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Original Article

Potential application value of microvessel-slice spiral computed tomography perfusion combined with microvessel density measurement in evaluating pathological grading of solitary non-small cell lung cancer

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Abstract: This study aims to investigate the potential application value of microvessel-slice spiral computed tomography (MSCT) perfusion imaging combined with microvessel density (MVD) measurement in evaluating the degree of differentiation of solitary non-small cell lung cancer (sNSCLC). The MSCT perfusion test results of 46 sNSCLC patients, confirmed through surgery and clinical pathological examination, were retrospectively analyzed for parameters, including blood flow (BF), blood volume (BV), mean transit time, and permeability surface (PS). Then, the values were compared with the postoperative MVD measurement results in order to analyze differences between the CT perfusion parameters and MVD in the tumor tissues with different degrees of differentiation. In the 46 sNSCLC cases, BV showed statistical significance in identifying high, moderate, and poor degrees of differentiation of NSCLC. Meanwhile, PS showed statistical significance in identifying high and poor degrees of differentiation ($P<0.05$), in which BV had a maximum difference. A statistically significant difference in MVD was found between the different degrees of differentiation of NSCLC. BF, BV, and PS were positively correlated with MVD. MSCT perfusion imaging could indirectly reflect the angiogenesis of lung lesions in vivo, thus exhibiting reference values for the preoperative determination of the degrees of differentiation of NSCLC.

Keywords: Non-small cell lung cancer, X-ray compute, perfusion imaging, microvessel density

Introduction

Solitary pulmonary lesion is defined as a single pulmonary lesion with a diameter of ≤4.5 cm. It is not associated with pulmonary lymphadenectasis, lung atelectasis, or pneumonia, among which primary bronchopulmonary carcinoma accounts for a sizeable ratio, with an increasing trend. Regarding its pathological types, 80-85% of cases are non-small cell lung cancer (NSCLC) [1], including squamous cell carcinoma, adenocarcinoma, and large cell undifferentiated carcinoma. The 5-year survival rate of NSCLC patients is only 15% [2]. Since 1997, lung cancer has ranked highest in incidence and mortality rates among all cancers. With the aggravation by population aging, industrialization, pollution, and destruction of human living environments, particularly with the continuous increase in the smoking population, the incidence and mortality rates of lung cancer in China in the next 20 years will continue to increase. Tumor angiogenesis is closely related with tumor differentiation; that is, the lower the degree of differentiation, the stronger the intensity of tumor angiogenesis and the higher the degree of malignancy [3]. With the progress of medical imaging research studies in recent years, computed tomography (CT) perfusion imaging has been known to quantitatively reflect the characteristic changes of lesions or tumor microcirculation [4]. Meanwhile, as the criterion standard parameter of angiogenesis, microvessel density (MVD) could quantitatively

reflect the status of tumor angiogenesis [5]. Certain researchers found that CT perfusion parameters could reflect the blood supply of lung tumors, thus reflecting the degree of differentiation and staging of lung cancer to a certain extent [6, 7]. Therefore, measuring CT perfusion parameters could guide further clinical treatment and judgment of prognosis. Currently, a large number of domestic and foreign research studies have investigated the potential application value of CT perfusion imaging in the evaluation of the efficacy of cancer chemotherapy [8-11]. Thus, identification of a method for early and accurate evaluation of the degree of differentiation of malignant cancers would have great significance for clinical treatment and prognosis evaluation for such cancers [12]. This study used microvessel-slice spiral computed tomography (MSCT) perfusion imaging to quantitatively evaluate tumor angiogenesis, with the aim of analyzing differences between MSCT perfusion parameters and MVD in solitary non-small cell lung cancer (sNSCLC) patients with different degrees of differentiation. In addition, we investigated the diagnostic value of in vivo MSCT perfusion imaging for pathological grading.

