Diffusion kurtosis imaging: correlation analysis of quantitative model parameters with molecular features in advanced lung adenocarcinoma

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Abstract

Background: Due to development of magnetic resonance-based functional imaging, it is easier to detect micro-structural alterations of tumor tissues. The aim of this study was to conduct a preliminary evaluation of the correlation of non-Gaussian diffusion kurtosis imaging (DKI) parameters with expression of molecular markers (epidermal growth factor receptor [EGFR]; anaplastic lymphoma kinase [ALK]; Ki-67 protein) in patients with advanced lung adenocarcinoma, using routine diffusion-weighted imaging as the reference standard.

Methods: Data from patients with primary lung adenocarcinoma diagnosed at Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS) from 2016 to 2019 were collected for retrospective analysis. The pathologic and magnetic resonance imaging data of 96 patients who met the inclusion criteria were included in this study. Specifically, the $K_{app}$ and $D_{app}$ parameters measured from the DKI model; apparent diffusion coefficient (ADC) value from the diffusion-weighted imaging model; and the $EGFR$, $ALK$, and Ki-67 biomarkers detected by immunohistochemistry and/or molecular biology techniques after biopsy or surgery were evaluated. The relations between quantitative parameters (ADC, $K_{app}$, $D_{app}$) and pathologic outcomes ($EGFR$, $ALK$, and Ki-67 expression) were analyzed by Spearman correlation test.

Results: Of the 96 lung adenocarcinoma lesions (from 96 patients), the number of $EGFR$- and $ALK$-positive and high Ki-67 expressing lesions were 53, 12, and 83, respectively. The $K_{app}$ values were significantly higher among patients with $EGFR$-positive mutations ($0.81 \pm 0.12$ vs. $0.66 \pm 0.10$, $t = 6.41$, $P < 0.001$), $ALK$ rearrangement-negative ($0.76 \pm 0.12$ vs. $0.60 \pm 0.15$, $t = 4.09$, $P < 0.001$), and high Ki-67 proliferative index (PI) ($0.76 \pm 0.12$ vs. $0.58 \pm 0.13$, $t = 4.88$, $P < 0.001$). The $D_{app}$ values were significantly lower among patients with high Ki-67 PI ($3.19 \pm 0.69 \mu m^2/ms$ vs. $4.20 \pm 0.83 \mu m^2/ms$, $t = 4.80$, $P < 0.001$) and $EGFR$-positive mutations ($3.11 \pm 0.73 \mu m^2/ms$, $t = 3.59$, $P = 0.002$). The differences in mean $D_{app}$ ($3.73 \pm 1.26 \mu m^2/ms$ vs. $3.26 \pm 0.68 \mu m^2/ms$, $t = 1.96$, $P = 0.053$) or ADC values ($1.19 \pm 0.37 \times 10^{-3} \mu m^2/s$ vs. $1.50 \pm 0.53 \times 10^{-3} \mu m^2/s$, $t = 3.38$, $P = 0.001$) and high Ki-67 PI ($1.28 \pm 0.39 \times 10^{-3} \mu m^2/s$ vs. $1.67 \pm 0.77 \times 10^{-3} \mu m^2/s$, $t = 2.88$, $P = 0.005$). $K_{app}$ was strongly positively correlated with $EGFR$ mutations ($r = 0.844$, $P = 0.008$), strongly positively correlated with Ki-67 PI ($r = 0.882$, $P = 0.001$), and strongly negatively correlated with $ALK$ rearrangements ($r = -0.772$, $P = 0.001$). $D_{app}$ was moderately correlated with $EGFR$ mutations ($r = -0.650$, $P = 0.024$) or Ki-67 PI ($r = -0.734$, $P = 0.012$). ADC was moderately correlated with Ki-67 PI ($r = -0.679$, $P = 0.033$).

