BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2016
VOL. 73, NO. 1, 32–37
http://dx.doi.org/10.1080/09674845.2016.1146434

Value of Ki-67 and computed tomography in the assessment of peripheral lung adenocarcinoma

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ABSTRACT

Purpose: This study was designed to determine whether proliferation antigen Ki-67 and/or a computed tomography (CT) value could be used to evaluate the clinical-pathological features of peripheral lung adenocarcinoma.

Materials and methods: A total of 116 eligible lung cancer patients were enrolled. Nodule size, lymph node metastasis, differentiation, Ki-67 expression and CT findings were assessed. The relationship between clinic parameters and the CT feature was analysed statistically.

Results: The percentage of lesions that had ground-glass opacity or localised air bronchogram was significantly greater in low CT value group (<30, $p < 0.05$). No significant association was observed between CT value and size in the subgroup with CT value > 0 ($p = 0.66$). As a proliferative marker of lung cancer, Ki-67 was present in a total of 115 (99.9%) of the 116 evaluable primary lung cancers. There was a statistically significant correlation between the Ki-67 index and CT value ($p < 0.05$). Compared to CT value, Ki-67 index possessed higher sensitivity to predict the differentiation and lymph node metastasis of peripheral lung adenocarcinoma, adding of CT value would enhance its specificity.

Conclusion: Combination of Ki-67 expression and CT value determination was useful for the classification of differentiation and metastatic or proliferative potential of peripheral lung adenocarcinoma.

ARTICLE HISTORY
Received 5 December 2015
Accepted 21 January 2016

KEYWORDS
Computed tomography (CT); lung adenocarcinoma; CT value

Introduction

Lung cancer is the most common cause of cancer-related death worldwide, resulting in more than 2 million deaths worldwide each year. To date, various prognostic factors have been reported, such as TNM stage, aberrant expression of some molecules and physiologic status.[1]

In lung adenocarcinoma cases, pulmonary nodules are a common incidental finding on thoracic computed tomography (CT) scans, and the investigation and surveillance of such nodules comprises a significant workload for clinical teams. CT findings are well-known important prognostic indicators. For example, the ground-glass opacity (GGO) ratio is strongly associated with survival; GGO-dominant tumours have a 5-year survival rate of nearly 100%. In addition, spiculation, notch and air bronchograms have also been reported to be associated with a good prognosis.[2,3] However, the observation of an solitary pulmonary nodules (SPN) with special feature CT findings is relatively uncommon. In order to further understand the role of CT in the diagnosis and differential diagnosis of pulmonary nodules, it is necessary to balance the weight of various radiological signs in the identification of pulmonary nodules. Recent studies have emphasised the importance of mass measurement and the solid portion of the tumour for evaluation of tumour aggressiveness and temporal progression.[4–6]

Apart from CT scanning, immunohistochemistry (IHC) analysis is able to add prognostic value to the current staging system through identifying markers of tumour aggressiveness. The Ki-67 proliferation index, one of the biological markers used in histopathological evaluation, being an important criterion in the differentiation of benign and malignant tumours, such as lung squamous cell carcinoma. For example, the Ki-67 labelling index could be linked to tumour infiltration patterns of lung squamous cell carcinoma and cell proliferative activity in the minimal (T1) lung cancer.[7,8] The CT value of a substance is equal to the attenuation coefficient of the material and the difference between the water absorption coefficient and the water attenuation coefficient. The CT value of the substance reflects the density of the substance, that is, the higher the CT value of the substance is, the higher is the density of the substance. Although there are some reports on the association between radiological findings and histological grade of the tumour in NSCLC,[2,3] there are

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little data about the CT value of the primary tumour on preoperative SPN.

Here, our study aimed to assess the clinic application of Ki-67 expression combined with the CT value in the evaluation of lung adenocarcinoma, to determine its usefulness in the classification of differentiation and metastatic or proliferative potential of peripheral lung adenocarcinoma. We hypothesised that the combination of Ki-67 and the CT value would give improved sensitivity and specificity for lung adenocarcinoma than either scoring system alone.

