Isocitrate Dehydrogenase 1 Is a Novel Plasma Biomarker for the Diagnosis of Non–Small Cell Lung Cancer

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Abstract

**Purpose:** Effective biomarkers for the diagnosis of non–small cell lung cancer (NSCLC) are needed. We previously showed that isocitrate dehydrogenase 1 (IDH1) is significantly increased in NSCLC tumors. This study aimed to examine the plasma levels of IDH1 in a large patient population to evaluate its effectiveness in NSCLC diagnosis.

**Experimental Design:** The plasma levels of IDH1, CA125, Cyfra21-1, and CEA were assayed by ELISA. Blood samples were obtained from 1,422 participants (943 patients with NSCLC and 479 healthy controls). The samples were randomly divided into a training set and a test set. Receiver operating characteristic and binary logistic regression analyses were applied to evaluate diagnostic efficacy and establish diagnostic mathematical models.

**Results:** Plasma IDH1 levels were significantly higher in patients with NSCLCs than in healthy controls ($P < 0.001$). The diagnostic use of IDH1 in lung adenocarcinoma [area under curve (AUC): 0.858 and 0.810; sensitivity: 77.1% and 76.2%; specificity: 82.9% and 76.6%; in the training set and test set, respectively] was significantly greater than that of CA125, Cyfra21-1, or CEA ($P < 0.001$). The model combining IDH1 with CEA, CA125, and Cyfra21-1 was more effective for lung adenocarcinoma diagnosis than IDH1 alone (sensitivity and specificity in the training set: 75.8%, 89.6%; test set: 86.3%, 70.7%). In addition, the plasma levels of IDH1 could contribute to the diagnostic model of lung squamous cell carcinoma.

**Conclusions:** IDH1 can be used as a plasma biomarker for the diagnosis of NSCLCs, particularly lung adenocarcinoma, with relatively high sensitivity and specificity. 

Introduction

Lung cancer is the leading cause of cancer death worldwide (1). The goals of this study were to detect and determine the level of isocitrate dehydrogenase 1 (IDH1) in the plasma of patients with non–small cell lung cancer (NSCLC), to evaluate its diagnostic significance, and to show the feasibility of combining IDH1 with existing biomarkers on a large scale.

Reports from United States and China indicate that lung cancer has a high mortality rate; this mortality rate is usually ascribed to late diagnosis (2, 3). With the increases in aging populations and environmental pollution worldwide, the upward trend in lung cancer incidence will continue, and there will be an increased demand for improved lung cancer diagnosis. Current methods for the clinical diagnosis of lung cancer include chest X-ray, computed tomographic (CT) scans, and other imaging techniques. Although imaging data play an important role in diagnosis, they are limited by high false-positive rates and their inability to detect hidden lesions, subclinical lesions, and small metastases. In the National Lung Screening Trial (NLST), 53,454 participants at high risk for lung cancer were enrolled. The participants were randomly divided into different groups and underwent annual screenings with either low-dose CT (26,722 participants) or single-view posteroanterior chest radiography (26,732 participants) for 7 years. Although the use of low-dose CT reduced the mortality attributed to lung cancer, 96.4% of the positive screening results in the low-dose CT set and 94.5% in the radiography set were found to be false-positives (4). Furthermore, the harmful effects of radiation and the expense and overdiagnosis associated with CT scans have been points of controversy. In addition to CT scans, there are many invasive diagnostic methods for...
auxiliary diagnosis, such as bronchoscopy and needle biopsy, but these methods are painful and time-consuming. Hence, a top priority in lung cancer research should be the identification of a noninvasive, nonradiative, inexpensive, and rapid diagnostic method with high sensitivity and specificity.

At present, a large number of tumor-specific circulating proteins have been identified in the plasma of patients with cancer including carcinoembryonic antigen (CEA) in colon cancer, cancer antigen 125 (CA125) in ovarian cancer, and prostate-specific antigen (PSA) in prostate cancer. These proteins are produced by cancer cells, the tumor microenvironment, or the host response, and they could be used for diagnosis, to define the therapeutic response, or to monitor disease recurrence (5). However, there are currently no good biomarkers for NSCLC diagnosis. According to the National Academy of Clinical Biochemistry guidelines, some existing NSCLC biomarkers, such as CEA and Cyfra21-1, have been used in clinical practice, whereas others, such as CA125, have been recommended for further validation (6). However, these biomarkers have low sensitivity, ranging from only 50% to 60%, with specificities of approximately 90% (7–9).

