks following CT. The results of PET-CT were regarded as negative if there was no or very low metabolic activity within lesions. A maximum standardised uptake value (SUV_{max}) of 2.5 was used as an arbitrary tumour limit. Two tumour areas of the same lesion selected from each patient were recorded if one was $SUV_{max} = 2.5-5$, the other was $SUV_{max} > 5$ by CT guided biopsy (Figs. 1, 2).

Immunohistochemistry

All the specimens were fixed in 10% neutral buffered formalin and processed routinely for VEGF and EGFR immunohistochemistry. In all cases, each of the formalin-fixed and paraffin-embedded tissue blocks was cut into five pieces with 2- μ m spacing and transferred onto slides. One slice was stained with HE, and the others were used for IHC studies. The expressions of VGFR and EGFR were detected by immunohistochemistry method (pv-6000 two-step) (Zhongshan Golden Bridge Biotechnology Corporation, Beijing, China). Phosphate buffered saline (PBS) was used in place of primary antibodies as a negative control, and positive results were used as a positive control.

Judgment of results

VEG-positive cells were stained with brown-yellow granules or masses, specifically in the cytoplasm or cell membrane. EGFR-positive expression was seen in cytomembrane or cytolymph. Both the intensity and percentage of positive cells were measured. The staining intensity was determined for the following four classes: 0 = undetectable; 1 = faint buff; 2 = moderate buff; and 3 = high buff

or sepi. Stained cell sections in each case were randomly selected and five high-power fields were counted under the microscope up to 400. We counted 200 cells in each region for a total number of 1000 cells, calculated the percentage of positive cells, and then calculated the score. Using the percentage of stained cells \times staining intensity, the integrated scoring was assessed. Cell expression was stratified as follows: 0 (negative) for less than 10% positive cells, 1 (weak) for less than 25% positive cells, 2 (moderate) for less than 50% positive cells, and 3 (strong) for more than 50% positive cells. The product of the staining intensity and positive cell scores determined the final result for each section (Figs. 3A-D).

Statistical analysis

Using SPSS11.5 statistical analysis software, the two samples were compared by *t*-test, and multiple samples were compared by chi-squared analysis. Bivariate correlation analysis was used to evaluate the SUV_{max} , respectively, correlated with VGFR and EGFR expression. Continuous variables were expressed as median \pm standard deviation and the difference had statistical significance $p < 0.05$.

Results

VGFR-positive expression was found in 21 cases (70%). VGFR expression had no difference with regard to age, gender, pathology, and differentiation ($p > 0.05$). The expression of VGFR was statistically significantly different with regard to tumour sizes and clinical stage ($p < 0.05$) (Table 1).

EGFR expression showed no difference with age, gender, pathology, and differentiation ($p > 0.05$). The expression of EGFR showed statistically significant differences with regard to tumour size and clinical stage ($p < 0.05$) (Table 2).

The SUV_{max} correlated positively with VGFR expression and the higher VEGF expression score, SUV values greater ($r = 0.879$, $p = 0.000$).