Materials and methods

Study subjects

The study subjects comprised 116 patients (63 male and 53 female, median age 63 years) with lung adenocarcinoma who subsequently underwent thoracotomy or selected video-assisted thoracic surgery (VATS) between 2012 and 2014. Pretreatment staging procedures included physical and blood examinations, bronchoscopy, CT of the thorax. All patient records/information was anonymised and de-identified prior to analysis in the May of 2015, and all authors had access to identifying information during or after data collection. Subjects with autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus), chronic infections (e.g. human immunodeficiency virus infection, tuberculosis), bone marrow involvement, anticoagulant and antithrombotic drug using, or those who had received immunosuppressive treatment were excluded. The study was approved by the ethics committee of The First Affiliated Hospital of Soochow University to be free informed consent given by participants.

CT image acquisition

CT scans were used to assess tumour size, density and CT value. The resulting images by a Philips CT scanner (Holland) were displayed at a width of 1000 Hounsfield units (HU) and a level of –750 HU in the lung window and at a width of 400 HU and a level of 45 HU in the mediastinal window. The resulting images by a Siemens CT scanner (Germany) were displayed at a width of 1580 HU and a level of –670 HU in the lung window and at a width of 360 HU and a level of 55 HU in the mediastinal window. Their scan parameters were standardised for equilibrium of data analysis.

Histology

Archived paraffin blocks belonging to the patients were sectioned at 4 microns and placed on polylysine glass slides. The Ki-67 antibody (Genetech, clone GM001) binding was detected using a bond polymer refining kit (Roche VENTANA). The grading was performed according to the staining diffusivity and intensity. The extent of the staining was categorised into five semi-quantitative classes based on the percentages of membrane positive tumour cells. Immunostaining was independently examined by two clinical pathologists who were unaware of the patient outcome.

Statistical analysis

Statistical analysis was performed with SPSS statistical software (Version 19.0; SPSS Inc., Chicago, IL, USA). χ^2 test and two-sided Fisher exact test were applied to determine the strength of association between the categorical variables. Receiver operating characteristic (ROC) curves were calculated to select the cut-off level of Ki-67 index and CT value indicating differentiation and lymph node metastasis. All tests were two sided with a *P*-value of less than 0.05 being considered statistically significant.

Results

A total of 116 eligible patients were enrolled. Baseline characteristics of the subjects were listed in Table 1.

CT image

At initial presentation (Table 1), the lung tissue abnormalities consisted of GGO nodules with little solid component (11/116), those with 'inhomogeneous air inflation' (17/116) and those tissues with a 'solid nodule/mass' (88/116). Lesion diameters were 0.4–5.0 cm (Median = 2.7 cm, Mean ± SEM = 2.88 ± 0.11 cm, IQR = 2.0 cm). A total of 79 lesions (79/116) were less than 3 cm (T1), the remaining cases (37/116) being more than 3 cm (T2). The CT value in the 116 cancer cases was ranged <0 to 93 (Median = 30, Mean ± SEM = 24.44 ± 1.71, IQR = 35). Patents presenting with GGO tissues with little solid component exhibited a negative CT value, inhomogeneous air inflation tissues demonstrated low CT value, whereas solid nodule/mass tissues had an increased CT value (Table 2, $p < 0.05$). Based on the median CT value, we divided the cases into three groups: <0, 0–30 and >30.

Clinical results

The statistical evaluation results of the relationship between the CT value of primary lesion and size, lymph node metastasis, Ki-67 index are shown in Figure 1, Table 2. No significant association was observed between CT value and size in the cases with CT value > 0. There was also no statistically significant relationship between age, gender and CT value. ROC curves were used to calculate the cut-off level of Ki-67 index and CT value indicating tumour differentiation and lymph node metastasis.

Table 1. Patients' clinical characteristics and CT findings of tumours.

Factor	Number
Age Mean (range)	63 (36–83)
Gender	
Male	63
Smoking	44
Female	53
Smoking	2
Co-morbidities	
Hypertension	27
Diabetes	16
COPD	13
Chronic hepatitis B	5
Size	
≦3 cm	79
>3 cm	37
Lymph node metastasis	
positive	38
negative	78
Differentiation	
Poor	47
Moderate-Well	69
CT image	
GGO	11
Heterogeneous density	17
Solid mass	88
Ki-67 index (median = 20%)	
≦20%	67
>20%	49

Notes. GGO, ground-glass opacity: COPD, chronic obstructive pulmonary disease.

Table 2. The relationship between the clinic parameters and CT value.