We recently conducted a quantitative analysis of potential biomarkers in NSCLC tumor tissues, which revealed that IDH1 is highly expressed and represents a biomarker for poor prognosis in NSCLCs (10). This protein attracted our attention because it plays an important role in the tumor-associated redox process. Redox reactions play important roles in carcinogenesis and cancer therapy (11–13). This was the first report that IDH1 is upregulated in lung cancer, providing new insight into its involvement in the disease. As such, IDH1 was chosen for further study. IDH1, which normally localizes to the cytoplasm and peroxisomes, is a 47-kDa enzyme comprising 414 amino acids that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate, producing the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH). NADPH is an essential cofactor for the regeneration of glutathione (GSH) and also plays critical roles in the maintenance of the reduced state of thioredoxin (14). Both GSH and reduced thioredoxin are important antioxidants that enable mammalian cells to resist oxidative damage, thus suppressing apoptosis and enhancing cell survival and growth. IDH1 knockdown by RNA interference suppressed the proliferation of an NSCLC cell line and inhibited the growth of xenograft tumors in vivo (10). This suggests that IDH1 may play a critical role in lung cancer. Most recent studies on IDH1 in tumors have focused on mutations in the IDH1 gene and have shown that somatic IDH1 mutations occur in more than 70% of grade II/III gliomas and secondary glioblastomas (15). Dong and colleagues reported that the R132H mutation in IDH1 results in a gain of function (16). The mutation of R132 to Leu, His, Cys, or Ser abolishes magnesium binding and blocks the conversion of isocitrate to α-ketoglutarate. Instead, α-ketoglutarate is converted to R(−)-2-hydroxyglutarate. Elevated levels of R(−)-2-hydroxyglutarate are correlated with an elevated risk of malignant brain tumors, making IDH1 mutants a therapeutic target (16). However, no IDH1 mutations have been detected in NSCLC tissues (10). Because our previous retrospective investigation only evaluated a small cohort of patients with lung cancer (100 cases), we could not precisely estimate the diagnostic efficiency of IDH1 in different subtypes or stages of lung cancer. As such, further analysis of the sensitivity and specificity of IDH1 as a diagnostic biomarker in NSCLCs in a larger population is needed.

Here, we measured the levels of IDH1, CEA, CA125, and Cyfra21-1 in a large clinical population to study the diagnostic efficiency of these markers in NSCLCs and to build models to improve NSCLC diagnosis.

Materials and Methods

Patients

Patients with NSCLCs (1,000) and healthy individuals (500) were continuously enrolled from the Department of Thoracic Surgery and Department of Cancer Prevention in the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences from 2007 to 2011. The patients and healthy individuals were enrolled according to the following criteria: patients had no antineoplastic therapy, radiotherapy, or chemotherapy before surgery or cancer diagnosis within 3 years before the collection of the samples; healthy controls had not received a diagnosis of malignancy or benign tumors after routine examinations including chest X-rays, liver function tests, or complete blood tests (blood routine examination, blood biochemical analysis, tumor markers analysis, virus index analysis, and blood coagulation system analysis); 57 patients with NSCLCs and 21 healthy individuals were excluded because of missing information or a history of malignant disease. The remaining 943 patients with NSCLCs (median age, 61 years; range, 26–86 years) and 479 sex- and age-matched healthy controls (median age, 56 years; range, 41–70 years) were enrolled. All patients underwent surgery, and blood samples were collected 3 days before surgery with patients’ informed consent. Among the patients, 489 had lung squamous cell carcinomas.
Table 1. The characteristics of the patient population