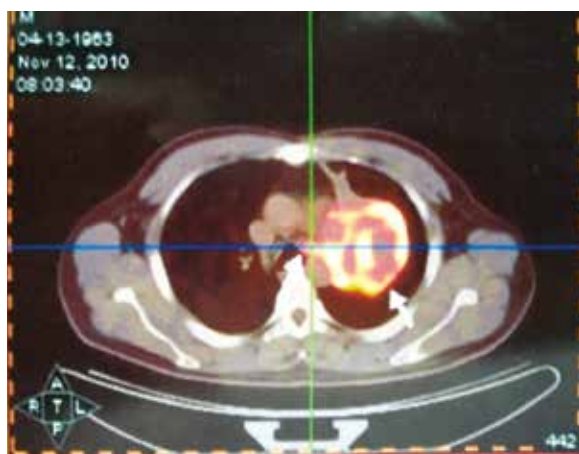


Fig. 1. PET-CT Fused image

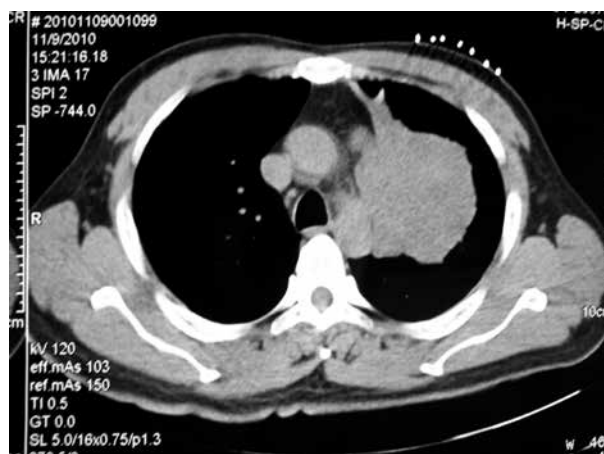


Fig. 2. CT-guided biopsy

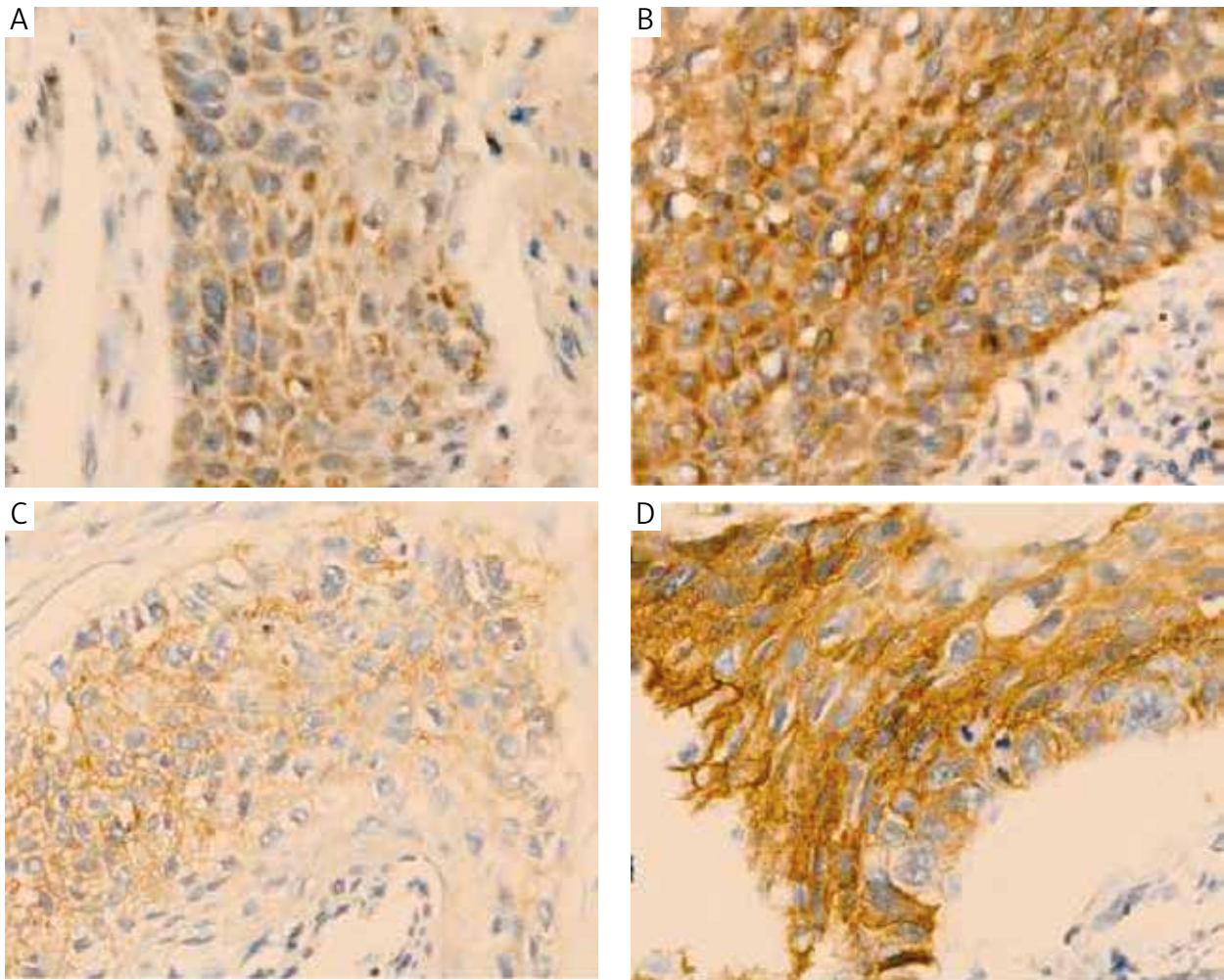


Fig. 3. A) VEGFR low expression (400×); **B)** VEGFR high expression (400×); **C)** EGFR low expression (400×); **D)** EGFR high expression (400×)

The SUV_{max} correlated positively with EGFR expression and the higher EGFR expression score, SUV values greater ($r = 0.839, p = 0.000$).