	Ki-67(%)*		Size(cm)**	
CT value (median = 30)	>20	≦20	>3	≦3
<0	3	27	0	30
0–30	15	23	15	23
>30	31	17	22	26

Note. The clinic parameters of subjects from the group with CT value > 30, 0–30 and <0 were comparable.
*$p < 0.05$, significant difference among three subgroups.
**$p = 0.66$, no significant difference between the groups with CT value > 30 and 0–30.

The optimal 'Ki-67 index cut-off' indicating differentiation and lymph node metastasis was found to be 12.50 and 9.00, respectively. Briefly, the data for sensitivity were found to be 95.7 and 97.4%, specificity was found to be 53.6 and 35.9%, respectively. The area under the ROC curves was 0.77 (CI 95% 0.69–0.86) and 0.69 (CI 95% 0.60–0.79), respectively. The positive predictive value and negative predictive value of 'Ki-67 index cut-off' indicating differentiation were 94.9 and 58.4%, respectively. The positive predictive value and negative predictive value of 'Ki-67 index cut-off' indicating lymph node metastasis were 42.5 and 96.5%, respectively.

The optimal 'CT value cut-off' indicating differentiation and lymph node metastasis was found to be 26.5 and 30.5, respectively. The data for sensitivity were found to be 89.4 and 68.4%, specificity was found to be 68.1 and 71.8%. The area under the ROC curves was 0.81 (CI 95% 0.73–0.89) and 0.72 (CI 95% 0.62–0.81), respectively. The positive predictive value and negative predictive value of 'CT value cut-off' indicating differentiation were 72.9 and 60.9%, respectively. The positive predictive value and negative predictive value of 'CT value cut-off' indicating lymph node metastasis were 54.2 and 82.3%, respectively.

Furthermore, the combination of 'Ki-67 index cut-off' and 'CT value cut-off' indicating differentiation and lymph node metastasis was also analysed. The sensitivity was found to be 40.6 and 34.6%, specificity was found to be 97.9 and 100%. The area under the ROC curves was 0.69 (CI 95% 0.59–0.79) and 0.67 (CI 95% 0.58–0.77), respectively. The positive predictive value and negative predictive value of 'combination' indicating differentiation were 96.5 and 75.9%, respectively. The positive predictive value and negative predictive value of 'combination' indicating lymph node metastasis were 75.9 and 100%, respectively.

Taken together, the CT value appeared to be significantly associated with differentiation and lymph node metastasis upon combination to analysis of Ki-67 index. It was suggested that more patients with well-differentiated cancer and without lymph node metastasis had a lower CT value and Ki-67 index, whilst patients with peripheral lung adenocarcinoma having high CT value

