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Full Length Research Paper

Role of time-density curve of ct enhancement in combination with pathological examination in the diagnose of lung cancer

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The study aimed to investigate the relationship between the expressions of cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF), microvessel density (MVD) and the appearances on enhanced CT scan in lung cancer patients. Findings on dynamic CT scan were evaluated respectively in 35 patients without lung cancer (control) and 25 lung cancer patients receiving chemotherapy or radiotherapy followed by complete resection. Immunohistochemistry was used to detect the expressions of COX-2, VEGF and MVD and the relationships between CT enhancement value, histological types, clinic stages, lymph node metastasis, differentiation grades and the expressions of COX-2, VEGF and MVD were evaluated. The expressions of COX-2, VEGF and MVD (t = 11.615, 14.187, 10.524, respectively; P<0.05) and the CT enhancement value (q = 21.59, 67.81, respectively; P<0.01) in the lung cancer group were significantly higher than those in the control group. In addition, the expressions of COX-2, VEGF and MVD were related to the CT enhancement value, histological types, clinic stages and lymph node metastasis, but have no relevance with the differentiation grades among lung cancer patients. Dynamic CT enhancement scans can reflect the vascularity of lung cancer.

Key words: Lung cancer, immunohistochemistry, cyclooxygenase-2, vascular endothelial growth factor, microvessel density, CT enhancement value.

INTRODUCTION

Angiogenesis correlates well with the tumor occurrence (Folkman and Beckner, 2000). Numerous studies have shown that the angiogenesis in the lung cancer is related to the occurrence, development and invasion of lung cancer. Cyclooxygenase-2 (COX-2) (Li et al., 2004b) and vascular endothelial growth factor (VEGF) (Li et al., 2004a) are important factors involving in the development of lung cancer. The expressions of both factors in the lung cancer are closely related to the occurrence, development and prognosis of lung cancer. Microvessel density (MVD) is also an important parameter reflecting the biological behavior of tumors (Wu et al., 2003). The blood supply to the lung cancer is different from benign disease due to the angiogenesis (Zielinski and Kulig, 1984). Thus, lung cancer can be identified based on the density of diseases following injection of contrast materials. Dynamic CT is a measurable technique which is safe to evaluate the blood supply to the lung cancer and has the potential to investigate the angiogenesis in the tumors (Li et al., 2003).

In the present study, dynamic CT scans were performed in 25 patients with lung cancer and the CT values were analyzed. In addition, immunohistochemistry was employed to detect the expressions of COX-2, VEGF and the MVD in the tumors.

The relationship between these three parameters and the pathological types were also evaluated. Our results

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Table 1. CT plain value of four diseases [(Hounsfield unit (HU)].

Diseases	n	CT value ($\bar{x}$ ± s)
Lung cancer	25	38.04±2.91
Pneumonia	15	36.6±4.19
Tuberculosis	10	34.9±3.18
Benign tumor	10	35.7±3.83

(F test, F = 1.832 P>0.05).

may provide evidence of biological behaviors of lung cancer and the basis for the diagnosis of lung cancer.

MATERIALS AND METHODS

Clinical information

A total of 60 patients from July 2004 to February 2006 included in the study and were assigned into two groups. One contained 25 patients with lung cancer and another contained 35 patients with benign disease as a control group. The diagnosis of lung cancer was based on the pathological examination. There were 42 males and 18 females with the age range of 22 ~ 74 years. The pathological classification was done with the Mountain's TNM criteria. Two pathologists who were blind to the study evaluated the pathological features and consensus was achieved. The cancer tissues were collected in the surgery, embedded in paraffin and cut into sections with direction as consistent as CT scan. Then, the CT images were compared with the pathologic sections and the relationship between pathologic findings and findings on CT scan.

Conditions for CT scan

None of the patients received chemotherapy or radiotherapy before surgery. Following plain CT scan, dynamic CT scan (Siemens sensation 10) was carried out to confirm the optimal slice where the lesion could be best presented. The conditions for CT were 120 kV, 200 mA, 0.5 s, matrix: 512 × 512, FOV: 30 cm, slice thickness: 2 mm; slice distance: 2 mm (pitch: 1.5:1) and standard image reconstruction was done. The conditions for enhancement scanning were similar to those aforementioned. A total of 90 to 100 ml of 350 mg/ml Omnipaque was injected at 3 ml/s through the elbow vein and scanning was done 30 s later, and postponed scanning 1, 2, 3, 4, 5 and 7 min later. The central slice of the lesion was used for the measurement of CT value. The dynamic enhanced peak value and time were used to delineate the density curve (T-DC). Three experienced doctors who were blind to the pathology evaluated the images.

