Stage I lung adenocarcinoma: the value of quantitative CT in differentiating pathological subtypes and predicting growth of subsolid nodules

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Abstract
The aim of this study was to investigate feasibility of quantitative computed tomography (CT) measurements in predicting invasiveness and growth of nodular ground glass opacities (nGGOs).

A set of 203 patients (group A) with nGGOs that were confirmed stage-I adenocarcinomas and 79 patients (group B) with nGGOs that were completely followed up were included. Lesions diameters, volume (VOL), maximum (MAX), mean (MEN), and standard deviation (STD) of CT attenuation were measured. P53 labeling index (LI) was evaluated through immunohistochemistry in group-A patients. Multivariate linear stepwise regressions were performed based on group-A lesions to calculate P53-LI prediction from CT measurements. The receiver operating characteristic (ROC) curve analyses were performed to assess the performance of P53-LI prediction in predicting invasiveness and growth of nGGOs. The Cox regression analysis was conducted to identify correlation between P53-LI prediction and volume doubling time (VDT) of lesions in group B.

Diameter, VOL, MEN, STD, and the P53 LI showed significant differences between lesions of different pathological invasiveness (P < .01). By multivariate linear regressions, MEN and STD were identified as independent variables indicating P53 LI (P < .001); thus, an equation was established to calculate P53-LI Prediction: P53LI Prediction = 0.013 × MEN + 0.024 × STD + 0.741 (R square = 0.411, P < .001). The P53-LI Prediction showed good performance, similar as the actual one, in differentiating pathological invasiveness of nGGOs. In addition, the P53-LI Prediction demonstrated excellent performance in predicting growth of nGGOs (AUC = 0.833, P < .001) and independently forecasted VDT of nGGOs (β = 1.773, P < .001).

The P53-LI Prediction that was calculated from preoperative quantitative CT measurements of nGGOs indicates lesions’ invasiveness and allows for predicting growth of nGGOs.

Abbreviations: AIS = adenocarcinoma in situ, CT = computed tomography, INV = nonlepidic-predominant adenocarcinoma, LI = labeling index, LPA = lepidic-predominant adenocarcinoma, MAX = maximum CT attenuation, MEN = mean CT attenuation, MIA = minimally invasive adenocarcinoma, nGGOs = nodular ground glass opacities, STD = standard deviation of CT attenuation, VDT = volume doubling time, VOL = volume.

Keywords: adenocarcinoma, ground glass opacity, high-resolution computed tomography, lung cancer, P53

1. Introduction
Subsolid nodules, also known as nodular ground glass opacities (nGGOs), are challenging, given their poorly defined margins, heterogeneous internal features, and varying attenuations.[1,2] Pathologies of nGGOs are commonly associated with lung adenocarcinomas, whose classification were recently revised by the International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS), aiming to provide more specific diagnosis and better stratification.[3–6] Pathologic differentiations of nGGOs have been imperative and attractive for thoracic surgeons, as different subtypes necessitate different treatments and will present with distinctive prognosis.[7–9]

The p53 suppressor gene plays a critical role in tumor genesis and progression. Immunohistochemical assessment of mutant P53 has been commonly used in clinical diagnosis and prognosis of lung cancer.[7–9] However, the P53 labeling can only be obtained invasively either from operation or from biopsy. The evaluation of nGGOs may benefit particularly from computed tomography (CT), which has contributed to an increased detection of lung cancer at earlier and more curable stages.[10]

Many CT characteristics of nGGOs have been proposed for differentiating benign or indolent nGGO from malignant and invasive one, such as the diameter, volume, mass, attenuation, and heterogeneity, etc.[11–13] Understanding the correlation between quantitative CT parameters and histologies of nGGOs may enable noninvasive characterization of suspected primary lung adenocarcinoma and may aid in decision as to whether lung
resection is undertaken. Therefore, it is important to obtain an index that reflects both radiological and pathological characteristics of invasive nGGOs, in order to identify optimal target population.

In this study, we first established a prediction model incorporating parameters from CT image with immunohistochemical P53 labeling for assessing pathological invasiveness of nGGOs based on a set of patients who had experienced operations. Then, we tested the prognostic value of this model in another set of GGO patients who had been followed up for 5 years.

