Significant correlation between urinary N\textsuperscript{1}, N\textsuperscript{12}-diacetylspermine and tumor invasiveness in patients with clinical stage IA non-small cell lung cancer

Yusuke Takahashi\textsuperscript{1,2*}, Hirotoshi Horio\textsuperscript{1}, Koji Sakaguchi\textsuperscript{1,4}, Kyoko Hiramatsu\textsuperscript{3} and Masao Kawakita\textsuperscript{3}

Abstract

Background: To select optimal candidates for limited lung resection, it is necessary to accurately differentiate the non-invasive tumors from other small-sized lung cancer. Urinary N\textsuperscript{1}, N\textsuperscript{12}-diacetylspermine (DiAcSpm) has been reported to be a useful tumor marker for various cancers. We aimed to examine the correlation between preoperative urinary DiAcSpm levels and specific clinicopathological characteristics such as the histological tumor invasiveness in patients with clinical stage IA non-small cell lung cancer (NSCLC).

Methods: We defined non-invasive tumors as NSCLC showing no vascular invasion, lymphatic permeation, pleural invasion, or lymph node metastasis. Preoperative urine samples were obtained from 516 consecutive patients with NSCLC resected at our institution between April 2008 and January 2013. Urinary DiAcSpm values were determined for all preoperative urine samples using the colloid gold aggregation procedure. Among these patients, 171 patients with clinical stage IA NSCLC met the criteria of our study cohort. Finally, we investigated the correlation between non-invasive tumor and urinary DiAcSpm levels.

Results: The median urine DiAcSpm for males was 147.2 nmol/g creatinine and 161.8 nmol/g creatinine in females. These median values were set as the cut-off values for each gender. Patients with higher urinary DiAcSpm levels frequently had significantly elevated serum CEA (p = 0.023) and greater lymph node metastasis (p = 0.048), lymphatic permeation (p = 0.046), and vascular invasion (p = 0.010). Compared with patients with non-invasive tumors, patients with invasive tumors had a tumor size >2.0 cm (p = 0.001), serum CEA >5.0 mg/dL (p < 0.001), high urinary DiAcSpm (p = 0.002), and a tumor disappearance rate (TDR) <0.75 (p < 0.001). Multivariate analysis revealed that a tumor size < 2.0 cm (RR = 2.901, 95% CI; 1.372-6.136, p = 0.005), high urinary DiAcSpm (RR = 3.374, 95% CI; 1.547-7.361, p = 0.002), and TDR < 0.75 (RR = 4.673, 95% CI; 2.178-10.027, p < 0.001) were independent predictors for invasive tumors.

Conclusions: We successfully showed that there was a significant correlation between urinary DiAcSpm levels and pathological tumor invasiveness in patients with clinical stage IA NSCLC. Further research would elucidate the clinical usefulness of DiAcSpm levels as a predictor of tumor invasiveness.

Keywords: Tumor invasiveness, Urine diacetylspermine, Clinical stage IA, Non-small cell lung cancer

* Correspondence: yusuketakahashigts@gmail.com

\textsuperscript{1}Department of Thoracic Surgery, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, 3-18-22 Hon-komagome, Bunkyo-ku, Tokyo, Japan

\textsuperscript{2}Department of General Thoracic Surgery, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo, Japan

Full list of author information is available at the end of the article

© 2015 Takahashi et al; licensee BioMed Central. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
Surgery is one of the major therapeutic choices for patients with primary lung cancer. Specifically in clinical stage IA non-small cell lung cancer (NSCLC), the standard treatment remains lobectomy and systematic hilar and mediastinal lymph node dissection [1]. Recent advancements in diagnostic techniques have increased the accuracy and frequency of detection of small-sized lung tumors [2]. Using these advancements, a number of researchers have attempted to prove the effectiveness of limited lung resection; however, their studies have shown a higher local recurrence rate after limited resection, even though a negative surgical margin had been confirmed pathologically [3-5]. Possible explanations for local recurrence following limited resection may include insufficient surgical margins, misdiagnosis of nodal involvement, or intrapulmonary lymphatic spread [6]. Limited resection is often performed in patients with peripheral small-sized lung cancer, although two randomized control trials comparing limited resection with standard lobectomy in patients with clinical T1aN0M0 NSCLC are currently taking place in Japan. In order to select optimal candidates for limited resection it is necessary to accurately differentiate between non-invasive tumors that have been confirmed histologically and other small-sized lung cancers. Several researchers aiming to better characterize these tumors have reported that a greater proportion of ground-glass opacity (GGO) was a significant predictor of non-invasive lung cancer [7,8]. Moreover, a recent report described that the presence of a micropapillary component was independently associated with an increased risk of recurrence in patients with stage I NSCLC treated with limited resection [9].