Immunohistochemistry

Reagents for immunohistochemistry with PV method were purchased from the ZhongShan Biotech Co., Ltd (Beijing). Immunohistochemistry was performed using PV method. The tissues embedded in paraffin were from The Second Affiliated Hospital of Harbin Medical University and had been pathologically diagnosed.

Scoring of the pathological features

The COX-2 and VEGF expressions were scored according to the staining intensity and the number of positive cells (Wang et al., 2000). Five fields were randomly selected from each section followed by evaluation. The staining intensity was scored as follows: 0, no staining; the buff accounted 1, the palm yellow accounted 2, tan accounted 3. The number of positive cells was scored as follows: 0, ≤5%, 1, 5 ~ 25%; 2, 25 ~ 50%, 3, >50%. The multiple of both scores was as follows: 0, (-); 1 to 2, (+); 3 to 4, (++); 5 to 6, (+++). The detection of MVD was performed according to the method described by Weidner (1995).

Statistical analysis

Student t test, F test, q test, linear correlation and linear regression were used in the statistical analysis which was done using SAS software (edition 8.1). A value of P<0.05 was considered statistically significant.

RESULTS

CT plain value

There was no marked difference in the values of plain CT among lung cancer, tuberculosis, pneumonia and benign tumors. Thus, normal CT could not be applied to diagnose the lung cancer (Table 1).

CT enhancement value

Significant differences in the CT enhancement value

Table 2. CT enhancement value of four diseases.

Disease	n	CT enhancement value (HU)
Lung cancer	25	33.68±8.74
Benign tumor	10	14.30±2.11
Pneumonia	15	40.60±3.68
Tuberculosis	10	6.30±1.16

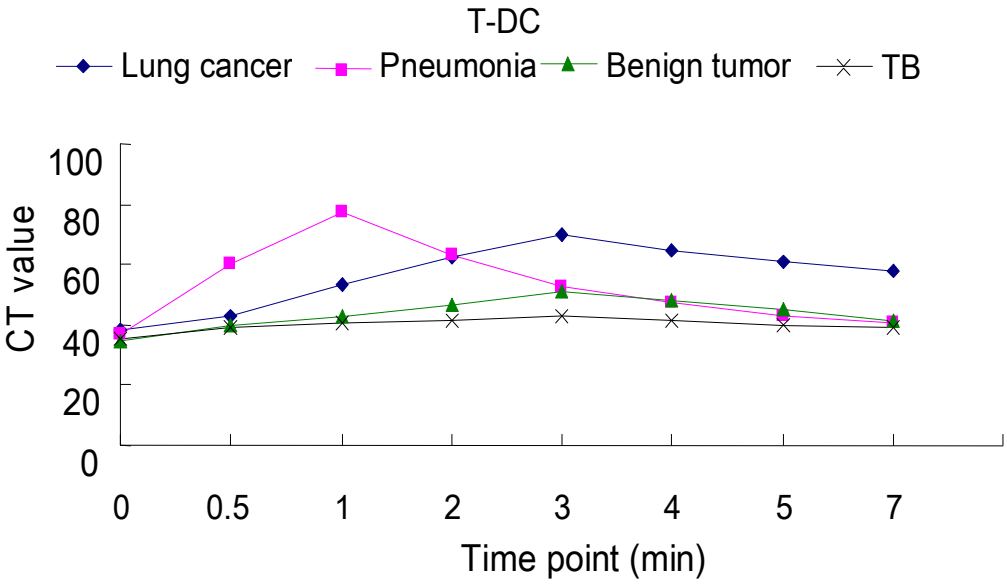

Figure 1. The time-density curve of different diseases.

were noted among four diseases (P<0.01). The CT enhancement value of lung cancer was markedly higher than that of benign tumor and tuberculosis but lower than that of pneumonia. The CT enhancement value of lung cancer was not markedly different from that of pneumonia (q = 2.04, P>0.05) (Table 2).