Materials and methods

Clinical data

The MSCT perfusion test results of 46 sNSCLC patients, confirmed through surgery and clinical pathological examination, examined in our hospital between December 2010 and December 2012 were retrospectively analyzed. The inclusion criteria were as follows: solitary pulmonary lesion, diameter of ≤4.5 cm, without lung hilar and mediastinal lymphadenectasis, without significant inflammation or atelectasis around the lung lesion, and without satellite lesion. All of the patients initially underwent CT perfusion scanning, and results were then confirmed by surgery and clinical pathological examination. The exclusion criteria were as follows: (1) presence of significant benign calcification (calcified lesions ranged more than 10%) or space occupied by fat components; (2) the patient could not cooperate in the examination after breathing training; (3) presence of pure ground-glass-like dense nodules; (4) presence of a space-occupying lesion with a maximum diameter of <1 cm; (5) the patient could not tolerate high injection rates of contrast agent; (6) history of allergy; and (7) presence of a space-occupying lesion that disrupts breathing, so that the perfusion value could not be calculated.

The 46 cases included 29 cases of adenocarcinoma and 17 cases of squamous cell carcinoma (10 highly, 21 moderately, and 15 poorly differentiated cases). Of the patients, 32 were male and 14 were female. Their ages ranged from 35 to 76 years, with a mean age of 62.1 years. The diagnoses of all the cases were confirmed by performing a surgical pathological examination. Anti-tumor therapy was not performed before perfusion imaging. All the patients provided informed consent for participation in this study. All the patients initially underwent 64-layer spiral CT normal and perfusion scanning (GE Lightspeed), followed by lung (subsegment) resection by the same chief physician, who was the director of the thoracic surgery department of our hospital, 1 week after the MSCT examination.

CT perfusion imaging

GE Lightspeed VCT (64-layer 128-slice CT) was used for routine CT scanning with a 5-mm thickness and spacing. Four consecutive layers were selected as the target from the site of the lesion with the largest diameter. CT perfusion scanning was performed by using the toggling-table technique. The scanning parameters were as follows: 80 kV; 60-80 mA; dose-length product, 187.97 ± 39.35 mGy; effective dose, 2.65 ± 0.51 mSv; non-ionic contrast agent ioversol (300 mgI/mL; Jiangsu Hengrui Co.); and total dose, 50 mL, injected through the cubital vein with an injection flow rate of 4 mL/s. The GE ADW4.5 workstation and perfusion 4 perfusion software packages were used for mapping, analysis, and calculation. The corresponding blood flow (BF), blood volume (BV), mean transit time (MTT), and permeability surface (PS) were also determined. The circular maximum region of interest (ROI) was selected from the central area of the space-occupying lesions according to the following criteria: sampling range diameter of >1.0 cm; a distance of 2-3 mm from the tumor edge; absence of necrosis and avoidance of the artifact area and lesion edge, among others; and an average number of 3-5 ROI could be selected when the tumor

Table 1. CT perfusion parameters among different differentiation degrees of NSCLC

Perfusion parameter	Differentiation degree		
	Poor	Moderate	High
BV	14.33 ± 4.66	10.66 ± 2.39	7.69 ± 1.87
BF	93.64 ± 51.11	82.23 ± 49.20	64.47 ± 31.51
MTT	15.67 ± 9.62	19.73 ± 23.21	12.77 ± 6.48
PS	27.60 ± 10.98	20.21 ± 7.21	17.54 ± 11.17

Table 2. Comparison of *P* values of CT perfusion parameters among different differentiation degrees of NSCLC

Differentiation levels		P			
		BV	BF	MTT	PS
Poor	Moderate	0.004*	0.504	0.451	0.074
Poor	High	0.000*	0.058	0.544	0.007*
Moderate	High	0.012*	0.264	0.168	0.477

Note: *P<0.05.

diameter was >3 cm. All measurements were completed and averaged by three independent physicians (with a job title of attending physician or higher). If the results obtained by these three physicians had a huge difference, these should be verified, consulted, and remeasured, or a senior physician with a higher job title would be requested to redetermine the measurements.