Methods
Urine samples were obtained before treatment from 516 consecutive patients who were diagnosed with operable NSCLC at Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital between April 2008 and January 2013. Among these patients, 171 consecutive patients with clinical stage IA NSCLC were consistent with our study cohort. We received prior approval to use patient urine samples from the ethical committees at Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital and Tokyo Metropolitan Institute of Medical Science. Informed consent was obtained from all patients and approved by the Institution Review Board.

Determination of urinary DiAcSpm using the colloid gold aggregation procedure
Urine samples were supplemented with 3 mmol/L NaN3 and stored at −20°C, as previously described [12]. Urinary DiAcSpm was measured by the colloidal gold aggregation procedure using a JCM BM-6010 automatic biochemical analyzer (JEOL, Tokyo, Japan). The colloidal gold aggregation procedure relies on the binding specificity of bovine serum albumin (BSA)-acetylspermine conjugate, a DiAcSpm mimic, to colloidal gold-antibody complexes resulting in a stable red-purple solution. Addition of the BSA-acetylspermine conjugate into the solution induces a color change from red-purple to grey due to aggregation of the colloidal gold particles. DiAcSpm, a monovalent antigen that cannot cross-link multiple gold particles, competes with the BSA-acetylspermine conjugate for binding to the colloidal gold-antibody complexes. Therefore, when a urine sample containing DiAcSpm is added to the colloidal system, DiAcSpm competes with the colloidal gold-antibody complexes and suppresses color change. Thus, by using this competitive colloid system, the concentration of DiAcSpm in a urine sample can be determined by measuring the color change of the solution. Auto DiAcSpm® (Alfresa Pharma Co., Osaka, Japan), a reagent that can be used in automated clinical analyzers, is commercially available. The concentration of DiAcSpm determined by the colloidal gold aggregation procedure have shown to closely correspond with those determined by mass spectrometric analysis [13]. Urine creatinine levels were measured enzymatically using the NESAUTO® VLII CRE reagent (Alfresa Pharma Co., Osaka, Japan) on a JCM BM-6010 automated biochemical analyzer (JEOL, Tokyo, Japan).

Clinicopathological assessment
All clinicopathological data were retrieved from patient medical records. Preoperative evaluation included a physical examination, blood chemistry analysis, measurement of tumor markers, bronchoscopy, chest radiography, computed tomography (CT), brain MRI, and bone scintigraphy. Integrated positron emission tomography scan and CT scan (PET/CT) were also performed where appropriate. Clinical lymph node metastasis was
defined as enlarged lymph nodes measuring > 1 cm on the short axis by CT scan and/or hypermetabolic lymph nodes on PET/CT scans. Histological confirmation of lymph node metastasis was made using endobronchial ultrasound-guided transbronchial needle aspiration of enlarged lymph nodes. All patients underwent a lobectomy or bilobectomy and systematic lymph node dissection for resection of the primary lesion.

All surgical specimens underwent thorough pathological examination. Each tumor was diagnosed according to the current histological classification of the World Health Organization [14] and was staged according to the tumor node metastasis classification of the International Union against Cancer, 7th edition [15]. Vascular and pleural invasion and lymphatic permeation were evaluated using both hematoxylin and eosin (HE) section staining and Victoria Blue van Gieson (VvG) section staining.

Non-invasive tumor was defined as NSCLC showing no vascular invasion, pleural invasion, lymphatic permeation, or lymph node metastasis. The following clinicopathological information was collected from patient medical records: age (categorized into two groups, ≤69 years and >69 years, according to the median age), gender, tumor size, preoperative serum CEA level (dichotomized at the normal upper limit of 5 mg/dL), pathological lymph node involvement, vascular invasion, pleural invasion, lymphatic permeation, histological type, and pathological stage.

Of the 171 NSCLC lesions, 140 adenocarcinomas were identified and classified according to the adenocarcinoma classification newly proposed by the International Association for the Study of Lung Cancer, American Thoracic Society, and the European Respiratory Society (IASLC/ATS/ERS) [16]. The adenocarcinomas were divided into three groups: (1) adenocarcinoma in situ (AIS), (2) minimally invasive adenocarcinoma (MIA), and (3) invasive adenocarcinoma (I-ADC). Then, we evaluated the correlation between histological invasiveness of the tumors, defined according to the above classification, and the specific clinicopathological factors described above.