Conclusions: The $K_{app}$ value of DKI parameters was strongly correlated with different expression of $EGFR$, $ALK$, and Ki-67 in advanced lung adenocarcinoma. The results potentially indicate a surrogate measure of the status of different molecular markers assessed by non-invasive imaging tools.

Keywords: Lung adenocarcinoma; Magnetic resonance imaging; Diffusion kurtosis imaging; Epidermal growth factor receptor; Anaplastic lymphoma kinase; Ki-67 protein
Introduction
Lung cancer remains the leading cause of cancer-related mortality worldwide. The 5-year overall survival rate for all patients diagnosed with lung cancer is relatively low, about 15% to 20% regardless of the tumor stage and treatment received.[1-3] The recognition of specific molecular alterations in certain lung cancer sub-types, has facilitated tailored therapy and ushered in the era of “personalized” oncologic practice in the last decade.[4] Within the family of lung carcinomas, the molecular foundation of lung adenocarcinoma is currently best understood; approximately 60% of all lung adenocarcinomas have an oncogenic driver mutation that, in many cases, predicts treatment response and correlates with certain clinicopathologic features.[5,6]

As for the imaging analysis of lung adenocarcinoma, computed tomography (CT) usually constitutes the first modality in evaluation and staging; other functional modalities such as positron emission tomography-CT or thoracic magnetic resonance imaging (MRI) have emerged to be important supplementary tools.[7] Diffusion-weighted imaging (DWI), one of the widely applied functional MRI techniques, has shown potential for improved cancer detection, prediction of cancer aggressiveness, and evaluation of pathologic sub-types.[8] However, a limitation of DWI is that it works on the assumption that water diffusion is Gaussian in behavior, which is unlikely to be the case in micro-structurally complex tissues.[9] In such tissues, diffusion kurtosis imaging (DKI), a more recently described non-Gaussian technique, potentially better reflects water diffusivity in tissues with ultrahigh b values. The DKI model is sensitive to deviations of tissue diffusion from a Gaussian pattern and has been shown to be robust for parameter quantification, in addition to being more accurate to assess micro-structural complexity in a tissue than conventional DWI.[10] Thus, the application of DKI has been successfully investigated in previous diffusion studies involving various human organs.[11-14]

In the current study, we evaluated the correlation of DKI parameters with the status of micro-structural molecular markers such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and Ki-67 protein to investigate the link between non-invasive imaging parameters and clinicopathologic features, with the aim of providing more valuable information to increase the accuracy of detection, staging, and treatment monitoring for patients with advanced lung adenocarcinoma.

Methods
Ethical approval
This study was approved by the Institutional Review Board of the Cancer Institute & Hospital, Chinese Academy of Medical Sciences (No. NCC2017ZDXM-001) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Patients
Between July 2016 and June 2019, a total of 157 consecutive patients diagnosed with lung adenocarcinoma who underwent MRI examination at the Department of Diagnostic Radiology, Cancer Hospital, Chinese Academy of Medical Sciences were included in this study. MRI examinations were performed to evaluate and preoperatively stage these lesions, and the DKI sequence was used for clinical application along with routine conventional MRI. The following inclusion criteria were applied in this study: (1) patients with tumors that were histopathologically confirmed as primary lung adenocarcinomas by subsequent resection or biopsy; (2) patients did not receive any therapy or surgery prior to MRI examination; and (3) patients who underwent routine MRI, DWI, and DKI in the same scanner. The following exclusion criteria were applied: (1) patients with lesions that showed ground-glass opacity (GGO) on MRI; and (2) patients with lesions smaller than 2 cm. Finally, a total of 96 patients (42 males and 54 females) with histopathologically confirmed primary lung adenocarcinoma were enrolled in this retrospective study for data analysis.