Time-density curve

Repeated measures analysis of variance was performed for analysis. The result expressed by each curve was different (F = 37.543, P<0.05) (Figure 1). The time-density curve of lung cancer showed the CT value increased gradually over time. The pneumonia raised and declined soon. We could see obvious peak from both of them. The benign tumor presented a low and even type. Tuberculosis presented a flat type. Both benign tumor and tuberculosis had no peak. The time to peak occurrence in the lung cancer was 3 ~ 4 min in 90.9% of lung cancers and 2 ~ 3 min in 9.1% of lung cancers. The time to the peak of pneumonia occurred mostly at 1 min after contrast injection. The time was 1 min in 56.4% of pneumonia and 30 s in 32.7% of pneumonia.

Expressions of VEGF, COX-2 and the MVD

Expressions of VEGF, COX-2 and MVD were different between lung cancer and benign diseases. The proportion of VEGF/COX-2 positive cells and MVD in the lung cancer was 64.8 ± 3.8%, 76.4 ± 14.1% and 62.5 ± 14.6/HP, respectively showing significant difference (P<0.05) (Table 3). However, there were no remarkable differences in these parameters among lung benign tumor, pneumonia and tuberculosis. The expressions of VEGF and COX-2 and the MVD in the lung adenocarcinoma were dramatically higher than those in the lung squamous carcinoma. These findings showed the lung cancer of different pathological types had different biological behaviors and pathologic features. The expressions of VEGF, COX-2 and MVD were not different between well and poorly differentiated lung cancers showing that VEGF and/or COX-2 induced

Table 3. Relationships between VEGF, COX-2, MVD and CT enhancement value, clinic stage and pathological grade in the lung cancer patients.

		n	VEGF		COX-2		MVD	
			%	P value	%	P value	cells/HP	P value
Pathological types	Squamous cell carcinoma	9	71.2±11.4	F = 8.94<0.05	52.1±10.2	F = 7.493<0.05	50.2±11.6	F = 4.816<0.05
	Adenocarcinoma	13	87.3±12.6		79.8±12.7		68.9±12.3	
	Adenosquamous carcinoma	2	75.7±3.5		56.2±3.1		54.2±2.8	
Differentiation	High differentiation	13	73.4±12.3	F = 0.967>0.05	64.2±12.8	F = 1.216>0.05	61.3±12.2	F = 1.159>0.05
	Low differentiation	12	75.6±11.9		67.1±11.9		63.5±11.3	
Clinical stages	I	16	74.3±12.4	F = 5.439<0.05	54.8±13.7	F = 8.147<0.05	54.2±12.8	F = 4.361<0.05
	II	7	86.7±10.5		70.3±9.9		65.1±10.8	
	III	2	88.9±4.3		73.4±3.1		67.3±2.7	
CT enhancement value	≥20HU	22	87.1±13.8	t = 2.558<0.05	74.2±13.1	t = 2.493<0.05	67.8±13.9	t = 2.384<0.05
	<20 HU	3	71.9±3.5		58.6±4.2		52.7±3.7	
Lymphatic nodule metastasis	Positive	8	87.2±11.2	t = 2.482<0.05	68.7±11.8	t = 2.413<0.05	68.1±11.7	t = 2.487<0.05
	Negative	17	72.1±15.3		53.2±14.6		53.4±13.7	

Table 4. Comparison of mean enhancement peak value in pathological types (q test).

Pathological types	n	Enhancement peak value ($\bar{x}$ ± s)
Adencarcinoma	13	41.26±6.28
Squamous carcinoma	9	24.15±5.73
Adenosquamous carcinoma	2	37.0±1.17

angiogenesis is a common phenomenon in lung cancer regardless the pathological stage (Table 4). Moreover, patients with lymph node metastasis had significantly higher expressions of VEGF, COX-2 and MVD, which indicates that patients having elevated expressions of VEGF, COX-2 and MVD in the lung cancer may be susceptible to metastasis (Table 5). In stage II and III lung cancer, the expressions of VEGF, COX-2 and MVD were dramatically higher than those in stage I cancer. Furthermore, the expressions of VEGF, COX-2 and MVD were different in cancers with different CT enhancement values (Table 6). In addition, correlation analysis showed there were positive correlations between any two of COX-2, VEGF and MVD.

Linear regression analysis was done to explain the relationship of CT enhancement value of lung cancer and the expressions of VEGF, COX-2 and MVD with the equations of Y 1 = -31.4 + 99.88X (X: CT enhancement value, Y1: COX-2, R2 = 0.93, P<0.01), Y2 = -34.28 + 115.27X (Y2: MVD, R2 = 0.60, P<0.01) and Y3 = -15.79 + 69.17X (Y3: VEGF, R2 = 0.90, P<0.01). These results revealed

Table 5. Comparison of enhanced peak value in different clinical stages of lung cancer (q test).