Measurement of tumor disappearance rate on chest CT
We calculated a tumor disappearance rate (TDR) using the following tumor dimension measurements on high-resolution chest CTs [17]: pDmax, which is the maximum dimension of a tumor on pulmonary window setting images; pDperp, the largest dimension perpendicular to the maximum axis on pulmonary window setting images; mDmax, the maximum dimension of a tumor on mediastinal window setting images; and mDperp, the largest dimension perpendicular to the maximum axis on mediastinal window setting images. Using these measurements, the TDR was then calculated using the following formula:

\[
    \text{TDR} = 1 - (\frac{mDmax \times mDperp}{pDmax \times pDperp})
\]

The TDR threshold was set at 0.75, as previously described [18].

Statistical analysis
Two-category comparisons were performed using the Pearson Chi-Square (\(\chi^2\)) Test and the Fisher’s Exact Test for categorical variables, and the Mann–Whitney U Test was performed for continuous variables. All statistical tests were two-sided, and \(p < 0.05\) were considered statistically significant. To determine the impact of factors considered significant predictors of survival by earlier univariate analysis, a multivariate analysis was performed on these predictors using a logistic regression model. All statistical analyses were performed using SPSS software (version 20; SPSS Inc., Chicago, Ill).

Results
Patient characteristics
Our cohort consisted of 84 males and 87 females. Ages ranged from 36 to 89 years, with a median age of 69 years. Tumor size from the resected specimens ranged from 0.8 to 3.5 cm, with a median of 1.8 cm. Within the study cohort, there were 137 adenocarcinomas, 24 squamous cell carcinomas, and 10 with other histology (2 adenosquamous carcinomas, 2 large cell carcinomas, 2 non-small cell carcinomas, 2 large cell neuroendocrine carcinomas, 1 clear cell carcinoma, and 1 carcinoid). There were 136 identified as pathological stage IA tumors, 121 as stage IB, 7 as stage IIA, and 7 as stage IIIA. Tumor stage was increased in 35 patients due to additional diagnoses following surgery. Discoveries that contributed to the up-staging included lymph node metastasis in 15 patients, an actual tumor size > 3 cm in 8 patients, and pleural invasion in 26 patients. Further, lymphatic permeation was seen in 24 patients and vascular invasion in 39 patients. A summary of patients and their pathological characteristics is presented in Table 1. The median urine DiAcSpm was 147.2 nmol/g creatinine (range: 3.7-3918.8) in males and 161.8 nmol/g creatinine (range: 66.0-867.6) in females. These median values were set as the cut-off values for urine DiAcSpm for each gender, as previous reports have demonstrated that the urine DiAcSpm values for healthy women are higher than those for men [12].

Measurement of tumor disappearance rate on chest computed tomography
When we applied 0.75 as the cut-off value for TDR where TDR was used to test the correlation with pathological
invasiveness, we achieved sensitivity and specificity values of 59.2% and 74.6%, respectively, with the best predictive accuracy of 70.2%. For urinary DiAcSpm, we applied cut-off values of 147.2 for males and 161.8 for females and achieved 69.4% sensitivity and 57.4% specificity which yielded a prediction accuracy of 60.8% for pathological invasiveness. When the TDR and urinary DiAcSPM were inversely combined, the predictive value of non-invasive tumor was 94.9% (37 of 39).

**Table 1 Baseline characteristics of initial study cohort (n = 171)**

| Characteristic                        | Value          |
|--------------------------------------|----------------|
| **Age**                              | Median (range) |
|                                       | 69 (36–89)     |
| **Gender**                           |               |
| male                                 | 84            |
| female                               | 87            |
| **Smoking history**                  |               |
| Never-smoker                         | 58            |
| Smoker                               | 113           |
| **Synchronous multiple lung cancer** | yes           |
|                                       | 4             |
| no                                    | 167           |
| **Histological type**                |               |
| adenocarcinoma                       | 137           |
| squamous cell carcinoma              | 24            |
| large cell carcinoma                 | 2             |
| large cell neuroendocrine carcinoma  | 2             |
| adenosquamous carcinoma              | 2             |
| carcinoid                            | 1             |
| clear cell carcinoma                 | 1             |
| NSCLC, NOS                           | 2             |
| **Lymphatic permeation**             |               |
| negative                             | 147           |
| positive                             | 24            |
| **Vascular invasion**                |               |
| negative                             | 132           |
| positive                             | 39            |
| **Pleural invasion**                 |               |
| negative                             | 145           |
| positive                             | 26            |
| **Pathological stage**               |               |
| IA                                    | 136           |
| IB                                    | 21            |
| IIA                                   | 7             |
| IIB                                   | 0             |
| IIIA                                  | 7             |