Discussion

With the popularity of CT, magnetic resonance (MR), and other equipment, three-dimensional conformal radiotherapy (3-DCRT) and intensity-modulated radiation therapy (IMRT) have been widely recognised and applied. Target radiation doses may have been upgraded to improve the efficacy of radiotherapy to a certain extent. However, there are still some deficiencies using conventional CT for intensity-modulated conformal radiotherapy planning. It may determine the target area of tumour cell distribution and radiation sensitivity differences in different regions by functional imaging such as PET and single photon emission computed tomography (SPECT) imaging. The rapid development of these technologies has resulted in the production of biological target volume (BTV) and biological intensity-modulated radiation therapy (BIMRT), and can provide the basis for individualised treatment to determine BTV and BIMRT for non-small cell lung cancer (NSCLC). Application of intensity-modulated radiotherapy with different irradiation doses for different biological tar-

gets can improve local control rates and survival rates of NSCLC, as well as reduce the exposure doses of surrounding lung tissue, heart, oesophagus, spinal cord, etc., in order to reduce radiation complications.

^{18}F -FDG-PET is a kind of functional or metabolic imaging using 18-fluorodeoxyglucose as tracers. Standardised uptake value (SUV) as a semi-quantitative index reflecting the organisation of glucose metabolic rate, plays an important role in diagnosis [13], staging [14, 15], efficacy evaluation [16, 17], and prognosis [18] of NSCLC. PET-CT has a very important clinical significance for radiation treatment planning to determine the target. Giraud *et al.* [19] reported that two radiotherapy doctors outlined a separate target, and the compliance of the target was 84% with the use of PET-CT, while it was only 37% with the use of CT in line. Steenbakkers *et al.* [20] reported that doctors from 11 different agencies outlined GTV in 22 patients with NSCLC using CT, and a year later the overall difference of the target outlined by reusing PET-CT decreased from 45% to 18%.

As shown in Fig. 4, a lesion of lung cancer had different features on the CT image and the ^{18}F -FDG-PET image. On the CT image, the lesion of lung cancer had no difference of CT value within the mass. While on the ^{18}F -FDG-PET image, from the centre to the edge of the mass, the lesion

Table 1. Relation between EGFR expression and clinical pathological factors in NSCLC

	<i>n</i>	VEGF $\bar{x} \pm s$	<i>P</i>	<i>n</i>	SUV value $\bar{x} \pm s$	<i>P</i>
Sex						
male	12	5.19 ±0.96	0.217	18	8.71 ±2.09	0.188
female	9	5.81 ±1.24		12	9.90 ±2.73	
Age						
< 60 years	12	5.40 ±1.22	0.786	19	9.12 ±2.44	0.848
≥ 60 years	9	5.53 ±1.00		11	9.30 ±2.43	
Pathology						
adenocarcinoma	7	4.80 ±1.14	0.053	11	8.25 ±2.41	0.102
others	14	5.78 ±0.97		19	9.73 ±2.27	
Differentiation						
poor	7	5.73 ±1.06	0.437	10	10.11 ±2.12	0.138
moderate-well	14	5.32 ±1.14		20	8.73 ±2.44	
Tumour size						
≥ 5 cm	6	4.58 ±1.15	0.017	10	7.71 ±1.60	0.014
< 5 cm	15	5.81 ±0.90		20	9.93 ±2.41	
Stage						
I-II	8	4.43 ±0.78	0.000	11	7.94 ±2.19	0.027
III-IV	13	6.09 ±0.74		19	9.91 ±2.25	

Table 2. Relation between EGFR expression and clinical pathological factors in NSCLC

	<i>n</i>	EGFR $\bar{x} \pm s$	<i>P</i>	<i>n</i>	SUV value $\bar{x} \pm s$	<i>P</i>
Sex						
male	12	5.37 ±0.96	0.144	18	8.71 ±2.09	0.188
female	9	6.02 ±0.98		12	9.90 ±2.73	
Age						
< 60 years	12	5.53±1.18	0.538	19	9.12 ±2.44	0.848
≥ 60 years	9	5.80 ±0.73		11	9.30 ±2.43	
Pathology						
adenocarcinoma	7	5.16 ±0.96	0.115	11	8.25 ±2.41	0.102
others	14	5.89 ±0.96		19	9.73 ±2.27	
Differentiation						
poor	7	5.62 ±0.89	0.941	10	10.11 ±2.12	0.138
moderate-well	14	5.66 ±1.08		20	8.73 ±2.44	
Tumour size						
≥ 5 cm	15	5.84 ±0.95	0.171	20	9.93 ±2.41	0.014
< 5 cm	6	5.17 ±1.03		10	7.71 ±1.60	
Stage						
I-II	8	4.94 ±0.92	0.007	11	7.94 ±2.19	0.027
III-IV	13	6.08 ±0.79		19	9.91 ±2.25	

had obvious differences in SUV. The different SUVs reflect the different metabolic rates of the lung cancer cells.