MVD assay

For all the patients, surgical resection was performed and complete pathological specimens were obtained. The sections corresponding to the CT images were obtained according to the orientations in vivo. The ROIs consistent with the pathological specimens were selected as much as possible. Then, the tissue MVD was labeled and measured with a vascular endothelial cell surface antigen CD34 monoclonal antibody. By using MaxVision quick immunohistochemistry, MVD was calculated as follows: first, the full field section was observed at low magnification (×40), and the vision was selected according to the maximum stained endothelial cells; second, the microvessel number was counted in 3 visions (×400), and the average value was calculated as MVD by using the Weidner method [13]. The results were determined by using a double-blind method. All the measurements were independently completed and averaged by three pathologists (with a job title of attending physician or higher). If the results obtained by these three doctors had a huge difference, these should be verified, consulted, and remeasured, or a senior physician with a higher job title would be requested to redetermine the measurements.

Pathological grading

The criteria for identifying the degree of differentiation of NSCLC were as follows: high differentiation: the tumor cells were cubic or columnar and regularly sized, with glandular and tubular shapes; moderate differentiation: with irregular glands and tubes, partial formation of incomplete glandular cavity, and obvious heteromorphism; poor differentiation: with unobvious glandular and tubular structures, or almost without duct-like structure, appearing as solid lumps or cords. The criteria for identifying the degree of differentiation of squamous carcinoma were as follows: high differentiation: with keratosis phenomenon or cancer pearl inside the carcinoma nest, or an intercellular bridge; poor differentiation: the cancer appeared as a solid piece or nest, without keratosis phenomenon and intercellular bridge; moderate differentiation: with partial keratosis phenomenon, intercellular bridge, and solid piece or nest appearance. The diagnosis was independently completed by three pathologists (with a job title of attending physician or higher). If the results of these three physicians had a major difference, these were verified, consulted, and remeasured, or a senior physician with a higher job title would be requested to redetermine the measurements.

Statistical analysis

All data were analyzed by using the SPSS16.0 statistical package. The measurement data were expressed as mean ± standard deviation. The reference value used was the 95% confidence interval (CI), with a test level of $\alpha = 0.05$. A *P* value of <0.05 was considered as statistically significant. The comparison between the CT perfusion parameters and MVD used the Pearson correlation method. The identification

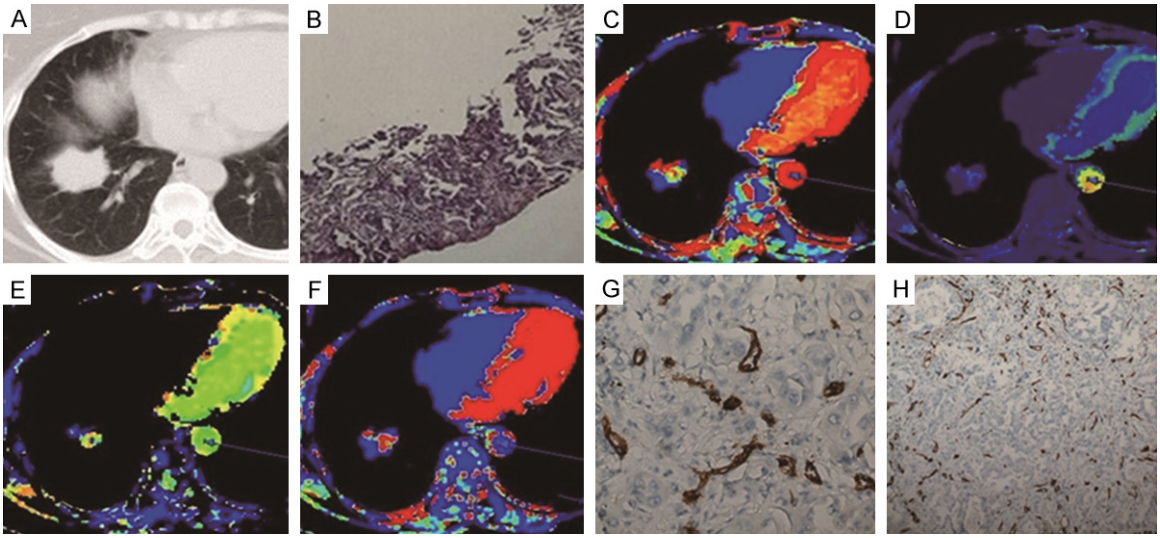

Figure 1. Moderately differentiated adenocarcinoma. A. Routine CT image; B. Pathological image; C. BV pseudocolor image, BV: 13.55 ml/100 g; D. BF pseudocolor image, BF: 48.03 ml/(100 g·min); E. MTT pseudocolor image, MTT: 18.24 s; F. PS pseudocolor image, PS: 11.45 ml/(100 g·min); G. Pathological image of CD34 monoclonal antibody labeled MVD (×100); H. Pathological image of CD34 monoclonal antibody labeled MVD (×400).