**Table 2 Correlation between urine DiAcSpm and clinicopathological factors**

| Factors                  | Urine DiAcSpm level (nmol/g creatinine) | p-value * |
|--------------------------|----------------------------------------|-----------|
| **Age (years)**          |                                        |           |
| ≤ 69                     | 47                                     | 0.286     |
| > 69                     | 38                                     |           |
| **Smoking history**      |                                        |           |
| Never-smoker             | 32                                     | 0.335     |
| Smoker                   | 53                                     |           |
| **Tumor size (cm)**      |                                        |           |
| ≤ 2.0                    | 55                                     | 0.348     |
| > 2.0                    | 30                                     |           |
| **Serum CEA level (mg/dL)** |                                      |           |
| ≤ 5.0                    | 79                                     | 0.023     |
| > 5.0                    | 6                                      |           |
| **TDR**                  |                                        |           |
| ≥ 0.75                   | 25                                     | 0.867     |
| < 0.75                   | 60                                     |           |
| **Histological type**    |                                        |           |
| adenocarcinoma           | 68                                     | 0.970     |
| non-adenocarcinoma       | 17                                     |           |
| **Lymph node metastasis**|                                        |           |
| N0                       | 82                                     | 0.087     |
| N1-2                     | 3                                      |           |
| **Lymphatic permeation** |                                        |           |
| negative                 | 78                                     | 0.046     |
| positive                 | 7                                      |           |
| **Vascular invasion**    |                                        |           |
| negative                 | 73                                     | 0.010     |
| positive                 | 12                                     |           |
| **Pleural invasion**     |                                        |           |
| pl0                      | 75                                     | 0.287     |
| pl1-2                    | 10                                     |           |

*Fisher’s exact test, DiAcSpm = diacetylspermine, CEA = carcinoembryonic antigen level.

Correlation between clinicopathological characteristics and urinary DiAcSpm

We evaluated the data to determine whether there was a correlation between urinary DiAcSpm and clinicopathological characteristics (Table 2). The high urinary DiAcSpm group often showed significantly elevated serum CEA (p = 0.023), lymph node metastasis (p = 0.048), lymphatic permeation (p = 0.046), and vascular invasion (p = 0.010) compared with the low urinary DiAcSpm group.
The Mann–Whitney U test was used to evaluate differences between the absolute values of urinary DiAcSpm and serum CEA level (≤5.0 mg/dL vs. >5.0 mg/dL), lymph node metastasis (N0 vs. N1-2), vascular invasion (positive vs. negative), lymphatic permeation (positive vs. negative), histological type (adenocarcinoma vs. others), tumor size (≤2.0 cm vs. >2.0 cm), and TDR (≥0.75 vs. <0.75). The results of these tests are presented in Table 2 and are as follows: The urinary DiAcSpm from the normal serum CEA group and N0 group was significantly lower than the elevated serum CEA group (p=0.044) and N1-2 group (p=0.014), respectively. Urinary DiAcSpm from the negative vascular invasion group was significantly lower than the positive vascular invasion group (p = 0.002). Urinary DiAcSpm from the negative lymphatic permeation group was significantly lower than the positive lymphatic permeation group (p = 0.038). In contrast, there were no significant differences in urinary DiAcSpm in groups with adenocarcinoma (p = 0.585), tumor size ≤2.0 cm and >2.0 cm (p = 0.249) or TDR (≥0.75 and <0.75 (p = 0.489).

Correlation between pathologically confirmed tumor invasiveness and clinicopathological factors
We investigated the relationship between pathologically confirmed tumor invasiveness and clinicopathological factors (Table 3). There were 122 cases of non-invasive tumors and 49 cases of invasive tumors. Tumor size >2.0 cm (p = 0.001), serum CEA >5.0 mg/dL (p < 0.001), high urinary DiAcSpm (p = 0.002), and TDR >0.75 (p < 0.001) were more frequently observed in patients with invasive tumors than in those with non-invasive tumors. Pathologically confirmed tumor invasiveness was not significantly affected by age, gender, smoking history, or histological type.