Biological target volumes are tumour targets with different radio-sensitivity in the region determined by a series of biological factors. These biological factors include the lack of oxygen, blood supply, proliferation, apoptosis and cell cycle regulation, change of oncogenes and suppressor genes, invasion, and metastasis. Epidermal growth factor receptor and VEGF are important biological factors in the decision of BT. Vascular endothelial growth factor is also known as vascular permeability factor (VPF). Now it has been confirmed that VEGF and its family members are essential for the induction factor in tumour angiogenesis and other physiological and pathological angiogenesises.

Tumour growth must rely on new blood vessels. Vascular endothelial growth factor can strongly induce angiogenesis and plays an important role in sustaining the survival of endothelial cells. Many experimental studies and clinical data have proven that the radiation sensitivity of tumour cells is negatively correlated with the expression level of VEGF. Epidermal growth factor receptor (erbB1) is one of four members of the ErbB receptor family. They have a common structure, including an external ligand binding domain, a transmembrane domain, and intracellular tyrosine kinase activity for signal transduction. A ligand binding, such as epidermal growth factor (EGF) or transforming growth factor α (TGF- α), cause EGFR to dimerise or heterodimerise with other erbB family members. This

leads to activation of receptor tyrosine kinase signalling cascade and produces different effects including migration, maturation, differentiation, metastasis, angiogenesis, and apoptosis [21]. Domestic studies have shown that in patients with VEGF- and EGFR-positive expression of non-small cell lung cancer, SUV increased with VEGF and EGFR expression strength, but there is no in-depth study about the correlation between the two. This is the first study of the correlation between SUV and VEGF and EGFR expression scores in NSCLC. The expression of VEGF and EGFR in non-small cell lung cancer showed no statistically significant difference with regard to age, sex, tumour histological type, or tumour differentiation degree, but showed statistically significant differences with regard to tumour size and TNM stage. The greater the tumour diameter and the later the clinical stage, the higher the expression of VEGF and EGFR. The positive expressions of VEGF and EGFR were significantly higher than the negative expressions of VEGF and EGFR in lung cancer. The differences were statistically significant. This study also found that the expression scores of VEGF and EGFR were significantly correlated with the SUV in NSCLC. The expression scores of VEGF and EGFR were higher, and the SUVs were higher. The differences were statistically significant.

Heterogeneity of tumours means differences in the tumour cells within the same lesion caused by different tumour cell lines. Heterogeneity of tumours has its main differences in the histology, antigen, immunity, hormone receptors, metabolism, growth rate, sensitivity to radiotherapy and chemotherapy, invasion, and metastasis. The study showed that SUV values were unequal within the same tumour tissue, and both VEGF and EGFR expressions were different in different SUV regions of the same tissue, which were associated with tumour heterogeneity. Therefore, it may provide a reference on the delineation of BTV according to the heterogeneity of the expression of tumour radiotherapy-related genes (VEGF and EGFR). According to the results of this study, in which both VEGF and EGFR expression are positively correlated with the SUV in NSCLC, in theory the same tissue can be given different radiation doses according to different SUV values. The higher SUV-value areas in the same tissue can be given greater radiation doses in non-small cell lung cancer. This study may gradually transform non-small cell lung cancer radiotherapy into BIM-RT, and provide theoretical guidance to determine doses of intensity-modulated radiation therapy.

The authors declare no conflict of interest

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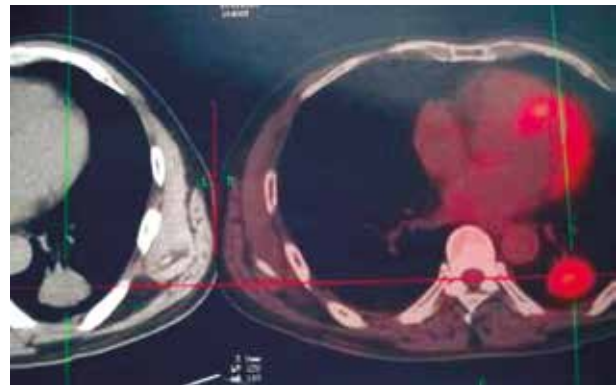


Fig. 4. A lesion of lung cancer shown on CT image (on the left) and on 18FDG-PET-CT image (on the right)

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