Imaging protocol
All MRI scans were performed on a 3.0-T whole-body scanner equipped with a 32-channel coil (Discovery MR750; GE Healthcare, Milwaukee, WI, USA). The routine lung imaging protocol included the following sequences: axial propeller T2-weighted imaging with fat suppression (T2WI/FS), axial fast spin echo T1-weighted imaging, DWI, and DKI. DWI was obtained by using respiratory-gated, single-shot, spin-echo, echo-planar technology with b values of 0, 800 s/mm². DKI was performed in the axial plane with b values of 0, 500, 1000, 1500, and 2000 s/mm². Details of scanning parameters are shown in Table 1. The scanning range of all sequences was 2 cm above and below the target lesion.

Quantitative image analysis
All images were successfully acquired. Subsequently, parametrical maps of apparent diffusion coefficient (ADC), D_app, and K_app were calculated on an offline workstation (GE Advantage Workstation AW4.6; GE Healthcare). The mean ADC was determined on the basis of the assumption of a mono-exponential relationship between signal intensity S₀ and b value in the DWI model:

\[ S(b) = S_0 \times \exp(-b \times ADC) \]

DKI model quantifies the non-mono-exponentiality of the diffusion by means of a second-order Taylor series expansion. To obtain D_app and K_app parameters, we applied the signal intensity data of five b values based on Rosenkrantz et al’s study,[10] using the following equation:

\[ S(b) = S_0 \times \exp(-bD_{app} + b^2D_{app}^2K_{app}/6) \]

Three quantitative parameters, based on the above calculation models, could be partitioned into two categories: diffusion coefficient (D_app, ADC) and kurtosis...
Fat suppression Spectral adiabatic inversion recovery Spectral adiabatic inversion recovery

tumor specimen, and Genomic deoxyribonucleic acid was extracted from the
ments, and Ki-67 protein expression status in all lesions.
and 21 were ampli
substantial signal loss.[15] The size for each lesion was
artifacts; or (b) the high signal barely visible owing to
reader before further parameter analysis. Inadequate DKI
the experience of interpreting DKI data. The imaging
Histologic analysis was performed, according to the 2015
values were automatically measured.

diffusion-weighted imaging; DKI: Diffusion kurtosis imaging.

Statistics analysis
Quantitative variables, tested with the Kolmogorov-
fraction-PCR was used to detect some other specific
fusions, for instance, echinoderm microtubule-
K5.

Table 1: Imaging protocol for DWI and DKI sequences in 157 patients with lung adenocarcinoma.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DWI</th>
<th>DKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>Single-shot echo planar</td>
<td>Single-shot echo planar</td>
</tr>
<tr>
<td>Orientation</td>
<td>Axial bilateral</td>
<td>Axial bilateral</td>
</tr>
<tr>
<td>Repetition time (ms)</td>
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<td>4200</td>
</tr>
<tr>
<td>Echo time (ms)</td>
<td>56</td>
<td>68</td>
</tr>
<tr>
<td>Voxel size (mm³)</td>
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<td>2.0 × 2.0 × 4.0</td>
</tr>
<tr>
<td>Fat suppression</td>
<td>Spectral adiabatic inversion recovery</td>
<td>Spectral adiabatic inversion recovery</td>
</tr>
<tr>
<td>Field of view (mm²)</td>
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<td>384 × 384</td>
</tr>
<tr>
<td>Matrix</td>
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<td>128 × 128</td>
</tr>
<tr>
<td>Section thickness (mm)</td>
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<td>4</td>
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<tr>
<td>No. of sections</td>
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<td>28</td>
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<tr>
<td>No. of signals acquired</td>
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<td>2</td>
</tr>
<tr>
<td>Bandwidth (kHz)</td>
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</tr>
<tr>
<td>Scanning time (mins)</td>
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<td>4:16</td>
</tr>
<tr>
<td>b values (s/mm²)</td>
<td>0, 800</td>
<td>0, 500, 1000, 1500, 2000</td>
</tr>
</tbody>
</table>

DWI: Diffusion-weighted imaging; DKI: Diffusion kurtosis imaging.

