

Correlation study between flash dual source CT perfusion imaging and regional lymph node metastasis of non-small cell lung cancer

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Research article

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Abstract

Objective To explore the correlation of flash dual source computed tomography perfusion imaging (CTPI) and regional lymph node metastasis of non-small cell lung cancer (NSCLC), and to evaluate the value of CT perfusion parameters in predicting regional lymph node metastasis of NSCLC.

Methods 120 consecutive patients with NSCLC confirmed by postoperative histopathology were underwent flash dual source CT perfusion imaging in pre-operation. The CT perfusion parameters of NSCLC, such as blood flow (BF), blood volume (BV), mean transit time (MTT) and permeability (PMB) were obtained by the image post-processing. Then microvessel density (MVD), luminal vascular number (LVN), luminal vascular area (LVA) and luminal vascular perimeter (LVP) of NSCLC were counted by immunohistochemistry. These cases were divided into two groups that patient with regional lymph node metastasis group (group A, 58 cases) and without regional lymph node metastasis group (group B, 62 cases) according to their pathological results. The CT perfusion parameters and the microvessel parameters were contrastively analysed between the two groups. Receiver operating characteristic (ROC) curve was used to assess the diagnostic efficiency of CT perfusion parameters in predicting regional lymph node metastasis of NSCLC in pre-operation.

Results Group A presented significantly lower LVA, BF and higher MTT, PMB than Group B ($P < 0.05$), while BV, LVN, LVP and MVD were no significant difference ($P > 0.05$). Correlation analysis showed that BF was correlated with LVA and LVP ($P < 0.05$), while BV, MTT and PMB were not correlated with LVN, LVA and LVP ($P > 0.05$). All the perfusion parameters were not correlated with MVD. According to the ROC curve analysis, when $BF < 85.16$ ml/100 ml/min as a cutoff point to predict regional lymph node metastasis of NSCLC, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value were 60.8%, 81.7%, 71.5%, 75.6% and 69.5% respectively.

Conclusion Flash dual source CT perfusion imaging can non-invasively indicate the luminal vascular structure of tumor and BF can be used as one of the important indexes in predicting regional lymph node metastasis of NSCLC in pre-operation.

Background

Lung cancer has been the leading cause of cancer death, in which non-small-cell lung accounts for more than 80% of the total [1]. So accurate staging of non-small cell lung cancer (NSCLC) is vital for the prognosis of patients. And in this process, lymph node metastasis is one of the important factors [2]. At present, CT diagnosis of NSCLC with or without regional lymph node metastasis is mainly based on the threshold of 10 mm short diameter of lymph node, but there are a certain false negative rate and false positive rate [3, 4]. FDG-PET/CT is currently the most important noninvasive method for diagnosing lymph node metastasis [5–7]. But due to the fact that PET/CT equipment and inspection fees are very expensive, it has not been widely used, especially in China. Tumor angiogenesis is an important factor affecting tumor growth, invasion, metastasis and prognosis. It was reported that lymph node metastasis

of cervical cancer was closely related to tumor angiogenesis [8]. Microvessel density (MVD) and luminal vascular parameters including the luminal vessels number (LVN), the luminal vessels area (LVA) and the luminal vessels perimeter (LVP) are important indicators of tumor angiogenesis. But this method belongs to invasive examination, and can not dynamically observe the angiogenesis of living tissues. Computed tomography perfusion imaging (CTPI) can reflect noninvasively the angiogenesis of tumor. It has important application value in quantitative and qualitative research of tumor [9]. Previous studies have shown that the correlation between CT perfusion parameters and luminal vascular parameters is better than MVD [9–11]. Flash dual source CT with advanced radiation reduction technology can greatly protect the health and safety of patients, so it is more suitable for CTPI research. Therefore, this study intends to introduce the luminal vascular parameters (LVN, LVA and LVP) to quantitatively analyze the microvascular structure of NSCLC and to make correlation analysis with flash dual source CT perfusion parameters in order to provide the theoretical basis in studying the CT perfusion imaging characteristics of NSCLC with regional lymph node metastasis, then to evaluate better the value of flash dual source CT perfusion parameters in predicting regional lymph node metastasis of NSCLC in pre-operation.