<table>
<thead>
<tr>
<th></th>
<th>Training set</th>
<th></th>
<th>Test set</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>SCC</td>
<td>ADC</td>
<td>Healthy controls</td>
<td>SCC</td>
</tr>
<tr>
<td>No. of patients</td>
<td>245</td>
<td>227</td>
<td>240</td>
<td>244</td>
</tr>
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<td>Age, y (median range)</td>
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<td>60 (26–81)</td>
<td>56 (41–70)</td>
<td>61 (28–85)</td>
</tr>
<tr>
<td>Gender</td>
<td>Female/male</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>11/234</td>
<td>110/117</td>
<td>119/121</td>
<td>13/231</td>
</tr>
<tr>
<td>Lifetime smoking history (pack-years)</td>
<td>0</td>
<td>24</td>
<td>137</td>
<td>190</td>
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<tr>
<td></td>
<td>0–25</td>
<td>60</td>
<td>35</td>
<td>30</td>
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<tr>
<td></td>
<td>&gt;25</td>
<td>161</td>
<td>55</td>
<td>20</td>
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| TNM stage      |              |              |          |              |              |                  |
|                | I            | 70           | 111      | 74           | 117          |                  |
|                | II           | 97           | 27       | 84           | 29           |                  |
|                | III          | 77           | 67       | 83           | 64           |                  |
|                | IV           | 1            | 22       | 2            | 14           |                  |

| T stage        |              |              |          |              |              |                  |
|                | 1            | 11           | 33       | 18           | 29           |                  |
|                | 2            | 163          | 154      | 149          | 154          |                  |
|                | 3            | 61           | 30       | 66           | 25           |                  |
|                | 4            | 10           | 8        | 10           | 9            |                  |

| N stage        |              |              |          |              |              |                  |
|                | 0            | 111          | 124      | 111          | 138          |                  |
|                | 1            | 81           | 26       | 77           | 24           |                  |
|                | 2            | 50           | 73       | 54           | 64           |                  |
|                | 3            | 2            | 3        | 1            | 0            |                  |

*The relationship between the smoking history and IDH1 level (training and test sets: Pearson χ² test, SCC: P < 0.01; ADC: > 0.05; NSCLC: < 0.01).*

(SCC) and 454 had lung adenocarcinomas (ADC). NSCLC was defined on the basis of CT and confirmed by histopathology according to the World Health Organization Classification of Tumors of the Lung (17). Tumor stage was defined according to the 7th IASLC/AJCC staging system (18, 19). All samples were randomly separated into a training set and a test set (Supplementary Methods). The patient clinicopathologic information, including age, gender, pathology, differentiation, tumor–node–metastasis (TNM) stage, and history of smoking are shown in Table 1. The data collection and analyses were conducted by independent researchers. The study was approved by the medical ethics committee of the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences.

**Specimen characteristics**

Preoperative peripheral blood samples were collected in EDTA and anticoagulant-free tubes and processed according to standard protocols. Within 30 minutes of collection, the blood samples were centrifuged at 3,000 rpm at room temperature for 10 minutes, and the supernatants were divided into 200 μL aliquots, flash frozen, and stored at −80°C until testing.

**ELISA**

Plasma IDH1 assays were conducted by a commercial laboratory (USCN Life Science Laboratories, Inc.). All samples were assayed simultaneously. All laboratory personnel were blinded to the identities of the samples. A commercially available ELISA kit (E97839Hu 96 Test, USCN Life Science) was used according to the manufacturer’s recommendations. The samples and kit components were equilibrated to room temperature before the assay. Aliquots of 100 μL of the plasma samples in standard diluent were added to the appropriate wells and covered with a plate sealer. The plates were incubated for 2 hours at 37°C. The liquid was removed from each well, and an aliquot of solution A containing secondary antibodies (diluted 1:200) was added. The samples were then incubated for another 1 hour at 37°C. After 5 washes with buffer, 100 μL solution B containing hydrogen peroxide at a 1:200 dilution was added, and the samples were incubated for 30 minutes. Color development was achieved by adding 90 μL 3,3,5,5-tetramethylbenzidine per well as a substrate. To stop the reaction, 50 μL sulfuric acid was added. The optical density (O.D.) was measured at 450 nm on a Synergy 2 multimode plate reader (Biotek). The concentration of IDH1 was...
calculated with a quadratic polynomial fitting curve. When the concentration of IDH1 was less than 0.53 U/L, the lower limit of the standard curve, the value was set to zero. The mean intra-assay coefficient of variation from the quality control samples was 6.7% ($n = 20$).