Pathological analysis
Histological analysis was performed, according to the 2015
World Health Organization Classification of Tumors of the
Lung and Pleura,[16] using tissue samples obtained at
the time of either surgical resection or image-guided
biopsies. In this study, 29 patients underwent surgical
resection of the lesion, 52 patients underwent thoraco-
oscopy surgery, and CT-guided transthoracic core-needle
biopsy of lung tumors was performed in 15 patients.

An experienced pathologist evaluated the pathologic sub-
types and results of EGFR mutations, ALK rearrange-
ments, and Ki-67 protein expression status in all lesions. Genomic
deoxyribonucleic acid was extracted from the
tumor specimen, and EGFR tyrosine kinase exons 19, 20,
and 21 were amplified by a nested polymerase chain
reaction (PCR) using specific primers. ALK rearrange-
ments were detected by means of fluorescence in situ
hybridization or reverse transcription-PCR. Samples were
deemed to be fluorescence in situ hybridization-positive if
more than 15% of scored tumor cells showed split ALK 5’
and 3’ probe signals or had isolated 3’ signals. Reverse

Statistics analysis
Quantitative variables, tested with the Kolmogorov-
Smirnov test for normality analysis and with the Levene
test for variance homogeneity analysis, were expressed as
mean ± standard deviation. Differences between these sub-
groups were compared by using independent-samples t
test. Categorical data were presented as counts and
percentages, and then sub-groups were analyzed by a Chi-
squared test or the Fisher’s exact test. Receiver operating
characteristic (ROC) curve analysis was performed to
calculate the area under the curve (AUC) to determine the
accuracy of ADC, Kapp, and Dapp in differentiating lesions
with different immunohistochemical expression. Sensitivity,
specificity, positive predictive value, and negative
predictive value were generated using the optimal cutoff
values. The diagnostic accuracy could be assessed
according to the AUC: excellent, 0.9 < AUC ≤ 1.0; good,
0.8 < AUC ≤ 0.9; fair, 0.7 < AUC ≤ 0.8; poor, 0.6 < AUC ≤
0.7; fail, 0.5 < AUC ≤ 0.6.[18]

The Spearman correlation analysis was used to evaluate
the association of imaging parameters with molecular
markers’ expression. A correlation coefficient (r) of 1.0
was deemed to indicate perfect correlation; 0.8 to 0.9,
strong correlation; 0.6 to 0.7, moderate correlation; 0.3 to
0.5, fair correlation; and lower than 0.3, poor or no
correlation.[19] All statistical analyses were carried out
using SPSS 23.0 for Windows (IBM SPSS Inc, Chicago, IL,
USA). Two-sided P values of <0.05 were considered statistically significant.

Results

General information

Ninety-six lung adenocarcinoma lesions from 96 patients were included with a mean size of 4.1 ± 3.2 cm (range 2.1–7.3 cm). The 96 patients included 42 males (43.8%) and 54 females (56.2%) with a median age of 66 years (44–82 years). Of these, 53 (55.2%) showed EGFR-positive mutations, 12 (12.5%) showed ALK rearrangements, and 83 (86.5%) showed high Ki-67 expression. In our study, patients with ALK rearrangements tended to be younger than those without (78.0% vs. 22.0%, χ² = 4.669, P = 0.028). The two groups displayed no significant difference in the mean size (4.0 ± 2.8 cm vs. 4.1 ± 3.1 cm, t = 1.116, P > 0.05). Two (2.1%) lesions showed both EGFR gene mutations and ALK gene rearrangements. The most histologic sub-types were lepidic and acinar adenocarcinomas (accounting for 25%, respectively), and rather late pathologic stages of the tumors were recorded (stage IIIA-IV).

Summary of kurtosis metrics

Table 2 summarizes the ADC, K<sub>app</sub>, and D<sub>app</sub> metrics in lesions with different immunohistochemical expression.