Methods

Patients

This study has been approved by the ethics committee of Zunyi Medical University. And each patient or the patient's family was fully informed and signed the informed consent before performing the pre-operative CT examination. From January 2015 to May 2019, 120 consecutive patients with NSCLC confirmed by histopathology were enrolled in the study according to the following criteria: (i) All cases successfully underwent CT perfusion scanning preoperative and accepted surgical treatment; (ii) No treatment in pre-operation, such as chemotherapy or radiotherapy; (iii) less than 2 weeks between the time of the CT perfusion imaging and the operation; (iv) absence of severe heart, lung or kidney insufficiency. Of these cases, 77 patients is males and 43 patients is females. The mean age was 54.35 years (range, 25–74 years). The cases were divided into two groups that patient with lymph node metastasis group (group A, 58 cases) and without lymph node metastasis group (group B, 62 cases) according to their pathological results.

Equipment and methods

CT perfusion scan were performed by using SOMATOM Definition Flash dual source CT scanner (Siemens, Germany) with Care dose 4D technology and X-care technology. CT scan involved two steps. First, an unenhanced CT scan was performed to determine the section of perfusion scan with following scan parameters: 100 kV, 100 mAs, 0.5 pitch, 512 × 512 mm matrixs and slice thickness was 8 mm, scanned from the thoracic inlet to the bottom of the lung. Second, a perfusion CT scan in the condition of breathless which covered upper and lower poles of lung tumor (the scanning range is the upper and lower 3.5 cm centered at the center of lung tumor) was performed with following parameters: 80 kV, 120 mAs, 3 mm reconstruction slice thickness (reconstruction interval 2 mm) in B20f smooth Eva image

reconstruction method after injecting contrast medium (iohexol) 2–4 seconds. 50 ml iohexol (300 mg/ml) was injected via an antecubital fossa vein at a flow rate of 6 ml/second, followed by 20 ml of saline flush. In our study, CTDIvol is (60.89 ± 8.27) mGy and DLP is (536.28 ± 45.06) mGy.cm.

Image post-processing

CT perfusion imaging data of lung tumor were transmitted to workstation (Siemens Syngo Multimodality workplace) and post-processed by the volume perfusion computed tomography (VPCT) body software to obtain the perfusion parameters of NSCLC, such as blood flow (BF), blood volume (BV), mean transit time (MTT), permeability (PMB). The region of interest (ROI) of reference blood vessel was set in the thoracic aorta (if the plane of lung tumor was not present in the thoracic aorta, the arteria carotis or truncus brachiocephalicus was chosen). And the ROI of lung tumor was drawn freehand around the boundary (2–3 mm from the edge of the tumor) of the tumor (avoid necrosis area, calcification areas and blood vessels) in the maximum section (If any artifact interference, the section will not be regarded as the analysis section). Of 120 cases, 3 cases of necrosis and 1 case of calcification. Two experienced radiologists (both more than six years of experience and same level in interpreting CT perfusion imaging) were invited to do the experiments without knowing the patients' information and take the average value from them as the final data. The two experienced radiologists would be asked to calculate again if the difference between their data is beyond 10%.

Histopathologic study

After surgery, tumor tissue specimens were fixed in the inflated state by 10% buffered formalin, and embedded in paraffin. The tissue samples (3 μ m paraffin slice) that came from the maximum section of tumors which diagnosed to be NSCLC by hematoxylin and eosin staining were immunohistochemically stained by using the PV 2-Step method (PV-9000 2-step plus Poly-HRP Anti-Mouse/Rabbit IgG Detection System, Golden Bridge Co., Beijing, China) with CD34 (Golden Bridge Co., Beijing, China) monoclonal antibody according to the following steps: (i) removing paraffin of the tissue slices (ii) the slices were incubated in 3% nonionic hydrogen peroxide for 10 minutes and then washed with phosphate-buffered saline (PBS) three times (2 minutes each time). (iii) addition of anti-rat CD34 monoclonal antibody in a 1:50 dilution for 1 hour at room temperature and then washed with PBS three times (2 minutes each time). (iv) addition of reagent 1 (Polymer Helper) for 20 minutes at room temperature, then washed with PBS three times (2 minutes each time). Then addition of reagent 2 (Polyperoxidase-anti-mouse/rabbit IgG) as the method of reagent 1. (v) the slices were then stained with DAB solution and subsequently counterstained with hematoxylin. (vi) the slices were then rinsed with distilled water, dried and mounted. The tissue slices were stained with α -smooth muscle actin (SMA) in the same method as CD34 staining. The control group was set up with PBS solution instead of the first antibody respectively.