2. Methods

2.1. Patient selection

This study was approved by the Institutional Research Board. Informed consents were obtained from all patients included. First, for establishing prediction model of P53 labeling index (LI), we retrospectively selected 203 patients (group A) with single nGGO who were resected and pathologically confirmed stage-I adenocarcinomas between January 01, 2010, and May 30, 2016, from the lung cancer registration system of our hospital. Chest high-resolution CT (HRCT) images that were closest to the date of tumor resection were retrieved. Of the 203 patients, 74 were men and 102 were women. The mean age of entire cohort was $32.1 \pm 7.2$ years (standard deviation, SD). The mean interval between the date of the closest preoperative HRCT study and the date of operation was $13.7 \pm 6.2$ days.

Then, we enrolled another set of 79 patients (group B) with single nGGO who did not experience operation but were consequentially followed up for 5 years to test the prediction model. We closed the follow-up work when patient met anyone of the following: 1) lesion’s volume doubled, 2) death or imperative operation was done, 3) came to the end of study, 4) lost to follow up. Follow-up CT examinations were reviewed.

2.2. Chest CT acquisition and nodule analysis

All chest CT images were obtained with a 16-detector-row (Somatom Sensation 16; Siemens, Forchheim, Germany) CT scanner using the following parameters: $120$ kV, $100$ mA, collimation of $16 \times 0.75$ mm, beam pitch of $0.7$, and gantry rotation time of $0.5$ s. Raw data were reconstructed for HRCT with thickness of $1$ mm, interval of $0.75$ mm, and a bone algorithm for reconstruction of lung.

After a training session of $5$ cases, a radiological resident and a 12th-year postgraduate radiological student independently performed measurements of nGGOs, blinding to the pathological diagnosis. According to established method,[14] a series of regions that could predict lesions of nGGOs by using receiver operating characteristic curve (ROC) analyses. On the basis of data of group-B patients, the methodology was used for analyzing interobserver agreements between the 2 observers, with values greater than $0.75$ indicating good agreement.[20] All statistical analyses were performed using SPSS 20 (IBM SPSS Inc., Chicago, IL) based on mean values of each measurement obtained by the 2 observers, with a 2-sided $P$ value of $<0.05$ indicating statistical significance. Intergroup differences were analyzed by using the 1-way analysis of variance (ANOVA) with post-hoc test being performed by using the Kruskal–Wallis test. To establish equation for calculating P53-LI Prediction, multivariate stepwise linear regressions were conducted. Then, the P53-LI Prediction was compared with actual P53-LI in relation to their capacities in predicting invasiveness and growth of nGGOs by using receiver operating characteristic curve (ROC) analyses. On the basis of data of group-B patients, the interobserver agreements were excellent for automated measurements between the 2 observers with the ICC ranging from $0.811$ to $0.912$. All CT parameters of nGGOs showed

2.3. Pathological diagnosis and P53-LI evaluation of lesions in group-A patients

According to the newly introduced IASLC/ATS/ERS classification,[13] lesions’ pathological sections of group-A patients were reviewed by a pulmonary pathologist with more than 5 years of experience, blinding to imaging findings. First, the specimens were categorized as adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), lepidic-predominant adenocarcinoma (LPA), acinar, papillary/micropapillary, solid, and invasive adenocarcinoma. Then, AIS and MIA (AIS/MIA) were grouped together, while LPA, acinar, papillary/micropapillary, and solid histologic subtypes that had relatively high-grade patterns of invasion compared with AIS/MIA were grouped together as LPA for analysis. The invasive subtypes were considered as 1 group of invasive nonlepidic-predominant adenocarcinoma (INv).

Then, immunostaining with the antibody for P53 was performed on specimens of group-A patients according to established methodology.[17] The P53-positive nuclei were stained brown. An Olympus BX 50 microscope equipped with a cannon camera (Olympus, Tokyo, Japan) was used for microscopy and image capturing. Percentage of positive cells was quantitatively calculated by using Image Pro Plus 6.0 (Media Cybernetics, Washington, DC; Fig. 1), which has been widely used in biomedicine.[17–19]