Table 3. Comparison of MVDs among the sNSCLC cases with different pathological characteristics

Differentiation levels		P
Poor	Moderate	0.004*
Poor	High	0.000*
Moderate	High	0.012*

Note: *P<0.05.

of the degree of differentiation of the NSCLC patients used one-way analysis of variance, and the least-significant-difference method was used in the pairwise comparisons.

Results

CT perfusion

The CT perfusion parameters significantly differed between the different degrees of differentiation of NSCLC (**Table 1**). The intergroup comparisons are shown in **Table 2**, with a *P* value of <0.05 considered as statistically significant. The results showed that BF and MTT had no statistically significant differences, whereas BV showed statistical significance in identifying high, moderate, and poor degrees of differentiation of NSCLC. PS showed statistical significance in identifying high and poor degrees of differentiation of NSCLC (*P*<0.05), in which BV had the maximum difference.

Comparison of MVD

The MVD of squamous carcinoma in the sNSCLC patients was 28.16 ± 11.86 and 40.64 ± 10.24 for adenocarcinoma (**Figure 1**). MVD was 9.89 ± 2.21 in high differentiation, 7.09 ± 2.05 in moderate differentiation, and 45.52 ± 12.75 in poor differentiation, with statistically significant differences (*P*<0.05).

Comparison of MVD between the patients with different pathological characteristics

The analysis of the MVD values showed differences between the different degrees of differentiation of sNSCLC, in which BV showed the maximum difference (**Table 3**).

Correlation analysis of the CT perfusion parameters and MVD

According to the statistical analysis, BF, BV, and PS were positively correlated with MVD (*P*< 0.05), in which BV and PS had the highest correlation coefficients, whereas MTT had no correlation with MVD (*P*>0.05, **Table 4**).

Discussion

CT perfusion imaging is a noninvasive functional imaging method and could quantitatively reflect changes of lesions or tumor microcirculation through perfusion parameters. Thus, it

Table 4. Correlation analysis of CT perfusion parameters and MVD

Perfusion Parameter	MVD	
	P	r
BF	0.006*	0.273
BV	0.000*	0.697
MTT	0.431	0.080
PS	0.000*	0.563

Note: *P<0.05.

could detect pathological changes more accurately and earlier than the conventional imaging methods [4]. This study used the deconvolution method [14], which can obtain results similar to the actual hemodynamics, so the calculated perfusion parameters and functional images would better reflect the real situation inside the lesions. However, this method needed a long data acquisition time and is particularly sensitive to noise. Therefore, certain methods were needed to effectively inhibit the noise to ensure the accuracy of the calculation results, and it still needed to be continuously improved. Meanwhile, CT perfusion imaging uses the toggling-table technique, which could effectively improve the measurement accuracy of the CT perfusion parameters. However, its remarkable drawback was that it confers a high scanning radiation exposure. Therefore, we reduced the kV and mA values, avoided the non-inspected areas, narrowed the perfusion range, and covered other sensitive parts such as the thyroid gland. At the same time, the patients were informed of the radiation hazards before the examination and signed a written informed consent form.