Quantification of histologic microvessel parameters

Each slice was scanned at low magnification ($\times 40$ or $\times 100$) to determine six "hot spot" areas where the number of microvessels was at maximum including four peripheral areas and two center areas. In each area, one field of 0.20 mm² ($\times 200$ magnification, 0.512 mm \times 0.383 mm) was chosen randomly for the

purposes of counting and measure MVD, LVN, LVP and LVA. MVD were counted in the chosen field at high magnification ($\times 200$). Any CD34 highlighted endothelial cell or cell cluster that was apparently separate from peripheral tissues and connective tissues was counted as a single microvessel and branch construct with discrete breaks was also counted as a single microvessel. Taking the mean value of the data that came from the six fields above as the final MVD. For LVN, LVP and LVA, only microvessels with a discernible lumen and one or more complete layer(s) of -smooth muscle actin stained pericytes and smooth muscle cells (regarded as relatively mature tumor vessels) will be counted. Software (image pro plus 6.0) was used for vascular measurements. If the slices exist following situations: (i) the pathological tissue slip off; (ii) there are air bubbles; (iii) uneven staining; (iv) the background is not clean because the non-specific staining, will be excluded. Two pathologists (both more than 15 years of experience and same level in lung pathology) were invited to count each slice respectively. If the difference between the two results is more than 10%, they would be asked to do it again. The mean of the two or four results is the final histologic data.

Statistical analysis

All statistical analyses were performed by using statistical software (SPSS 18.0). $P < 0.05$ is considered to indicate a statistical significance. All data, including the perfusion parameters (BF, BV, MTT and PMB) and the microvessel parameters (MVD, LVN, LVP and LVA) were underwent consistency test within inter-observer and intra-observer. All continuous variables were underwent normality test. Normal distribution data were expressed as mean \pm SD, and abnormal distribution data were represented as median \pm interquartile range (IQR). The two-tailed Student t test or Wilcoxon test was used to compare the perfusion parameters and the microvessel parameters between the group A and the group B. Pearson or Spearman correlation analysis was used to investigate the relationships between the perfusion parameters and the microvessel parameters. Receiver operating characteristic (ROC) curve was used to assess the diagnostic efficiency of CT perfusion parameters in predicting regional lymph node metastasis of NSCLC.

Results

General characteristics of patients

Of these cases, group A includes 58 cases (33 cases of adenocarcinoma, 24 cases of squamous cell carcinoma, and 1 case of large cell undifferentiated carcinoma) and group B includes 62 cases (32 cases of adenocarcinoma, 26 cases of squamous cell carcinoma, 2 case of large cell neuroendocrine carcinoma and 1 case of sarcomatoid carcinoma). For N-stage, there were 62, 23, 35 and 0 cases in N0, 1, 2 and 3, respectively. For T-stage, there were 51, 46, 23 and 0 cases in T1, 2, 3 and 4, respectively (Table 1). In group A, the short diameter of lymph node was less than or equal to 10 mm in 11 cases (25 lymph nodes), while in group B, the short diameter of lymph node was greater than 10 mm in 8 cases (12 lymph nodes).