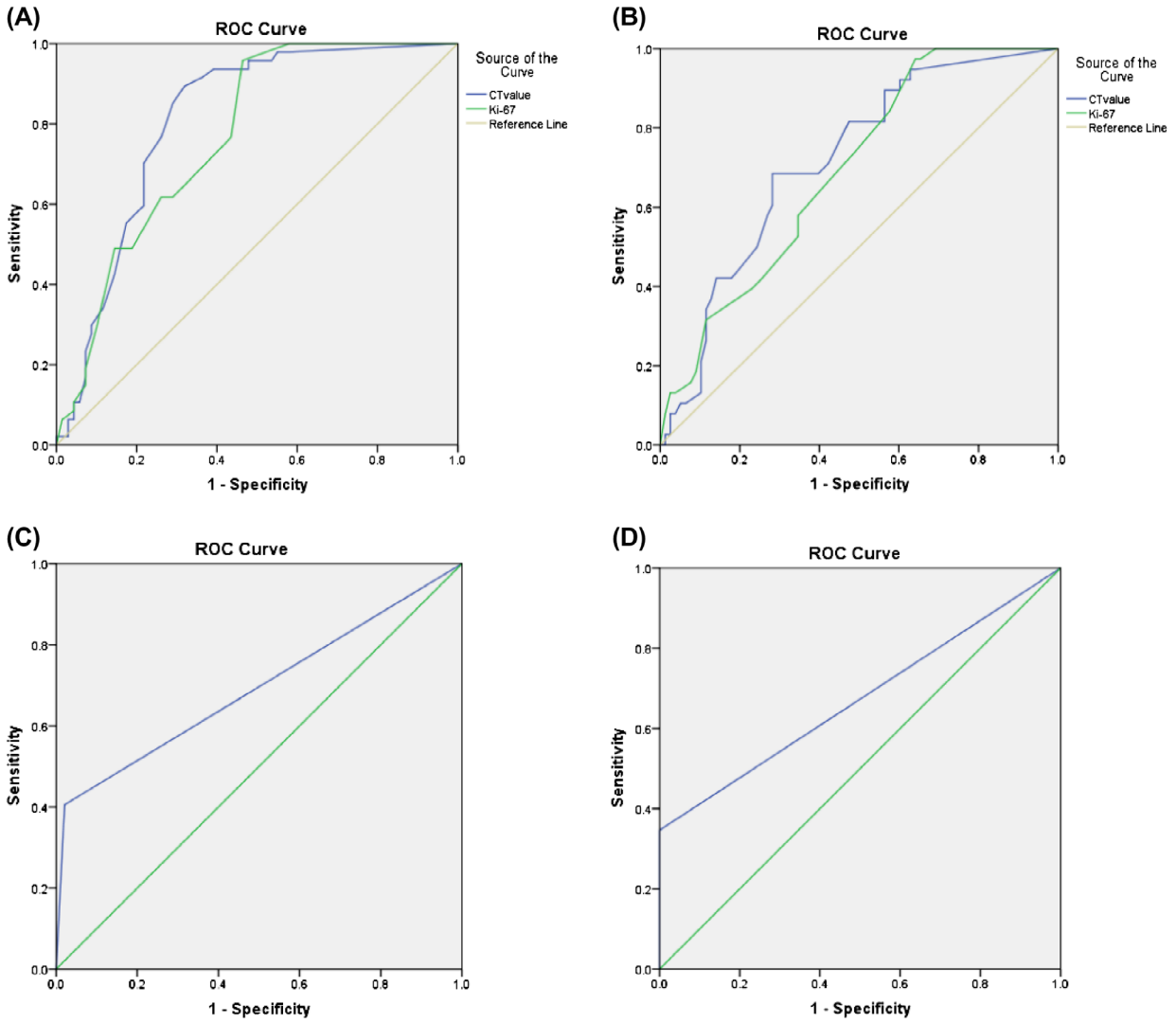

Figure 1. (A and B) the optimal 'Ki-67 index cut-off' indicating tumour differentiation (left) and lymph node metastasis (right) of lung adenocarcinoma, (C and D) the combination of 'Ki-67 index cut-off' and 'CT value cut-off' indicating differentiation (left) and lymph node metastasis (right) of lung adenocarcinoma.

and Ki-67 index exhibited poor-differentiated and metastatic potential.

CT value and cancer cell proliferation

Ki-67 positivity was present in 115 (99.9%) of the 116 evaluable primary lung cancers. The median of Ki-67 index among the cases was 20%. Based on the chi-square test, there were statistically significant relationships between the Ki-67 index and CT value (Table 2, $p < 0.05$), which suggested that the patients with lower Ki-67 index have a lower CT value and possess weak proliferative potential (Figures 2 and 3).

Discussion

The relationship between the positron emission tomography (PET-CT) parameters and biomarkers has been studied and correlated with tumour characterisation, such as its prediction of therapeutic response and prognostic implications in malignant pleural mesothelioma and a relationship of tumour glucose metabolism with NSCLC stage.[9,10] The CT and FDG-PET/CT are often part of routine diagnostic procedures of lung cancer, whose role had been to strengthen the clinical application of screening risk of lung cancer. Lee et al. have suggested that increased heterogeneity (higher entropy, lower uniformity) is associated with malignancy in non-small cell lung cancer, colorectal cancer and renal cell cancer.[11] Another study shows that anaplastic lymphoma kinase (*ALK*)-rearranged lung adenocarcinoma was absent of speculation on CT. Three-fourths of resected ground-glass nodules (GGN) tissue sections were positive for *EGFR, KRAS, ALK* or *HER2* mutations.[12] Quadruple-negative tumours were associated with a lack of GGN growth, whereas *EGFR* mutation-positive tumours displayed a correlation with growth.[13] These findings support the view that it is important to make full use of the CT scanning in the treatment of lung cancer.