Clinical stages	n	Enhancement peak value ($\bar{x}$ ±s)
Stage I	16	27.35±6.46
Stage II	7	36.42±6.29
Stage III	2	40.0±1.83

Table 6. Comparison of enhanced peak value in lymph node metastasis of lung cancer (t test).

Lymph node metastasis	n	Enhancement peak value ($_{Hu}$)
Yes	8	40.37±3.41
No	7	30.49±7.88

the associations between CT enhancement value and expressions of VEGF, COX-2 or MVD: the higher the CT enhancement value, the higher the expressions of VEGF, COX-2 and MVD. Thus, we can predict the malignant degree of lung cancer through CT enhancement value.

DISCUSSION

Role of CT enhancement value

In the present study, the peak CT enhancement value of pneumonia was 68 HU which was similar to that of lung cancer. This result was consistent with previous study (Zhang et al., 1997). Zhang and Kono (1997) investigated 65 lesions without calcify and results showed the enhancement values of both malignant tumor (41.9 ± 2.8 HU) and pneumonia (43.6 ± 7.7 HU) were similar. In the present study, the peak enhancement value of pneumonia was higher than that of benign disease and tuberculosis but was not different from that of lung cancer. Therefore, diagnosis of lung disease only based on the peak value will lead to difficult differentiation from pneumonia. The peak arrival times in CT enhancement scanning of lung cancer and pneumonia were 3 and 1 min, respectively, no obvious peak was found in the tuberculosis and lung benign tumor. Currently, some researchers devote themselves for research like that, but different equipments and techniques were used resulting in the difference in the peak varied. Yamashita et al. (1995) performed dynamic CT scanning of 18 lung cancer patients. Their results showed the peak arrival time was about 5 min. Zeng et al. (1997) used hand pushed method to carry out dynamic CT scanning and results revealed the time was 2 min. Zhang and Kono (1997) used injection machine with high pressure to perform scanning in 42 lung cancer patients and results indicated the time was around 1 min. The discrepancy among different studies may be attributed to the differences in the dose of contrast and injection speed (Yamashita et al., 1995; Liu et al., 1999). Injection at high speed may lead to a short peak arrival time. If the injection speed is slow (1 ~ 2 ml/s) or contrast density is too low, the occurrence of peak time might be postponed. In addition, lung cancers of different histologcal types had different peak time.

In the present study, the occurrence of arrival time in the lung adenocarcinoma was obviously earlier than that in the lung squamous carcinoma. Therefore, the histological types of lung cancer may be a main cause of the difference in the arrival time. In the present study, 90 to 100 ml of contrast were injected at 3 ml/s. Results revealed most of pneumonias achieved the peak at 1 min, but that of most of lung cancers was 3 min. Thus, 3 min was the best delay time. Thus, injection of 90 to 100 ml of contrast at 3 ml/s is recommended. Scanning was performed at 0.5 min after injection and then every 1 min. Using this strategy, we can easily acquire the peak and its accurate enhancement curve.

Appearances in the enhancement curve

The degree of the enhancement in diseases depends on the amount of contrast and the abundance of blood vessel in the lesion (Li and Xiao, 2000). After injection of the contrast, the image of pneumonia quickly enhanced and the speed was higher than that in lung cancer. Studies (Xiao et al., 1997) also revealed the blood supply of the lung cancer was mainly provided by the bronchus artery, which obviously extended and had arterial venous fistula. Moreover, there were numerous pathologic capillary vessels in the lung cancer which extensively integrated into a blood net resulting in the increase of vascular resistance and subsequent slow blood flow. In addition, the lung cancer has no lymphatic vessels. When

the contrast is injected, it is retained in the lesion and the removal of contrast delayed resulting in delayed time when compared with normal tissues. So the T-DC of lung cancer occurs slower and continuously increases. The benign tumor grew slow, had already wrapped a film and the blood supply resembled original tissues. So the amount of contrast in the benign tumor is less than that in the lung cancer and diffused slowly in the benign tumor. The pathologic feature of tuberculosis was the caseous necrosis that surrounds the fiber and its T-DC was flat.