Table 1
General characteristics of patients

Characteristics No. (%)
Location of the primary lesion
Right upper lobe 28 (23.33)
Right middle lobe 5 (4.17)
Right lower lobe 24 (20.00)
Left upper lobe 27 (22.50)
Left lower lobe 36 (30.00)
Histology
Adenocarcinoma 66 (55.00)
Squamous carcinoma 50 (41.67)
Large cell neuroendocrine carcinoma 2 (1.67)
Large cell undifferentiated carcinoma 1 (0.83)
Sarcomatoid carcinoma 1 (0.83)
Differentiation degree
Well-differentiated 89 (74.17)
Moderately differentiated 21 (17.50)
Poorly differentiated 10 (8.33)
Lymph node metastasis
N0 62 (51.67)
N1 23 (19.17)
N2 35 (29.16)
N3 0
T staging
T1 51(42.50)
T2 46(38.33)
T3 23(19.17)
T4 0

The microvessel parameters and CT perfusion parameters between group A and group B

All parameters, including the microvessel parameters (MVD, LVN, LVP and LVA) and CT perfusion parameters (BF, BV, MTT and PMB) were underwent consistency test within inter-observer and intra-observer. The Kappa value of the perfusion parameters within inter-observer and intra-observer is 0.86 and 0.81 respectively, and the Kappa value of the microvessel parameters within inter-observer and intra-observer is 0.85 and 0.82 respectively.

The microvessel parameters and CT perfusion parameters of group A and group B were compared. The result showed that group A presented significantly lower LVA, BF and higher MTT, PMB than group B ($P = 0.027, 0.006, 0.011$ and 0.048 , respectively). There were no significant difference in BV, LVN, LVP and MVD ($P > 0.05$) (Table 2, Fig. 1, 2).

Table 2
Difference of parameters between group A and group B

parameters	group A	group B	Z	P
BF(ml/100 ml/min)	66.13 ± 30.15	115.57 ± 80.21	-2.710	0.006
BV(ml/100ml)	8.58 ± 4.31	9.21 ± 3.54	-0.728	0.467
MTT(s)	15.85 ± 15.92	6.10 ± 2.57	-2.667	0.011
PMB(ml/100 ml/min)	21.18 ± 29.87	20.59 ± 7.91	-1.959	0.048
MVD(strip/field)	67.21 ± 46.69	70.85 ± 50.14	-0.447	0.637
LVN(strip/field)	6.70 ± 3.10	8.31 ± 3.87	-1.268	0.218
LVA(μm^2 /field)	4617.65 ± 1435.67	6541.37 ± 3235.76	-2.310	0.027
LVP(μm /field)	718.71 ± 216.75	942.09 ± 418.39	-1.951	0.052
Abbreviations: BF = blood flow; BV = blood volume; LVA = luminal vascular area; LVN = luminal vascular number; LVP = luminal vascular perimeter; MTT = mean transit time; MVD = microvessel density; PMB = permeability				

Relationship of CT perfusion parameters with microvessel parameters

Correlation analysis showed that BF was correlated with LVA and LVP ($r = 0.335, 0.383$, respectively; $P = 0.031, 0.012$, respectively), while BV, MTT and PMB were not correlated with LVN, LVA and LVP ($P > 0.05$). All the perfusion parameters were not correlated with MVD ($P > 0.05$) (Table 3).

Table 3
Relationship of CT perfusion parameters with microvessel parameters

parameters	BF BV MTT PMB							
	r	P	r	P	r	P	r	P
MVD	0.151	0.371	-0.126	0.431	-0.261	0.110	0.001	0.998
LVN	0.229	0.140	0.071	0.650	-0.051	0.761	0.052	0.750
LVA	0.335	0.031	0.160	0.313	-0.216	0.169	0.163	0.296
LVP	0.383	0.012	0.259	0.091	-0.138	0.379	0.070	0.679
Abbreviations: BF = blood flow; BV = blood volume; LVA = luminal vascular area; LVN = luminal vascular number; LVP = luminal vascular perimeter; MTT = mean transit time; MVD = microvessel density; PMB = permeability								

The efficiency of CT perfusion parameters in diagnosing regional lymph node metastasis of NSCLC

According to the above results, the CT perfusion parameter BF, which is different between group A and group B and correlated with the luminal vascular parameters was selected as the index to predict NSCLC with or without regional lymph node metastasis. ROC was used to test the ability of BF to diagnose regional lymph node metastasis of NSCLC. The area under ROC curves (AUC) for BF was 0.746 ($P < 0.05$). According to the ROC curve analysis, when $BF < 85.16$ ml/100 ml/min as a cutoff point to predict regional lymph node metastasis of NSCLC, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value were 60.8%, 81.7%, 71.5%, 75.6% and 69.5% respectively (Fig. 3).