2.4. Statistical analysis

The intraclass correlation coefficients (ICCs) were calculated for analyzing interobserver agreements between the 2 observers, with values greater than $0.75$ indicating good agreement.[20] All statistical analyses were performed using SPSS 20 (IBM SPSS Inc., Chicago, IL) based on mean values of each measurement obtained by the 2 observers, with a 2-sided $P$ value of $<0.05$ indicating statistical significance. Intergroup differences were analyzed by using the 1-way analysis of variance (ANOVA) with post-hoc test being performed by using the Kruskal–Wallis test. To establish equation for calculating P53-LI Prediction, multivariate stepwise linear regressions were conducted. Then, the P53-LI Prediction was compared with actual P53-LI in relation to their capacities in predicting invasiveness and growth of nGGOs by using receiver operating characteristic curve (ROC) analyses. On the basis of data of group-B patients, the multivariate Cox regression was conducted to identify independent variables that could predict lesions’ VDT.

3. Results

3.1. Basic, pathological information, and nodule measurements of group-A patients

Pathologically, $81$ lesions were diagnosed as AIS/MIA, while $78$ and $44$ lesions were, respectively, LEP and INv, in group-A patients. The differences of gender and age distributions among the pathological subtypes of nGGOs were not significant ($P > 0.05$, Table 1).

The interobserver agreements were excellent for automated measurements between the 2 observers with the ICC ranging from $0.811$ to $0.912$. All CT parameters of nGGOs showed
significant differences among pathological subtypes \((P < .01)\), except MAX, which failed to differentiate INV from LEP \((P_2 = .087; \text{Table 1})\).

### 3.2. Correlations between P53 LI and nodule measurements on CT

The multivariate linear stepwise regressions identified that MEN and STD were independent variables predicting P53 LI \((P \text{ values all } < .001)\), with STD having the largest standardized \(\beta\)-coefficient \(0.381\) indicating the largest power in predicting P53 LI, as summarized in Table 2. However, diameter, VOL, and MAX \((P \text{ values all } > .05)\) were excluded from the final model of the stepwise regressions, which means that they were not independent factors predicting P53 LI. Consequently, an equation for calculating P53-LI Prediction was established as follows:

\[
P53\text{LI Prediction} = 0.013 \times \text{MEN} + 0.024 \times \text{STD} + 9.741 
\]

\(R \text{ square} = 0.411, P < .01\).

### Table 1

| Variables | AIS/MIA | LEP | INV | P | P1 | P2 | ICC |
|-----------|---------|-----|-----|---|----|----|-----|
| Gender    |         |     |     |   |    |    |     |
| Male      | 40      | 34  | 15  | .258 | NA | NA | NA |
| Female    | 41      | 44  | 29  |    |    |    |     |
| Age, y    | 51.0 ± 8.6 | 53.1 ± 8.2 | 51.8 ± 7.6 | .267 | NA | NA | NA |
| CT measurements | | | | | | | |
| Diameter, mm | 13.1 ± 4.8 | 16.0 ± 6.3 | 21.0 ± 6.3 | <.001 | .004 | .001 | .841 |
| VOL, mm\(^3\) | 607.8 ± 394.7 | 1078.9 ± 601.1 | 1635.6 ± 548.3 | <.001 | <.001 | .002 | .862 |
| MAX, HU    | −79.8 ± 200.0 | 60.1 ± 127.8 | 177.8 ± 325.7 | <.001 | <.001 | .001 | .811 |
| MEN        | −464.0 ± 41.5 | −427.4 ± 40.7 | −401.9 ± 44.3 | <.001 | <.001 | .001 | .877 |
| STD        | 112.2 ± 30.6 | 153.1 ± 36.5 | 181.8 ± 33.4 | <.001 | <.001 | .001 | .912 |
| P53 LI (%) | 5.4 ± 2.0 | 8.0 ± 1.9 | 10.5 ± 1.6 | <.001 | <.001 | .001 | NA |
| P53 LI Prediction (%) | 6.1 ± 1.0 | 7.6 ± 0.9 | 8.7 ± 1.2 | <.001 | <.001 | .001 | NA |