Multivariate analysis
We performed a multivariate analysis to determine independent predictors of pathologically confirmed invasive tumors (Table 4). A tumor size >2.0 cm (Risk ratio (RR) = 2.871, 95% confidence interval (CI): 1.347-6.119, p = 0.006), high urinary DiAcSpm (RR = 3.374, 95% CI; 1.547-7.361, p = 0.002), and TDR > 0.75 (RR = 6.103, 95% CI; 1.962-18.981, p < 0.001) were independent predictors of invasive tumors. However, serum CEA was not an independent predictor of pathologically confirmed tumor invasive tumors.

Correlation between histological invasiveness defined in IASLC/ATS/ERS classification and clinicopathological characteristics
Of 171 NSCLC lesions, 140 stage IA adenocarcinomas were separately analyzed to determine the correlation between histological invasiveness, as defined by IASLC/ATS/ERS classification, and clinicopathological factors. We confirmed 87 cases of non-invasive adenocarcinomas and 53 cases of invasive adenocarcinomas. Male gender (p = 0.010), smoker (p = 0.033), tumor size >2.0 cm (p < 0.001), serum CEA >5.0 mg/dL (p = 0.006), high urinary DiAcSpm (p < 0.001), and TDR >0.75 (p = 0.023) were more frequently associated with patients with invasive tumors than in patients with non-invasive tumors (Additional file 1: Table S1). Further, a tumor size >2.0 cm (RR = 3.249, 95% CI; 1.380-7.650, p = 0.007), high urinary DiAcSpm (RR = 8.208, 95% CI; 3.470-19.417, p < 0.001), and TDR <0.75 (RR = 2.783, 95% CI; 1.090-7.108, p = 0.032) were independent predictors of invasive tumors (Additional file 1: Table S2). However, gender, smoking history, and serum CEA were not found to be significant independent predictors of histological invasiveness in clinical stage IA adenocarcinomas.
Discussion
The extent of pulmonary resections for small-sized NSCLC remains a considerable concern for thoracic surgeons. The major challenge is determining which subgroups of NSCLCs are suitable candidates for limited resection. Several investigators have reported that pathologically confirmed invasive factors was not a rare occurrence and tumors showed a considerable recurrence rate, even when a patient’s tumor was classified as clinical stage IA NSCLC [1,19]. Currently, there are randomized controlled trials underway to compare the differences in outcome between a lobectomy and a limited resection in patients with clinical stage IA NSCLC.

Previous reports have shown that pathologically confirmed invasive factors from resected NSCLCs were strongly correlated with more frequent nodal involvement and poorer outcomes [19,20]. Based on these findings, it is widely accepted that the ability to predict pathologically non-invasiveness is essential for identifying optimal candidates for limited resection. A number of retrospective studies have indicated that tumor non-invasiveness was frequently confirmed in resected lung cancers smaller than 2 cm [8,20]. However, there are reports indicating the occurrence of lymph node metastasis in 6% to 12% of small-sized NSCLC [19]. In the current study, positive lymph node metastasis was observed in 14 of 171 (8.2%) patients, which is consistent with previous reports. According to these results, it is suggested that tumor size alone cannot predict the pathologic invasiveness of NSCLC.

Several reports have demonstrated that the TDR on High-Resolution Computed Tomography (HRCT) was a significant predictor of pathologically confirmed invasiveness in small-sized NSCLC [8,19]. Alternatively, it has been reported that the percentage of solid opacity of a tumor on HRCT is a useful predictor of a non-invasive tumor [20-22]. These reports also demonstrated that the TDR alone is insufficient to perfectly predict non-invasive tumors.

In this study we have successfully demonstrated that urinary DiAcSpm is a useful marker that significantly correlates with pathologically confirmed non-invasive tumors; thus, it may be used to identify suitable candidates for limited resection. DiAcSpm is one of the minor polyamine components secreted in human urine. Polyamine excretion increases with tumor cell proliferation via the activation of intracellular polyamine metabolism and turnover [23]. Chen and colleagues demonstrated that an increase in DiAcSpm levels is associated with the stimulation of oxidative catabolism of polyamines [24]. Moreover, Kuwata et al. recently reported that DiAcSpm levels were elevated in tumor tissues from both primary sites and liver metastasis, suggesting that DiAcSpm may be produced from cancer cells themselves [25].