Discussion

Tumor angiogenesis and regional lymph node metastasis of NSCLC

Tumor angiogenesis is an important factor affecting tumor growth, invasion, metastasis and prognosis [12–14]. The lymph node metastasis of tumor cells has three common ways: (i) Tumor cells directly invade the lymphatic vessels; (ii) Tumor cells directly invade into the micro vessels, and then through the incomplete basement membrane into the tumor stroma, and then enter the lymphatic vessels leading to lymph node metastasis; (iii) Tumor cells that entered into the blood circulation may also enter into the stroma of tumor or tissue with blood flow and cause lymph node metastasis. Obviously, in the process, the value of tumor angiogenesis is significant [14, 15]. It was reported that lymph node metastasis of cervical cancer was closely related to tumor angiogenesis [8]. This study also showed that regional lymph node metastasis of NSCLC was related to the luminal vascular parameters, and the luminal vascular parameter LVA in group A are lower than group B, while no correlation with MVD. We believe that regional lymph node metastasis of NSCLC is more closely associated with the luminal vascular, and because of MVD including the vessels with and without lumen [16], the different proportion of them may cause the relationship between MVD and lymph node metastasis different too. Besides, the microvascular wall is mostly thin-walled and fissured, and even if some of the lumen is formed, it is also no function. Therefore, although MVD is considered to be a reliable indicator of tumor angiogenesis [17, 18], luminal vascular parameters may be a better indicator for the evaluation of tumor biological behavior.

The current situation of preoperative assessment of NSCLC with lymph node metastasis

At present, CT and PET-CT are the main non-invasive methods for preoperative assessment of NSCLC with lymph node metastasis. The size of lymph nodes is the main basis of CT in judging regional lymph node metastasis of NSCLC. The short diameter of lymph node is usually 10 mm as the threshold for the diagnosis of lymph node metastasis, but it is easy to lead to false positive and false negative [3, 4]. Moreover, the metastatic lymph nodes less than 10 mm in short diameter were also easily misdiagnosed in surgical operation and pathological examinations. This study showed that the short diameter of lymph node was less than or equal to 10 mm in 11 cases (25 lymph nodes) of lymph node metastasis group, while greater than 10 mm in 8 cases (12 lymph nodes) of non-lymph node metastasis group. This also

shows that it leads easily to false positivity and false negativity, if the size of lymph nodes is used to judge whether the lymph node are metastatic or not. The lymph node greater than 10 mm in short diameter is not necessarily metastasis, but less than 10 mm may also be metastasis.

FDG-PET/CT is currently the most important noninvasive method for diagnosing lymph node metastasis. Maximal standardized uptake value (SUVmax) is a good index marker for the diagnosis of metastatic lymph nodes, but there is no consensus on the optimal threshold of SUVmax, and some non-metastatic lymph nodes can also have high uptake of 18 F-FDG. it may also lead to false positive and false negative [19, 20]. In addition, due to the fact that PET/CT equipment and inspection fees are very expensive, it has not been widely used, especially in China.

The value of CT perfusion parameters in diagnosing regional lymph node metastasis of NSCLC in pre-operation

CTPI can provide qualitative and quantitative hemodynamic information, and it can reflect noninvasively the angiogenesis of tumor [9, 21, 22]. It has important application value in quantitative and qualitative research of tumor. This study showed that CT perfusion parameter BF was correlated with the luminal vascular parameters LVA and LVP. All the perfusion parameters were not correlated with MVD. BF refers to the flow rate of blood in unit time and volume. That is to say, the larger the LVA and LVP, the larger the blood flow rate and the blood volume of per unit volume and time. This demonstrates that the dual source CT perfusion parameters can indicate the luminal vessels of tumor, but as for MVD is uncertain. This may also be associated with the microvessels including some non-functional vessels.