### Analysis of tumor markers

The tumor markers CEA, CA-125, and Cyfra21-1 were analyzed with an Elecsys immunoassay analyzer (Roche Diagnostics). The upper normal limits for the tumor markers are 5 ng/mL for CEA, 35 U/mL for CA-125, and 3.3 ng/mL for Cyfra21-1.

### Statistical analysis

The standard curve was created in Curve Expert 1.3. SPSS (version 13.0) and MedCalc (version 9.6.2.0) were used for data analysis. The differences between plasma sample sets were evaluated by the Mann–Whitney $U$ test using continuous variables and nonparametric analyses by GraphPad Prism version 5 for Windows. Receiver operating characteristic (ROC) curves were plotted to assess the sensitivity, specificity, and areas under the curves (AUC) with a 95% confidence interval (CI). The optimum cutoff value for diagnosis was determined by maximizing the specificity at 95% in the training set. To assess whether the diagnostic efficiency of IDH1 in combination with CEA, CA-125, and Cyfra21-1 was superior to that of the 4 individual biomarkers alone, new variable models for NSCLCs were created on the basis of equations obtained by binary logistic regression. The regression equations for all comparisons are provided in the appendix (Supplementary Table S1). Differences were considered statistically significant when the $P$ value was lower than 0.05 (2-sided test).

### Results

We recruited a total of 1,422 participants (943 patients with NSCLCs and 479 healthy controls). The NSCLCs and healthy control samples were randomly divided into a training set of 712 samples and a test set of 710 samples (Fig. 1). The characteristics of the patients in the training and test sets are summarized in Table 1. The training and test set populations were comparable with respect to age and sex.

#### IDH1 plasma levels in NSCLC patients and healthy controls

We analyzed the plasma IDH1 levels (median ± IQR; IQR, interquartile range) in each participant using an ELISA assay. In the training set, the median plasma IDH1 levels of patients with ADCs and SCCs were 2.42 ± 1.82 and 1.87 ± 2.01 U/L, respectively, higher than that of the healthy controls (0.79 ± 0.33 U/L; Fig. 2A, $P < 0.0001$, Mann–Whitney $U$ test). In the test set, the median IDH1 levels of patients with ADCs and SCCs were 2.35 ± 1.51 and 2.03 ± 1.79 U/L, respectively, also higher than that of the healthy controls.

![Figure 2](https://www.aacrjournals.org/doi/10.1158/1078-0432.CCR-13-0132)
controls (0.95 ± 1.62 U/L; Fig. 2B, P < 0.0001, Mann–Whitney U test). In the whole set (i.e., the combined training and test sets), the median IDH1 levels in patients with ADCs and SCCs were 2.39 ± 1.77 and 1.96 ± 1.85 U/L, respectively, significantly higher than the level observed in healthy controls (0.88 ± 1.46 U/L; Fig. 2C, P < 0.0001, Mann–Whitney U test). In addition, the median level of plasma IDH1 in the patients with ADCs was significantly higher than that in the patients with SCCs (P = 0.012). These results indicate that plasma IDH1 can be used as a biomarker for the diagnosis of lung cancer and has a better diagnostic efficacy for identifying patients with ADCs than SCCs.

Next, we analyzed the correlation between the plasma IDH1 level and the clinical characteristics of patients with ADCs and SCCs. In the patients with ADCs, the median level of plasma IDH1 in patients with stage T2b–T4 (2.72 ± 2.23 U/L) was significantly higher than that in patients with stage T1a–T2a disease (2.31 ± 1.72 U/L; P < 0.05, Supplementary Fig. S1A). In contrast, although the median level of plasma IDH1 in patients with SCCs with stage T2b–T4 (2.11 ± 2.18 U/L) was higher than that in patients with SCCs with stage T1a–T2a (1.87 ± 2.58 U/L), this difference was not significant (Supplementary Fig. S1B). In addition, we did not observe any correlation between plasma IDH1 level and other patient clinical characteristics such as age, gender, smoking history, lymph node metastasis, or TNM stage.