We have previously reported that DiAcSpm is frequently elevated in patients with various cancers, including colorectal, breast, lung, prostate, testicular, renal, and pelvic cancer, with very low false-negative incidence [11,26]. It should be noted that compared to conventional tumor markers like CEA and CA19-9, urinary DiAcSpm level is more frequently elevated in the earlier stages of colorectal and breast cancer. In addition, it has been reported that poor prognosis of patients with urogenital malignancies is associated with an increase in urinary DiAcSpm [27]. Hiramatsu et al. also reported that there was a strong positive correlation between urinary DiAcSpm and disease progression [27], which is consistent with the current study results that high urinary DiAcSpm is positively correlated with tumor invasiveness (i.e., lymphatic permeation, vascular invasion, and lymph node metastasis). Therefore, we believe the results of the current study clearly indicate that urinary DiAcSpm level is significantly associated with pathologically confirmed tumor invasiveness in stage IA NSCLC.

The value for urinary DiAcSpm is usually normalized to creatinine (nmol DiAcSpm/g creatinine). Because DiAcSpm is not reabsorbed by the renal brush border, the glomerular clearance of DiAcSpm is comparable to that of creatinine [28]. This helps to explain why DiAcSpm, which is produced in tissues of early stage cancers and subsequently excreted into circulation, is recovered in the urine without significant loss and, therefore, may serve as a useful tumor marker that is highly sensitive for early stage cancers [11]. In one of our previous studies, we reported that the value for urinary DiAcSpm in a healthy male significantly differed from that in a healthy female [12]. We therefore separately employed the cut-off values for urinary DiAcSpm by gender.

| Variables | Risk factors | Risk ratio for invasive tumor | 95% CI | p-value* |
|-----------|--------------|-------------------------------|--------|----------|
| Tumor size (cm) | >2.0 | 2.871 | 1.347-6.119 | 0.006 |
| Urine DiAcSpm level (nmol/g creatinine) | high | 3.374 | 2.736-7.361 | 0.002 |
| Serum CEA (mg/dL) | ≥5.0 | 2.316 | 0.841-6.377 | 0.104 |
| TDR | < 0.75 | 6.103 | 1.962-18.98 | <0.001 |

*a Logistic regression analysis, CI = confidence interval, DiAcSpm = diacetylspermine, CEA = serum carcinoembryonic antigen level, TDR = tumor disappearance rate.
We considered using TDR as a complement to urinary DiAcSpm due to its high specificity for predicting non-invasive tumors. TDR was confirmed to be independently correlated with pathological tumor invasiveness. Thus, we have shown that the combination of TDR and urinary DiAcSpm was strongly correlated with pathological invasiveness. Our further analysis evaluating the correlation between clinicopathological characteristics and histological invasiveness, as defined in the IASLC/ATS/ERS classification of adenocarcinomas (Additional file 1: Tables S1 and S2), strongly supported the results from the analysis of the original patient cohort (Tables 2, 3, 4). In fact, the latter analysis revealed a stronger correlation between histological invasiveness and urinary DiAcSpm.

The mechanisms underlying an increase in urinary DiAcSpm value in cancer patients have not been fully understood, although a considerable amount of literature has reported the usefulness of urinary DiAcSpm as a novel tumor marker. The current study and further investigation may contribute to clarifying the mechanism and clinical significance of DiAcSpm.

There are several limitations that may exist in the current study. First, the study involved retrospective data collection in a small cohort of patients, and there is a possibility for bias due to selecting clinical stage IA. Further, although the HRCTs were retrospectively reviewed by two experienced observers who were blind to patient identification, image evaluation by the observers is subjective, thus, our evaluations may lack reproducibility. Secondly, we could not definitively confirm the appropriate use of limited resection, because our data did not include survival data. Therefore, in a future study, we should perform a follow-up with patients in the current cohort.

Conclusion
In conclusion, our data show that there is a significant correlation between urinary DiAcSpm and pathological tumor invasiveness in patients with clinical stage IA NSCLC. Future investigations should aim to elucidate the oncological significance and clinical usefulness of DiAcSpm.

Additional file

Additional file 1: Table S1. Relationship between histological invasiveness in IASLC/ATS/ERS classification and clinicopathological factors in adenocarcinoma cases. Table S2. Multivariate analysis for prediction of invasive adenocarcinoma among clinical Stage IA patients.