For the K<sub>app</sub> values, EGFR mutation-positive group was significantly higher than EGFR mutation-negative group (0.81 ± 0.12 vs. 0.66 ± 0.10, t = 6.41, P < 0.001); ALK rearrangement-negative group was significantly higher than ALK rearrangement-positive group (0.76 ± 0.12 vs. 0.60 ± 0.15, t = 4.09, P < 0.001); high Ki-67 expression group was significantly higher than low Ki-67 expression group (0.76 ± 0.12 vs. 0.58 ± 0.13, t = 4.88, P < 0.001).

For the D<sub>app</sub> values, EGFR mutation-negative group was significantly higher than EGFR mutation-positive group (3.59 ± 0.77 µm²/ms vs. 3.11 ± 0.73 µm²/ms, t = 3.12, P = 0.002); ALK rearrangement-positive group was not significantly different from ALK rearrangement-negative group (3.73 ± 1.26 µm²/ms vs. 3.26 ± 0.68 µm²/ms, t = 1.96, P = 0.053); low Ki-67 expression group was significantly higher than high Ki-67 expression group (4.20 ± 0.83 µm²/ms vs. 3.19 ± 0.69 µm²/ms, t = 4.80, P < 0.001).

For the ADC values, EGFR mutation-negative group was significantly higher than EGFR mutation-positive group (1.50 ± 0.33 × 10⁻³ mm²/s vs. 1.34 ± 0.31 × 10⁻³ mm²/s, t = 3.38, P = 0.001); ALK rearrangement-positive group was not significantly different from ALK rearrangement-negative group (1.34 ± 0.41 × 10⁻³ mm²/s vs. 1.19 ± 0.37 × 10⁻³ mm²/s, t = 0.53, P = 0.58); low Ki-67 expression group was significantly higher than high Ki-67 expression group (1.67 ± 0.77 × 10⁻³ mm²/s vs. 1.28 ± 0.39 × 10⁻³ mm²/s, t = 2.88, P = 0.005). Representative parametric maps are shown in Figures 1 and 2.

In patients with advanced lung cancer, according to the Spearman analysis, there was a strong positive correlation between K<sub>app</sub> and EGFR mutations or Ki-67 PI (r = 0.844, P = 0.008; r = 0.882, P = 0.001, respectively), and a strong negative correlation with ALK rearrangements (r = −0.772, P = 0.001). D<sub>app</sub> was moderately negatively correlated with EGFR mutations or Ki-67 PI (r = −0.650, P = 0.024; r = −0.734, P = 0.012), whereas ADC only had moderate negative correlation with Ki-67 PI (r = −0.679, P = 0.033). The correlations between D<sub>app</sub> and ALK rearrangements, ADC and EGFR mutations, ADC and ALK rearrangements, were not statistically significant (P = 0.137, 0.061, 0.612, respectively).

Table 3 summarizes the AUC for identification of lesions with different immunohistochemical findings for each of the metrics and the optimal thresholds for each of the metrics identified in the ROC analysis. With either scheme, K<sub>app</sub> had a mildly higher AUC for prediction of adverse final pathologic findings (AUC, 0.79–0.88) than ADC (AUC, 0.49–0.73) or D<sub>app</sub> (AUC, 0.60–0.86), and differences in performance between the metrics were not significant (P = 0.183, = 0.734, respectively). The ROC curves for the three classification schemes are depicted in Figure 3.
In this study, we found there was a strong positive/negative correlation between kurtosis coefficients ($K_{app}$) with these molecular markers’ status. Besides, the $K_{app}$ values in the EGFR mutation-positive group, ALK rearrangement-negative group, and high Ki-67 expression group were significantly higher than those in the control groups. Research shows $K_{app}$ represents a unitless parameter, larger $K_{app}$ indicates greater deviation from perfectly Gaussian diffusion behavior, and the $K_{app}$ parameter is likely associated with micro-structural complexity in vivo. When a genetic mutation arose in EGFR gene, it could have an impact on complicated micro-structures in biologic tissues such as membranes, myelin sheaths, and neural axons, leading to topologic rearrangement and complexity reflected by increased kurtosis and hence a higher $K_{app}$ value. As for the Ki-67 antigen, one of several cell cycle-regulating proteins, is proved to be associated with ribosomal RNA transcription, and numerous studies have suggested that tumor cells with high Ki-67 expression exhibited higher cellularity with nuclear atypia. Conversely, tumor cells with negative or low Ki-67 expression have loose cellularity and are typically associated with liquefactive necrosis and local fibrosis, resulting in few diffusion barriers. The $K_{app}$ parameter represents excessive diffusion kurtosis in the tissue. Thus, it is possible to use differences in $K_{app}$ values observed in our study to reflect the differences in micro-structural irregularity and heterogeneity between these sub-groups.