ROC analyses of IDH1, CEA, CA125, and Cyfra21-1 and the construction of diagnostic models for NSCLCs

ROC curves based on the ELISA results were plotted to determine the diagnostic efficiency of IDH1 plasma levels for NSCLCs (ADCs and SCCs). The efficiency of the existing clinical biomarkers, CEA, CA125, and Cyfra21-1, in distinguishing NSCLCs from healthy controls was also included (Figs. 3 and 4, Table 2). The measurements of the different individual markers and their predictive value and likelihood ratios in the diagnosis of NSCLCs (ADCs and SCCs) are summarized in Table 2.

Among the 4 ADC biomarkers, IDH1 displayed the highest AUC (Fig. 3A, training set: 0.858; 95% CI, 0.823–0.889; test set: 0.810; 95% CI, 0.771–0.884; Table 2) and satisfactorily discriminated patients with ADCs from healthy controls. Cyfra21-1 displayed the highest AUC in differentiating patients with SCCs from healthy controls (Fig. 3B, training set: 0.846; 95% CI, 0.811–0.877; test set: 0.833; 95% CI, 0.797–0.865; Table 2). IDH1 and CA125 had similar moderate capacities for differentiating patients with SCCs from healthy controls (P = 0.28, Table 2). CEA exhibited a low discriminatory capacity for both ADCs and SCCs, with AUC ranging from 0.519 to 0.600 (Fig. 3A and B, Table 2). IDH1 exhibited optimal efficacy for the diagnosis of all NSCLCs (Fig. 3C, training set: 0.817; 95% CI, 0.786–0.845; test set: 0.773; 95% CI, 0.741–0.804; Table 2). These results show that the diagnostic efficacy of IDH1 in ADCs and NSCLCs overall was greater than that of CEA, CA125, and Cyfra21-1 (P < 0.001, Table 2). On the basis of the ROC curve, we selected an optimum IDH1 cutoff value of 2.19 U/L for NSCLC diagnosis (training set: sensitivity, 48.7%; specificity, 95.0%; Table 2). Cyfra21-1 was most efficient for the diagnosis of SCCs but displayed a lower efficacy than IDH1 for NSCLCs (training set: sensitivity, 48.5%; specificity, 87.5%; Table 2).

We used binary logistic regression to investigate whether combining the markers could improve diagnostic accuracy. The combination of IDH1, CEA, CA125, and Cyfra21-1 improved the classification capacity and yielded a better optimal diagnostic efficacy for patients with ADCs (training set: AUC, 0.890; 95% CI, 0.858–0.917; test set: AUC, 0.838; 95% CI, 0.802–0.870; Table 2) than did IDH1 alone (P < 0.001, Fig. 4A). For patients with SCCs, the combination of IDH1, Cyfra21-1, and CA125 had AUCs of 0.914 value (95% CI, 0.886–0.938) in the training set and 0.893 (95% CI, 0.862–0.919) in the test set, superior to Cyfra21-1 alone (P < 0.001, Fig. 4B). In addition, the combination of IDH1, CA125, and Cyfra21-1 represented a more efficient diagnostic modality (training set: AUC, 0.896; 95% CI, 0.871–0.918; test set: AUC, 0.853; 95% CI, 0.825–0.879; Table 2) for NSCLCs than IDH1 or Cyfra21-1 alone (P < 0.001, Fig. 4C). Because CEA was ineffective in SCCs and NSCLCs, it was not included in model 2 or 3 (Supplementary Table S1).

Discussion

We previously showed that IDH1 mRNA and protein expression are elevated in tumor tissues compared with matched normal tissues (10). This suggests that IDH1 plasma level might be a useful biomarker for the diagnosis of patients with NSCLCs. We have 2 hypotheses for the elevated levels of IDH1 in blood, which are currently being investigated: IDH1 may be secreted extracellularly under exceptional circumstances or released into the blood by cell damage and cell death. In addition, plasma IDH1 levels were positively correlated with T stage in patients with NSCLCs, strongly suggesting that IDH1 might be a useful diagnostic biomarker.

In this report, we showed that the plasma IDH1 level was significantly elevated in 976 patients with NSCLCs compared with 479 healthy controls. We also tested 3 existing clinical biomarkers, CEA, CA125, and Cyfra21-1, in all the samples and compared their diagnostic efficacy to that of IDH1. Then, we established the AUCs for biomarker panels and determined the appropriate balance between sensitivity and specificity in the choice of cutoff point. To reduce the misdiagnosis rate, we set the specificity at 95% and compared the sensitivity between panels. We identified several multiprotein panels that offer high sensitivity and specificity and provide a significant improvement over the use of single markers for the discrimination of NSCLCs from healthy controls.