It was reported that CTPI plays an important role in evaluating lymph node metastasis of cancer in pre-operation [23, 24]. This study showed that the dual source CT perfusion parameters are related to the luminal vascular parameters of NSCLC, while the luminal vascular parameters are related to regional lymph node metastasis of NSCLC, which provides a theoretical basis for the evaluation of regional lymph node metastasis of NSCLC by CT perfusion parameters. In this study, CT perfusion parameter BF of NSCLC group with regional lymph node metastasis was lower than that of NSCLC group without regional lymph node metastasis. This showed that the dual source CT perfusion parameter BF have certain value in predicting regional lymph node metastasis of NSCLC.

ROC curve was used to evaluate the value of the dual source CT perfusion parameter BF in predicting regional lymph node metastasis of NSCLC. The result showed that BF was valuable in predicting regional lymph node metastasis of NSCLC in pre-operation. And the possibility of NSCLC with regional lymph node metastasis may be suggested when $BF < 85.16 \text{ ml}/100 \text{ ml}/\text{min}$. For these patients, the regional lymph node dissection should be performed more carefully, systematically and extensively to avoid missing the small metastatic lymph nodes.

Conclusion

This study showed that the luminal vascular parameters were the important indexes for the evaluation of tumor angiogenesis and were related to the regional lymph node metastasis of NSCLC. Flash dual source CT perfusion imaging can non-invasively indicate the luminal vascular structure of tumor and BF can be used as one of the important indexes in predicting regional lymph node metastasis of NSCLC in pre-operation. So the dual source CT perfusion imaging can be an effective supplement of traditional morphological image in diagnosing regional lymph node metastasis of NSCLC.

Small sample size and single-center study are the main limitations of this study. Therefore, further work is to expand the sample size and conduct multi-center research to improve the value of the CT perfusion parameters in predicting regional lymph node metastasis of NSCLC.

Abbreviations

AUC: area under the curve; BF: blood flow; BV: blood volume; CT: computed tomography; CTDIvol: volume computed tomography dose index; CTPI: computed tomography perfusion imaging; DLP: dose length product; LVA: luminal vascular area; LVN: luminal vascular number; LVP: luminal vascular perimeter; MTT: mean transit time; MVD: microvessel density; NSCLC: non-small cell lung cancer; PMB: permeability; ROC: receiver operating characteristic; ROI: region of interest; SMA: smooth muscle action; SUVmax: maximal standardized uptake value; VPCT: volume perfusion computed tomography

Declarations

Acknowledgments

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Authors' contributions

HTT participated in the design of the study, carried out the most studies (CT perfusion imaging, image post-processing, image analysis, statistical analysis and so on) and drafted the manuscript. SH and LXL carried out the CT perfusion imaging, image post-processing and image analysis. ZXJ, JKY, WF and SL participated in the CT perfusion imaging, image post-processing. CLN, WS and LQ participated in the data collection and arrangement. LBG conceived, designed and data analysis.

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Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information files.

Ethics approval and consent to participate

This study has been approved by the ethics committee of Zunyi Medical University. Each patient or the patient's family was fully informed and signed the informed consent before performing the pre-operative CT examination.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