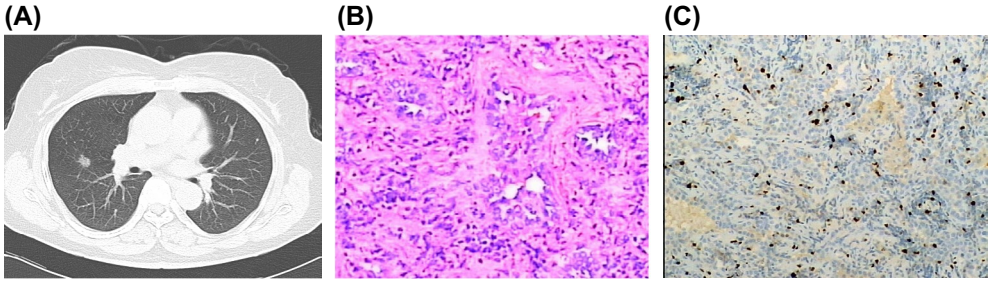

Figure 2. Pulmonary Ground-Glass Opacity Nodules in a 53-year-old woman. (A) CT scan shows opacity with ground-glass in the right lung, (B) Photomicrograph (HE) shows lepidic predominant adenocarcinoma with high differentiation, (C) IHC shows a low Ki-67 index.

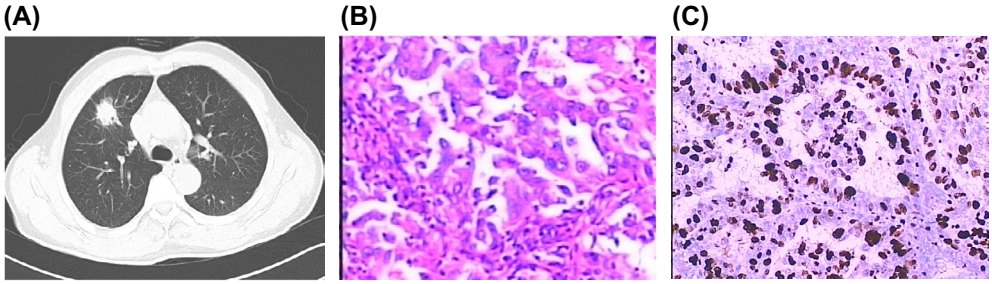

Figure 3. Adenocarcinoma with solid mass in a 60-year-old man. (A) CT scan shows a 3-cm solid mass in the right lung, (B and C) This poorly differentiated adenocarcinoma shows a high level of Ki-67 (55%).

According to the natural chronologic evolution of a lung cancer, it is generally accepted that as a GGO tissue increases in size, then the solid portion within the lesion tends to appear, and finally the solid portion increases in size and volume.[14] In this present study, the data showed that the CT value correlates with Ki-67 expression. Furthermore, the percentage of lesions that had GGO or localised air bronchogram was significantly greater in low CT value group, whereas solid nodule/mass lesions had a higher CT value. We also demonstrated that the CT value appears to be associated with differentiation and lymph node metastasis of lung adenocarcinoma. Taken together, along with an increasing CT value of SPN, lesions that appear as GGO with low CT value are likely to be to be poorly differentiated and invasive adenocarcinoma.

Ki-67 correlates with the proliferative activity of lung cancer cell and with FDG uptake. Among molecular markers, Ki-67 was linked with tumour doubling, and with increasing tumour size and progression. Here, the Ki-67 index could be used as a parameter of the characterisation of tumour malignancy *in vivo*. Analysis of Ki-67 index can also help distinguish invasive adenocarcinoma from preinvasive or minimally invasive adenocarcinoma with high sensitivity. Furthermore, in adding the CT value, Ki-67 index possessed higher specificity to predict the differentiation and lymph node metastasis of peripheral lung adenocarcinoma.

However, our study has several limitations. For, example, long-term follow-up was not conducted. Long-term follow-up regarding the influence of CT value and Ki-67 expression could provide a more detailed analysis of the recurrence rate and mortality. Taken together, it is believed that combination of CT value and Ki-67 index analysis can be used to evaluate the potential of differentiation, metastasis and carcinogenesis of lung adenocarcinoma, which might assist in recognition of disease progression and thus help in risk stratification and early intervention.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was supported by Project of Department of Health of Jiangsu Province [grant number H201208]; Natural Science Foundation of Jiangsu Province University [grant number 13KJB320021]; Jiangsu Provincial Special Program of Medical Science [grant number BL2012023]; Medical center of Suzhou (2016).

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