Relationship between CT enhancement value and findings in immunohistochemistry

It has been demonstrated that the metastasis and growth of tumors require the angiogensis (Tanigawa et al., 1997). Tumors of different histological types have different presentations in the angiogenesis. The blood vessels in the pneumonia, benign tumor and tuberculosis are normally structural lung blood vessel to respond appearances. The amount and quality of the newly generated blood vessels in the lung cancer vary in different pathological types. Yamashita et al. (1995) found that the degree of enhancement of lung cancer was more related to the microvessels (0.02 mm<d<0.1 mm) than with macrovessels (d>0.1 mm). These microvessels may be newly generated blood vessels of cancer which are different from those in the normal lung tissues. The occurrence of new blood vessels in the tumor determines the appearance of tumor on CT enhancement scan. Therefore dynamic CT can provide pathological information of the tumor based on the angiogenesis (Miles, 2002). Among numerous factors involving in the angiogenesis, VEGF is an important one which is secreted by the tumor cells. It can increase permeability of blood vessels and promote the angiogenesis (Li et al., 2004a). A great deal of newly generated blood vessels in the tumor is capillary vessels less than 0.1 ml in diameter. The base membrane of these vessels is not complete and made the contrast often exudates into the surrounding tissues. Thus, the contrast is remained in the tissues leading to the delayed time on CT scan. MVD is a critical indicator of angiogenesis and represents the amount of capillary vessels and small veins. Moreover, the detection of MVD is convenient and simple and has uniform procedures. Therefore, it has been widely used in the detection of angiogenesis of tumors after being developed by Weidner.

Our results also showed the positive relationship between VEGF and MVD in the tumor. The COX-2 is an important rate-limiting enzyme and can catalyze arachidonic acid to form prostaglandin. Our results revealed the tumor tissues had significantly increased expression of COX-2 which was related to the angiogenesis of tumor and involved in the infiltration and metastasis of cancers (Joo et al., 2003). In our study, positive association between COX-2 and MVD was also indicated. Some researchers (Davies et al., 2003) proposed that the increased expression of COX-2 may lead to increase of prostaglandin production and subsequently the elevation of PGE2 level. The later may stimulate the synthesis and secretion of VEGF finally resulting in the angiogenesis. Our findings also suggested the relationship between COX-2 and VEGF. Swensen et al. (1995) also found that there were a variety of newly generated blood vessels in the center of malignant lung tumor. In the study of Yamashita et al. (1995) results also revealed the relationship between MCD and the peak value on dynamic CT scanning of lung cancer. These findings were consistent with ours. In the present study, linear regression analysis was performed to investigate the relationship between peak value of CT value of lung cancer and the expressions of COX-2, VEGF or MVD. Our results indicated the CT peak value was positively related to the expressions of COX-2 and VEGF as well as MVD. Because of the presence of lymphatic vessel net, the blood circulation and lymphatic circulation have cross talk. Thus, the angiogenesis can directly influence the lymph node metastasis.

We can predict the infiltration and metastasis of tumors according to the CT peak value.

Conclusions

The characteristics of lung cancer include: the peak CT value of lung cancer is higher than tuberculosis and benign tumors but lower than pneumonia. The time-density curve of lung cancer has a flat slope and a long plateau. These characteristics are related to the angiogenesis of lung cancer and the active metabolism in the tumor. Our results also showed that the peak CT value of lung cancer occurs within 3 min which is important for the clinical application. If a lesion is noted in the plain CT, we better delayed at least 3 min after injection of contrast except for the normal chest regulations scan time in order to catch the peak value for the diagnosis of lung cancer. Otherwise, it may lead to misdiagnosis. Our results showed the expressions of COX-2, VEGF and MVD in the lung cancer were significantly different from those in the benign disease. At the meantime, the expressions of COX-2, VEGF and MVD in the lung cancer are related to the CT enhancement value. CT enhancement value in the lung cancer had level dissimilarity. Its expression of the VEGF, COX-2 and MVD had difference. Thus, dynamic CT scanning can be applied to evaluate the characteristics of angiogenesis of lung cancer before surgical intervention. Therefore, this strategy provides a mini-invasive method for the diagnosis of lung cancer. Lung cancers of different clinical stages and with or without lymph node metastasis vary in the expressions of VEGF, COX-2 and MVD.

These findings suggest the high expression of VEGF, COX-2 and high MVD may be factors of poor prognosis of lung cancer. In the present study, time-density curve was delineated with the peak values at different discontinuous time points. It could not make sure that the peak would exactly fall into the time points.

In addition, in the present study, the prognosis of lung cancer was indirectly determined by the lymph node metastasis. The direct relationship between CT peak value, the expressions of VEGF, COX-2, MVD and prognosis of lung cancer was not evaluated. More studies are required to reveal the specific mechanism.

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