Abbreviations
NSCLC: Non-small-cell lung cancer; GGO: Ground-glass opacity; DiAcSpm: N², N⁴-diacetylsperrmine; BSA: Bovine serum albumin; CT: Computed tomography; PET: Positron Emission Tomography scan; HE: Hematoxylin and eosin; VvG: Victoria blue van Gieson; CEA: Carcinoembryonic antigen; TDR: Tumor disappearance rate; CI: Confidence intervals; HR: Hazard ratio; IASLC/ATS/ERS: The international association for the study of lung cancer, american thoracic society, and european respiratory society; AIS: Adenocarcinoma in situ; MIA: Minimally invasive adenocarcinoma; I-ADC: Invasive adenocarcinoma.

Competing interests
The authors have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article. The authors have no potential conflicts of interest to disclose.

Authors’ contributions
YT had full access to all of the data in this study. HH takes responsibility for the accuracy of the data analysis and KS takes responsibility for the integrity of the data. YT: contributed to the design and coordination of the study, prepared the manuscript, and approved the final manuscript. KS: contributed to preparing the manuscript and read and approved the final manuscript. HH: contributed to preparing the manuscript and read and approved the final manuscript. MK: contributed to design of the study, preparing the manuscript, and read and approved the final manuscript.

Funding
This work was supported in part by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (#21590639).

Author details
1Department of Thoracic Surgery, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, 3-18-22 Hon-komagome, Bunkyo-ku, Tokyo, Japan. 2Department of General Thoracic Surgery, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo, Japan. Center for Medical Research Cooperation, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kami-kitazawa, Setagaya-ku, Tokyo, Japan. 4Department of Thoracic Surgery, Nagano Prefectural Suzuki Hospital, 1332 Oaza-suzaka, Suzaka, Nagano, Japan.

Received: 8 July 2014 Accepted: 3 February 2015
Published online: 18 February 2015

References
1. Ginsberg RJ, Rubinstein LV. Randomized trial of lobectomy versus limited resection for T1 NO non-small cell lung cancer. Lung Cancer Study Group. Ann Thorac Surg. 1995;60:15–23.
2. Yankelevitz DF, Reeves AP, Kostis WJ, Zhao B, Henschke CI. Small pulmonary nodules: volumetrically determined growth rates based on CT evaluation. Radiology. 2002;217:251–6.
3. Tsubota N, Ayabe K, Doi O, Mori T, Namikawa S, Taki T, et al. Ongoing prospective study of segmentectomy for small lung tumors. Study Group of Extended Segmentectomy for Small Lung Tumor. Ann Thorac Surg. 1998;66:1787–90.
4. Yoshida J, Nagai K, Yokose T, Nishimura M, Kakunuma R, Ohmatsu H, et al. Limited resection trial for pulmonary ground-glass opacity nodules: fifty-case experience. J Thorac Cardiovasc Surg. 2005;129:991–6.
5. Nakao M, Yoshida J, Goto K, Ishi G, Kawase A, Aokage K, et al. Long-term outcomes of 50 cases of limited-resection trial for pulmonary ground-glass opacity nodules. J Thorac Oncol. 2012;7:1563–6.
6. Ichinoe Y, Yano T, Tokoyama H, Inoue T, Asahi H, Katsuda Y. The correlation between tumor size and lymphatic vessel invasion in resected peripheral stage I non-small-cell lung cancer. A potential risk of limited resection. J Thorac Cardiovasc Surg. 1994;108:684–6.
7. Suzuki K, Asamura H, Kusumoto M, Kondo H, Tsuchiya R. “Early” peripheral lung cancer: prognostic significance of ground glass opacity on thin-section computed tomographic scan. Ann Thorac Surg. 2002;74:1635–9.
8. Shimada Y, Yoshida J, Hishida T, Nishimura M, Ishii G, Nagai K. Predictive factors of pathologically proven noninvasive tumor characteristics in T1aN0M0 peripheral non-small cell lung cancer. Chest. 2012;141:1003–9.
9. Nitadori J, Bograd AD, Kadota K, Sima CS, Rizk NP, Morales EA, et al. Impact of micropapillary histologic subtype in selecting limited resection vs
lobectomy for lung adenocarcinoma of 2 cm or smaller. J Natl Cancer Inst. 2013;105:1212–20.