In recent years, evaluation of biological behaviors of NSCLC under the conditions of primary tumor angiogenesis has become a new hot spot in medical imaging research. In 1971, Folkman [5] first proposed the concept of tumor angiogenesis, which considered that the growth and metastasis of solid tumors were dependent on the formation of new blood vessels within the tumors. Recent studies have also shown that the invasion and metastasis of cancer cells were closely related to their abilities of promoting angiogenesis, and that angiogenesis intensity, namely MVD, was considered to be the histological and quantitative indicator of angiogenesis, and closely related to the treatment, prognosis, and metastasis of tumors [15]. Currently, neovessels inside tumors are considered to cause changes in BV, perfusion volume, and PS, which forms the basis of CT enhancement [16]. Neovessels inside tumors produce high MVD but cannot be seen directly on images. The relevant information could be found from the aspect of tissue perfusion; that is, CT perfusion imaging of lung cancer could reflect, in essence, the blood flow characteristics of various types of lung cancers and intratumoral microvessel densities [17, 18]. Among the CT perfusion parameters, BF, BV, and MTT were related to the high MVD inside tumors, and PS was related to the high permeability of tumor vessels, which are the basis for CT perfusion imaging to detect the activities of tumor angiogenesis. Most scholars believed that MVD is positively correlated with BF and BV, with BF exhibiting the best correlation. BF is determined according to the tumor blood flow characteristics and intratumoral MVD. With a reduction in the degree of differentiation, BF would also decrease and thus could reflect local blood flow [18]. BV represents the number of functional capillaries. In addition, the higher the PS, the greater the amount of contrast agent exudated during the perfusion. The more the tumor blood vessels, the greater the amount of retained interstitial blood and the higher the BV. In this study, BV showed statistical significance in identifying high-moderate and moderate-poor, and high-poor differentiation of NSCLC. PS reflects the permeability of capillary endothelial cells. Malignant tumors have many immature newborn vessels, with incomplete vascular wall structures. Furthermore, various vascular growth factors produced by the cancer cells would promote an increase in microvascular permeability. Therefore, the contrast agent would more easily enter the tissue gaps through the incomplete capillary basal membranes. Through time, tumor angiogenesis would not always proceed. It might show a peak limit and a downward trend after reaching a certain level. However, this study selected solitary pulmonary lesions with a diameter of 4.5 cm or less. Even if these were malignant, they would still be in the early or mid-proliferative proliferation phase, with relatively small diameters. This study found that PS showed statistical significance in identifying high-poor degree of differentiation. MTT refers to the mean time during which blood flow passes the vascular system (arteries, capillary,

venous sinuses, and veins). A large number of arteriovenous direct accesses were observed, with different sizes, levels, and channels inside tumors. As the contrast agent injection rate and individual vascular circumstances affect MTT, with the tumor vessels increasing, BV would also be increased. Meanwhile, the interstitial pressure would also be increased, which might cause a decrease in intratumoral BV. Therefore, various factors caused the fact that MTT had no significant correlation with MVD.

MVD statistically significantly differed between the differently differentiated sNSCLC. BF, BV, and PS positively correlated with MVD ($P<0.05$), in which BV and PS had higher correlation coefficients, similar to the report in previous studies [19-23]. This study further confirmed the accuracy and reliability of MVD in evaluating tumor angiogenesis, indicating that CT perfusion imaging had certain clinical values in identifying the degrees of differentiation of NSCLC.

Malignancy grading is based on the degree of differentiation, atypia, and mitotic count. Different levels of malignancy have important significance for clinical treatment and prognostic evaluation of cancers. At present, no uniform standard of CT perfusion examination has been established for NSCLC histological grading. This study is the first to analyze the differences between enhanced MSCT perfusion indexes and tumor MVD for evaluating the degrees of differentiation of NSCLC. From the view that MSCT perfusion imaging could reveal the pathophysiological changes of tumors from the microcirculation level, we believe that the information it could provide is more valuable than that provided by conventional CT. Therefore, the combination of these two methods could obtain more accurate conclusions than the conventional CT staging methods. This study was a beneficial attempt in this field, despite its limitations such as small sample size, not including the pathological staging factors, among others. Future research studies are needed to obtain better results in terms of the aforementioned limitations.

In summary, BV, PS, and MVD had statistical significance in the pathological grading of sNSCLC. MSCT perfusion imaging could noninvasively reflect the internal angiogenesis status and functions inside tumors. Therefore, they can be used as important supplementary parameters for tumor pathological grading and be considered as useful for the treatment and evaluation of prognosis of tumors.

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Disclosure of conflict of interest

None.

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