There are already several good markers for the diagnosis of SCCs, such as Cyfra21-1 and SCCA, whereas good markers for ADCs are lacking (6). Cyfra21-1 was shown to have good diagnostic capability in a small cohort of patients with SCCs, with a high sensitivity (69%–79%) but an unsatisfactory efficacy, similar to other ADC diagnostic markers.
As such, we focused on ADC markers such as CEA and CA125. CA125 is used mainly for ovarian cancer screening and diagnosis (23, 24), whereas CEA is used for the diagnosis and prognosis of several types of cancer, including colon cancer (25, 26). Both CEA and CA125 were useful for monitoring recurrence and evaluating prognosis in NSCLCs but only performed moderately in diagnosing NSCLCs (27–33). We also included Cyfra21-1 because it is useful for the diagnosis of both SCCs and overall NSCLCs. Finally, we included CEA, CA125, and Cyfra21-1 in our panel. Our results confirmed that Cyfra21-1 is a good marker for SCC diagnosis, with a much better sensitivity than IDH1, CEA, or CA125 (Table 2). In contrast, for ADC diagnosis, IDH1 showed higher sensitivity than CEA, CA125, or Cyfra21-1 (Table 2). In the training set, the combination of IDH1 with CEA, CA125, and Cyfra21-1 improved the diagnostic sensitivity to 63.4% in ADCs, 63.3% in SCCs, and 60.4% in NSCLCs, with a specificity of 95%. These results were further validated in the test set (Table 2). Our analyses of a large cohort of 943 NSCLC patient established that IDH1 is a highly effective auxiliary diagnostic marker for ADCs.

Figure 3. ROC curve analyses of the use of IDH1, CEA, CA125, and Cyfra21-1 to differentiate NSCLC cases and controls. A, ROC curves for IDH1, CEA, CA125, and Cyfra21-1 for patients with ADCs versus controls in the training (left) and test sets (right), respectively. B, ROC curves for IDH1, CEA, CA125, and Cyfra21-1 for patients with SCCs versus controls in the training (left) and test sets (right), respectively. C, ROC curves for IDH1, CEA, CA125, and Cyfra21-1 for patients with NSCLCs versus controls in the training (left) and test sets (right), respectively.
In this study, the SCC and NSCLC groups had a significantly greater smoking history than the control group (Table 1). However, there were no significant differences between the smoking histories of the ADC group and the control group (Table 1). Pearson correlation analysis indicated no correlation between IDH1 levels and smoking history (pack-years) in any of the groups (Supplementary Table S2). We also stratified the patients and controls in our study according to smoking history. The AUCs of the ROC curves were similar for smokers and non-smokers (Supplementary Table S3).