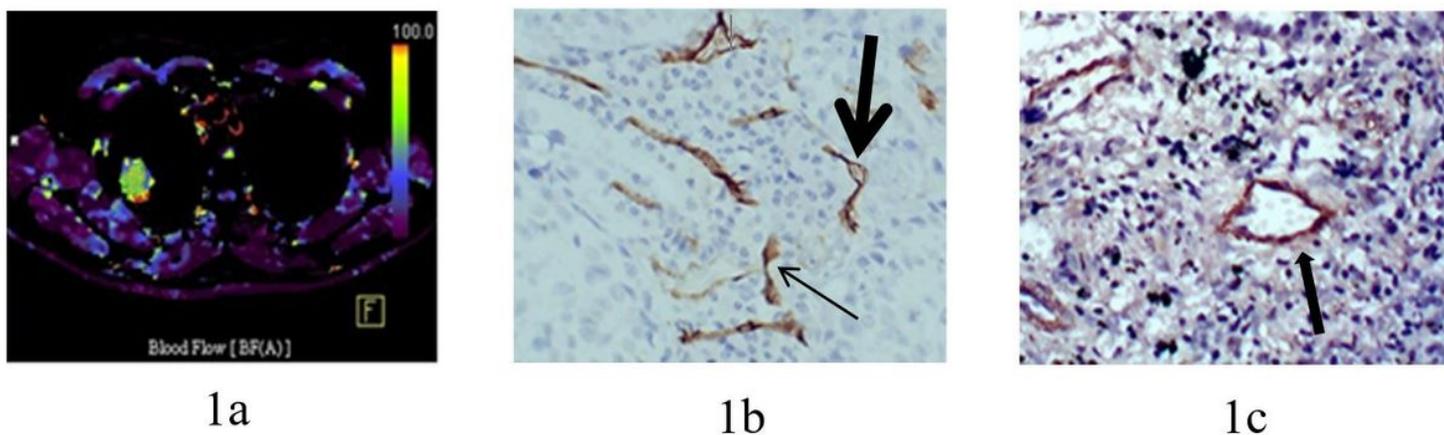
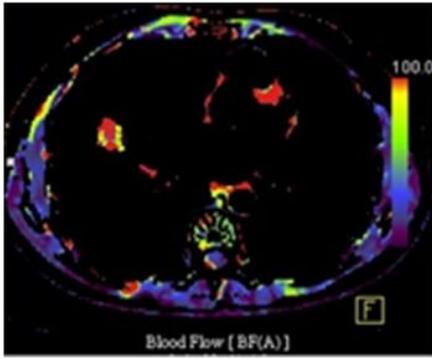
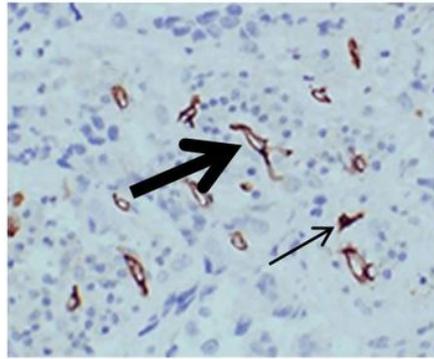


Figure 1

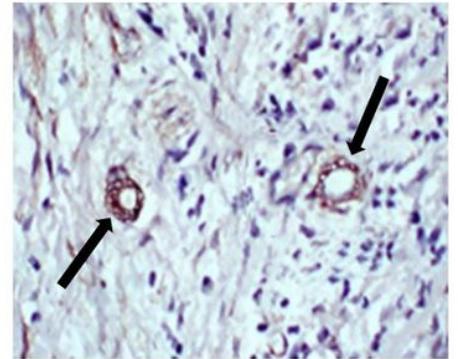
A 52-year-old man with squamous cell carcinoma at the upper lobe of right lung with right hilar lymph node metastasis. (a) Functional map of perfusion showed that blood flow value was low (48.36 ml/100ml/min). (b) CD34 staining showed microvessels with luminal vessels (thick arrow) and without luminal vessels (thin arrow) and the latter accounted for the main part ($\times 200$). (c) SMA staining showed fewer microvessels covered with completed layers of smooth muscle cells (arrows) ($\times 200$)



2a



2b



2c

Figure 2

A 65-year-old man with adenocarcinoma at the middle lobe of right lung without regional lymph node metastasis. (a) Functional maps of perfusion show that blood flow value was high (105.23 ml/100ml/min). (b) CD34 staining showed microvessels with luminal vessels (thick arrow) and without luminal vessels (thin arrow) and the former accounted for the main part ($\times 200$). (c) SMA staining showed more microvessels covered with completed layers of smooth muscle cells (arrows) ($\times 200$)