Unless otherwise indicated, numerical variables were recorded as mean ± standard deviation. Age, MEN, STD, P53 LI, and P53 LI Prediction were analyzed by 1-way ANOVA analysis and least significant difference (LSD) test. Diameter, VOL, and MAX were analyzed by Kruskal–Wallis test and Tamhane T2 test. P indicates the P values for 1-way ANOVA or Kruskal–Wallis analyses of all nGGOs; P1 indicates the P values for LSD test or Tamhane T2 test of AIS/MIA vs LEP; P2 indicates the P values for LSD test or Tamhane T2 test of LEP vs INV. AIS = adenocarcinoma in situ, HU = Hounsfield unit, ICC = intraclass coefficients, INV = invasive adenocarcinoma, LEP = lepidic-predominant adenocarcinoma, LI = labeling index, MAX = maximum, MEN = mean, P53 LI = P53 LI, VOL = volume.
The P53-LI Prediction was calculated as $6.1 \pm 1.0$ for AIS/MIA lesions, $7.6 \pm 0.9$ for LEP lesions, and $10.5 \pm 1.6$ for INV lesions with significant intergroup differences ($P < .001$; Table 1). Furthermore, we compared the diagnostic performance of P53-LI Prediction with the actual one by comparing their AUCs (Fig. 2). The P53-LI Prediction showed a little higher AUCs than the actual P53 LI either in differentiating LEP from AIS/MIA ($AUC = 0.870$ vs $0.831$, $P = .361$; Fig. 2A) or in differentiating INV from LEP ($AUC = 0.846$ vs $0.762$, $P = .127$; Fig. 2B), although not statistically significant. The optimal thresholds of P53-LI Prediction for differentiating LEPs from AIS/MIA and for differentiating INV from LEPs were 6.6% and 8.0%, respectively, with corresponding sensitivity being 87.2% and 81.8%, and specificity being 72.8% and 75.6%, respectively (Fig. 2).

### 3.3. Follow-up, demography, and nodule evaluation of patients in group B

In patients from group B, 38 (55.1%) were male and 31 (44.9%) were female, with age being 51.9 ± 7.99 years (mean ± SD). There was no significant difference in sex ratio or age distribution between stable and growing lesions ($P = .689$ and .063, respectively; Table 3). During follow-up, 8 (10.1%) cases were censored because of imperative operation ($n = 3$), lost in follow-up ($n = 2$), and death ($n = 3$). Finally, 61 patients were successfully followed up, with a follow-up rate of 89.9%. Twenty nGGOs (29.0%) were stable during the 5-year period, while 49 (71.0%) lesions showed a growing pattern. Of the 49 patients with growing nGGOs, 7 cases were closed to follow-up before the end of the 5-year period because of imperative operation ($n = 3$) and death ($n = 2$). Three cases failed to determine VDT. Eventually, 39 nGGOs presented with doubling volumes during the period of follow-up, with VDT being 27.6 ± 19.37 (Table 3).

The growing lesions showed significantly larger initial VOL, MAX, MEN, and STD than the stable ones ($P < .05$), while initial diameter was not significantly different between stable and growing lesions ($P = .102$, Table 3). On the basis of the equation established above, the P53-LI Prediction calculated were significantly higher in growing lesions than in stable lesions ($P < .001$, Table 3). The ROC analysis showed that the AUCs of nGGOs' initial diameter, VOL, MAX, MEN, STD, and P53-LI Prediction were $0.612, 0.666, 0.753, 0.729$, and $0.833$, respectively, in predicting lesions growth. Obviously, the P53-LI Prediction owned the best prediction performance.

In addition, we performed stepwise Cox regression analysis to identify relationships between initial CT measurements of nGGOs and their VDT obtained during the follow-up period. Results are summarized in Table 4. Only Ki67-PI Prediction was an independent variable that could forecast VDT of growing nGGOs ($\beta = 1.773$, $P < .001$). In lesions with similar characteristics with respect to the variables analyzed, a 1% increase of P53-LI Prediction forecasts a 5.888-fold increase of probability that nGGOs will double in volume within 5 years.

### 4. Discussion

In this investigation, the quantitative CT measurements of subsolid nodular lung adenocarcinoma discriminated 3 cohorts of IASLC/ATS/ERS-classified lesions, and were significantly correlated with the postoperative immunohistochemical P53 LI. Furthermore, calculated from these CT measurements, the P53 LI Prediction even showed a slightly higher diagnostic performance, although not statistically significant, than the actual P53 LI in differentiating pathological subtypes of nGGOs.
In recent years, advances in imaging technology have promoted a more precise and quantitative pattern in assessing nGGOs than traditional 1-dimensional or 2-dimensional methods. Hoop et al. [24] reported a similar percentage (47.8%, 33/69) of GGO lesions presented with a slow rate of growth (<2 mm per year). In this study, a similar percentage (47.8%, 33/69) of GGO lesions presented with a slow rate of growth (<2 mm per year). [24] In this study, a similar percentage (47.8%, 33/69) of GGO lesions presented with a slow rate of growth (<2 mm per year).