Although the CT scan, which is the currently recommended method of diagnosis, has several advantages, including its noninvasive nature, there are also some disadvantages to this method, such as radiation, cost, and low specificity (34–36). In contrast, our blood-based test is more practical than a CT scan because it uses a peripheral blood sample obtained by routine venipuncture. Our tests require less than 100 μL of plasma, do not require significant operator expertise, and are inexpensive and highly reproducible. In addition, our samples do not require special treatment such as extraction, purification, or reverse
| Table 2. The diagnostic efficiency of models in differentiating NSCLC cases and controls |
|----------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                  | Training set      | Test set          |                   |                   |                   |                   |                   |
|                                  | AUC (95% CI)      | SN (%)            | SP (%)            | PPV (%)           | NPV (%)           | Positive LR       | Negative LR       |
| ADC vs. control                  |                   |                   |                   |                   |                   |                   |                   |
| IDH1                             | 0.858 (0.823-0.889) | 56.0              | 95.0              | 91.4              | 69.5              | 11.19             | 0.46              |
| CEA                              | 0.600 (0.553-0.644) | 38.8              | 82.9              | 68.2              | 58.9              | 2.27              | 0.74              |
| CA125                            | 0.704 (0.661-0.746) | 14.1              | 99.2              | 94.1              | 55.0              | 16.92             | 0.87              |
| Cyfra21-1                        | 0.648 (0.603-0.691) | 30.8              | 87.5              | 70.0              | 57.2              | 2.47              | 0.79              |
| IDH1 + CEA + CA125 + Cyfra21-1   | 0.890 (0.858-0.917) | 63.4              | 95.0              | 92.3              | 73.3              | 12.69             | 0.38              |
| SCC vs. control                  |                   |                   |                   |                   |                   |                   |                   |
| IDH1                             | 0.778 (0.739-0.815) | 42.0              | 95.0              | 89.6              | 61.6              | 8.41              | 0.61              |
| CEA                              | 0.534 (0.486-0.579) | 15.1              | 82.9              | 47.4              | 48.9              | 0.88              | 1.02              |
| CA125                            | 0.747 (0.706-0.785) | 12.2              | 99.2              | 93.7              | 52.5              | 14.69             | 0.88              |
| Cyfra21-1                        | 0.846 (0.811-0.877) | 64.9              | 87.5              | 84.1              | 70.9              | 5.19              | 0.40              |
| IDH1 + CA125 + Cyfra21-1         | 0.914 (0.886-0.938) | 63.3              | 95.0              | 92.8              | 71.7              | 12.65             | 0.39              |
| NSCLC vs. control                |                   |                   |                   |                   |                   |                   |                   |
| IDH1                             | 0.817 (0.786-0.845) | 48.7              | 95.0              | 95.0              | 48.5              | 9.75              | 0.54              |
| CEA                              | 0.565 (0.528-0.602) | 26.5              | 82.9              | 75.3              | 36.4              | 1.55              | 0.89              |
| CA125                            | 0.726 (0.692-0.759) | 13.4              | 99.2              | 96.9              | 36.8              | 16.02             | 0.87              |
| Cyfra21-1                        | 0.731 (0.717-0.782) | 48.5              | 87.5              | 88.4              | 46.4              | 4.88              | 0.59              |
| IDH1 + CA125 + Cyfra21-1         | 0.896 (0.871-0.918) | 60.4              | 95.0              | 96.0              | 54.9              | 12.08             | 0.42              |
| Abbreviations: LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value. |
| aThe diagnostic cut-off value was 2.19 U/L. |
transcription (37). Thus, measuring IDH1 levels in patient blood can be a convenient, efficient, and reliable auxiliary method for the diagnosis of NSCLCs, particularly ADCs. Integrated analyses of plasma IDH1, CEA, Cyfra21-1, and CA125, in conjunction with imaging and clinicopathologic examination information, may provide a better panel of diagnostic tools for the rapid diagnosis of NSCLCs. This will enable the timely and effective treatment of NSCLCs and the improvement of cancer patient mortality.

However, because most of the participants in our study originated from northern China, common confounding variables and comorbid conditions cannot be eliminated. Because of the complexity of the causes of lung cancer and the potential differences in the genomic and molecular signatures of patients from different populations, future studies with an independent cohort of patients are needed. It will also be necessary to determine whether plasma IDH1 is a specific biomarker of NSCLCs by examining it in patients with other cancer types. Although the IDH1 levels in patients and controls are highly significantly different in our study, and the sensitivity and specificity of IDH1 are in patients and controls (Fig. 2). Therefore, at this stage, IDH1 can only be used as an adjunct approach for diagnosis. In addition, the molecular mechanisms and clinical implications of elevated IDH1 require further investigation.

This is the first large-scale study to report the clinically diagnostic relevance of IDH1 as a plasma marker for NSCLCs in a training set and a test set. Our results show that plasma IDH1 could be used to assist the diagnosis of NSCLCs, particularly ADCs. Hence, we recommend detecting IDH1 along with CEA, CA125, and Cyfra21-1 and using the presented models to aid in diagnosis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: N. Sun, F. Tan, X. Tan, J. He
Development of methodology: N. Sun, F. Tan, X. Tan, J. He
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Sun, Z. Chen, J. Li, X. Tan, M.Y.F. He, J. Sun, J. He
Writing, review, and/or revision of the manuscript: N. Sun, Z. Chen, F. Tan, Z. Liu, X. Tan, J. He
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N. Sun, B. Zhang, X. Tan, F. Zhou, N. Li, S. Wang, J. He
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