In addition, a moderate correlation between diffusion coefficient ($D_{app}$ and ADC) and Ki-67 PI or EGFR mutations was observed in the study. In the DKI model, $D_{app}$ is an adjusted ADC value that accounts for this non-Gaussian diffusion behavior. In our current study, the $D_{app}$ values were significantly lower among patients with high Ki-67 PI or positive EGFR mutations ($P < 0.001$, $P = 0.002$, respectively). It could be explained that the EGFR mutation-positive group and high Ki-67 expression group may have an impact on the restriction of water diffusion that can be reflected by decreasing $D_{app}$ values. Further details about this transition remain to be confirmed. In the subsequent ROC analysis for micro-environment assessment, both $K_{app}$ and $D_{app}$ were a little superior to ADC; thus, DKI has been shown to reflect the micro-structural characteristics of adenocarcinoma tissue slightly more accurately than conventional DWI. However, the diagnostic accuracy of these three parameters was not good enough (AUC ranges from 0.49 to 0.88). Upon correlating the $D_{app}$ and ADC values with ALK rearrangements, no statistically significant differences were observed with respect to the DKI $D_{app}$ values; however, there was a non-significant trend toward lower $D_{app}$ values among patients with negative ALK rearrangements than those with positive rearrangements. The differences in mean $D_{app}$ or ADC values between the groups with or without ALK rearrangements were not statistically significant; this may be because of the low rearrangement rate in our study. These results show that the DKI model affords a metric that reflects excess kurtosis in a tissue and contributes to further analysis of molecular biomarkers in lung adenocarcinoma.

Discussion

MRI is an attractive technique that provides an integral assessment of several morphologic and functional techniques to evaluate different tumor characteristics. In the current study, we evaluated DKI-derived parameters to characterize the micro-structural properties of advanced lung adenocarcinomas and correlated them with the corresponding histopathologic findings, to provide a better opportunity for radiologists to potentially gain further insights into the tissue characteristics and improve clinical management triage as compared with the use of standard DWI.
There were some limitations in this study. First, image quality of small lesions (for example, less than 2 cm) is more easily affected by the patient’s heart rate, fluctuation of heart rate, and breathing artifact, causing increased anatomic distortions; hence, our current study focuses on patients with relatively large lesions. Second, MRI scanners in common clinical use were still limited to obtain sufficient signal-to-noise ratio of GGO lesions at ultrahigh b value images (>1000 s/mm²), so GGO lesions are not included in this research sample. As a whole, our observations based on a small sampling of MRI examinations are still preliminary, and additional prospective studies are warranted to assess the utility of DKI metrics in predicting clinical outcomes.

We found that the DKI model contains specific information on the non-Gaussian diffusion behavior, provides additional parameters such as $K_{app}$, as an indicator of immunohistochemical findings, has a high value when assessing the patients with advanced lung adenocarcinomas, and shows slightly better diagnostic accuracies than the conventional DWI model. Using the DKI model during lung MRI is technically feasible in clinical routine, as it provides a practical clinical tool to quantify non-Gaussian water diffusion and probe the microscopic structure of biologic tissues.

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**Conflicts of interest**

None.

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