The 5-year disease-free survival rates of acinar- and papillary-predominant forms have been reported to be 84% and 83%, respectively, while those of micropapillary-solid-predominant adenocarcinomas have been reported to be 67% and 70%, respectively. [5] Therefore, it is necessary to develop a prediction model that can differentiate malignant or invasive nGGO from benign one in order to identify optimal target population for treatment.

The findings of the present study have great significance. As the multidisciplinary IASLC/ATS/ERS classification system was issued in 2011 in response to the need to better classify lesions and differentiate patients with varying survivals, pathological differentiation of nGGOs has been imperative and attractive for thoracic surgeons. [5,23,24] AIS comprises only lepidic noninvasive growth. MIA and LPA are also predominantly lepidic but have invasive foci, which is less than 5 mm for MIA and larger than 5 mm for LPA. [5] AIS, MIA, and LPA have 5-year disease-free survival rates of 100%, near 100%, and 90%, respectively. [5,23] The 5-year disease-free survival rates of acinar- and papillary-predominant forms have been reported to be 84% and 83%, respectively, while those of micropapillary-solid-predominant adenocarcinomas have been reported to be 67% and 70%, respectively. [5] Therefore, it is necessary to develop a prediction model that can differentiate malignant or invasive nGGO from benign one in order to identify optimal target population for treatment.

In this study, a similar percentage (47.8%, 33/69) of GGO lesions presented with a slow rate of growth (<2 mm per year). [24] In this study, a similar percentage (47.8%, 33/69) of GGO lesions presented with a slow rate of growth (<2 mm per year).

Several limitations of this study should be mentioned. First, the P53-LI Prediction was a virtual index that was calculated from MEN and STD of nGGOs. Therefore, its practicability needs to be confirmed. Second, we just established a prediction model from data of patients from group A, while tested it in another set of patients (group B). Thus, the result of this study might have suffered from impact of inter-patient variations. Researches that are performed with only 1 set of patients who have been fully followed-up to surgeries for establishing and testing the prediction model within only 1 patient group are needed. Third, this study was performed in a single institution with limited case sample. Thus, the result should only be explained within this study.

### Table 3

Demographic, radiological, and follow-up information of nGGOs from group-B patients (n=69).

| Variables                  | Stable | Growing |
|----------------------------|--------|---------|
| Gander                     |        |         |
| Male                       | 19     | 19      |
| Female                     | 14     | 17      |
| Age, y                     | 49.5±6.63 | 53.2±6.95 | .063 |
| Initial CT measurements    |        |         |
| Diameter, mm               | 14.0±6.61 | 16.9±6.66 | .102 |
| VOL, mm†                   | 714.4±618.61 | 1184.3±740.71 | .015 |
| MAX, HU                    | −103.8±194.52 | 90.7±215.87 | .001 |
| MEN                        | −471.0±35.58 | −424.9±42.14 | <.001 |
| STD                        | 111.6±42.79 | 156.6±40.91 | <.001 |
| P53 LI Prediction (%)      | 5.9±1.12 | 7.8±1.29 | <.001 |
| Volume-doubled lesions     | 0      | 39      |
| Doubling time, mo          | NA     | 27.6±19.37 |

| Variables                  | β-coefficients | SE  | Wald | HR (exp (β)) | P      |
|----------------------------|----------------|-----|------|--------------|--------|
| P53 LI Prediction          | 1.773          | 0.268 | 43.718 | 5.888        | <.001  |

Unless otherwise indicated, numerical variables were recorded as mean±standard deviation. IU=Hounsfield Unit, LI=labeling index, MEN and STD denote mean, mean, and standard deviation of CT attenuation within ground-glass opacity nodules, respectively. NA = not associated, VOL = volume. † Chi-square test. ‡ Student t test.
In conclusion, the P53-LI Prediction that was calculated from preoperative quantitative CT measurements of nGGOs indicates lesion invasiveness and allows for predicting growth of nGGOs.

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