10. Kawakita M, Hiramatsu K. Diacetylated derivatives of spermine and spermidine as novel promising tumor markers. J Biochem. 2006;139:315–22.

11. Hiramatsu K, Takahashi K, Yamaguchi T, Matsumoto H, Miyamoto H, Tanaka S, et al. N1, N2-diacetylspermidine as a sensitive and specific novel tumor marker for early- and late- stage colorectal and breast cancers. Clin Can Res. 2005;11:2986–90.

12. Hiramatsu K, Sakaguchi K, Fujie N, Saitoh F, Takahama E, Motiya SS, et al. Excretion of N1, N2-diacetylspermidine in the urine of healthy individuals. Ann Clin Biochem. 2013;51:1459–67.

13. Samejima K, Hiramatsu K, Takahashi K, Kawakita M, Kobayashi M, Trumoto H, et al. Identification and determination of urinaryacetylpolyamines in cancer patients by electrospray ionization and time-of-flight mass spectrometry. Anal Biochem. 2010;401:22–9.

14. Travis WD, Mueller-Hermelink HK, Harris CC. World Health Organization classification of tumours: pathology and genetics of tumours of the lung, pleura, thymus and heart. 3rd ed. Lyon: IARC Press; 2004.

15. Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol. 2007;2:706–14.

16. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol. 2011;6:244–85.

17. Takahashi Y, Ishii G, Aokage K, Hishida T, Yoshida J, Nagai K. Distinctive histopathological features of lepidoic growth predominant node-negative adenocarcinomas 3–5 cm in size. Lung Cancer. 2013;79:118–24.

18. Takahashi M, Shigematsu Y, Ohta M, Tokumasu H, Matsukura T, Hitai T. Tumor invasiveness as defined by the newly proposed IASLC/ATS/ERS classification has prognostic significance for pathologic stage IA lung adenocarcinoma and can be predicted by radiologic parameters. J Thorac Cardiovasc Surg. 2014;147:54–9.

19. Okada M, Nishio W, Sakamoto T, Uchino K, Tsubota N. Discrepancy of computed tomographic image between lung and mediastinal windows as a prognostic implication in small lung adenocarcinoma. Ann Thorac Surg. 2003;76:1828–32.

20. Yokose T, Suzuki K, Nagai K, Nishiwaki Y, Sasaki S, Ochiai A. Favorable and unfavorable morphological prognostic factors in peripheral adenocarcinoma of the lung 3 cm or less in diameter. Lung Cancer. 2000;29:179–88.

21. Yoshida J, Nagai K, Yokose T, Takahashi K, Nishimura M, Goto K, et al. Primary peripheral lung carcinoma smaller than 1 cm in diameter. Chest. 1998;114:710–2.

22. Matsuuma H, Oki I, Nakahara R, Suzuki H, Kasai T, Kamiyama Y, et al. Comparison of three measurements on computed tomography for the prediction of less invasiveness in patients with clinical stage I non-small cell lung cancer. Ann Thorac Surg. 2013;95:1878–84.

23. Russell DI, Levy CC. Polyamine accumulation and biosynthesis in a mouse L1210 leukemia. Cancer Res. 1971;31:248–51.

24. Chen Y, Kramer DL, Li F, Poter CW. Loss of inhibitor of apoptosis proteins as a determinant of polyamine analog-induced apoptoses in human melanoma cells. Oncogene. 2003;22:4964–72.

25. Kuwata G, Hiramatsu K, Samejima K, Iwasaki K, Takahashi K, Koizumi K, et al. Increase of N1, N2-diacetylspermidine in tissue from colorectal cancer and its liver metastasis. J Cancer Res Clin Oncol. 2013;139:925–32.

26. Sugimoto M, Hiramatsu K, Kamei S, Kinoshita K, Hoshino M, Iwasaki K, et al. Significance of urinary N1, N8-diacetylspermidine and N1, N2-diacetylspermine as indicator of neoplastic diseases. J Cancer Res Clin Oncol. 1995;121:317–9.

27. Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K, et al. Diagnosis and prognostic usefulness of N1, N8-diacetylspermidine and N1, N2-diacetylspermine in urine as novel tumor markers of malignancy. J Cancer Res Clin Oncol. 1997;123:539–45.

28. Miki T, Hiramatsu K, Kawakita M. Interaction of N1, N2-diacetylspermine with polyamine transport systems of polarized porcine renal cell line LLC-PK1. J Biochem. 2005;30:1943–6.