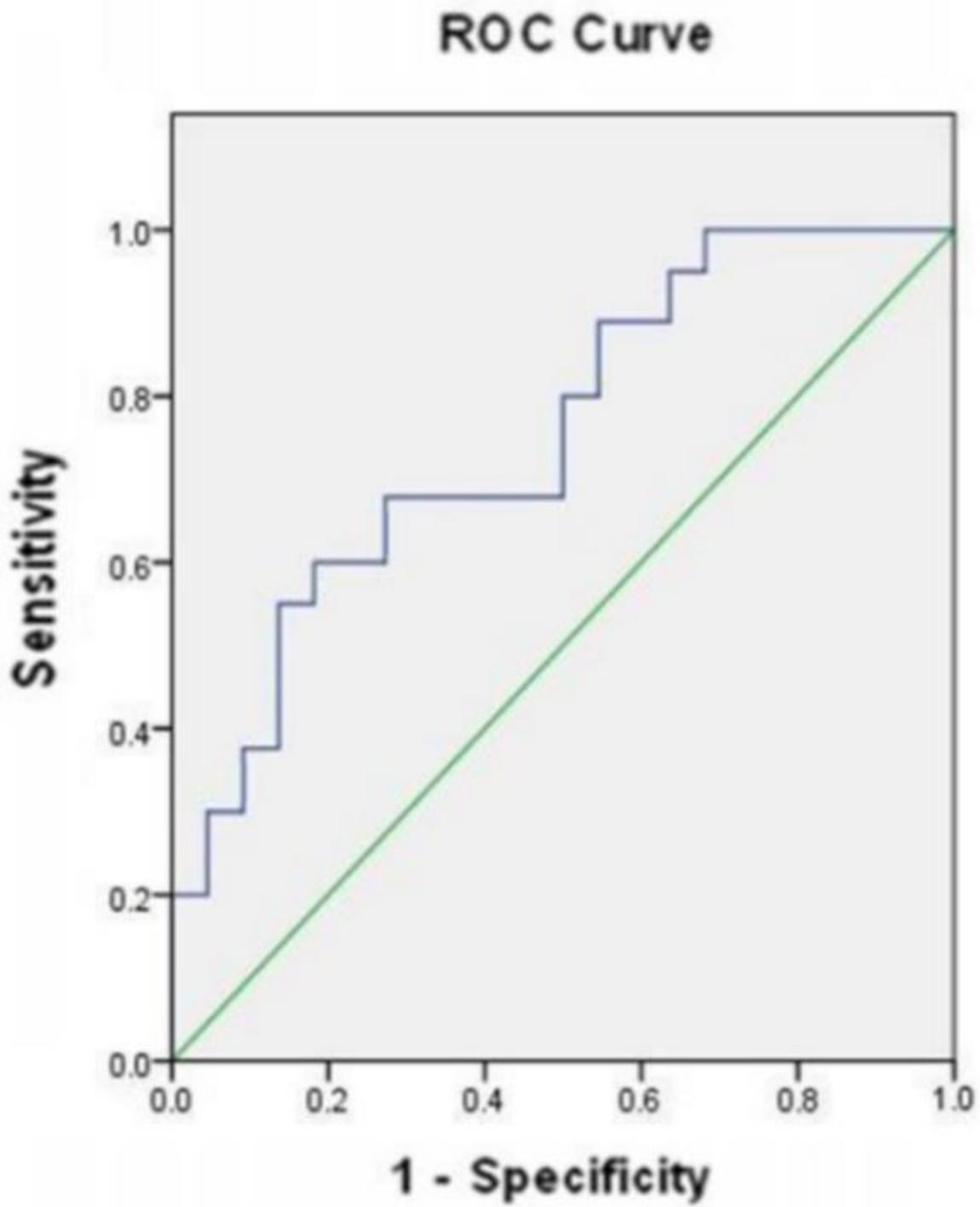


Figure 3

ROC curve of BF in predicting regional lymph node metastasis of NSCLC