ORIGINAL ARTICLE

Prognosis and morphometrical features of non-bronchioloalveolar cell adenocarcinoma: an assessment of the non-alveolar replacing area and high grade atypical area

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J Clin Pathol 2006;59:269–273. doi: 10.1136/jcp.2005.025908

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Accepted for publication 20 June 2005

Aim: It has become obvious that the prognosis of bronchioloalveolar cell carcinoma (BAC) in small peripheral adenocarcinoma of the lung is good, but most cases actually treated as pulmonary adenocarcinoma in hospitals tend to be non-bronchioloalveolar cell carcinoma (non-BAC). The prognoses of non-BAC are greatly varied. We studied the relationships between the morphometrical features and the prognoses of non-BAC.

Methods: In total, 69 cases of non-BAC measuring ≤ 20 mm in diameter across their greatest dimension were evaluated. We considered that the non-alveolar replacing area and the high grade atypical area were related to the prognosis, and therefore defined the ratio of both areas to the total area at the maximum face of the tumours as the non-alveolar replacing area ratio (NAAR) and the high grade atypical area ratio (HAAR), respectively. We thereafter analysed the relationships between both ratios and the prognosis.

Results: The NAAR and HAAR were significantly higher in the recurrent cases than in the recurrence free cases. We divided the non-BAC cases at the centre of both ratios into two groups, high and low. The 5 year survival rate of the high NAAR and high HAAR group was 48.3%, while that of the low NAAR and low HAAR group was 72.2%. The groups showing high rates for both ratios had significantly worse prognoses. A multivariate analysis indicated that the HAAR contributes most to the prognosis.

Conclusions: These results suggest that the NAAR and HAAR are closely associated with the prognosis of non-BAC.

Recently, the numbers of surgically resected cases of peripheral small sized lung cancer have increased with the expansion of mass check-ups and computed tomography (CT) examinations, and the characteristics of early adenocarcinoma have been gradually elucidated. Evidence suggests that the developmental form of peripheral small sized adenocarcinoma of the lung is important as a prognostic factor and that the prognosis of bronchioloalveolar cell carcinoma (BAC) is good. However, most cases that are treated in hospitals tend to be non-BAC, which has a worse prognosis. We focused on the broad spectrum of the lepidic development pattern along the external border, and of the nuclear atypia at the centre of non-BAC tumours, and quantified these characteristics and studied their relationships with the prognosis.

MATERIALS AND METHODS

In total, 896 cases of primary lung cancer were surgically treated at Kanagawa Prefectural Cancer Center and Kanto Rosai Hospital between May 1991, and December 1998. Of these, we studied 113 with peripheral adenocarcinoma ≤ 20 mm in diameter at the maximum face of the tumour, using formalin fixed specimens. No preoperative treatment such as chemotherapy or radiotherapy had been performed on the patients. We studied the clinical characteristics and histopathological features, metastases to lymph nodes, intrapulmonary metastasis, development pattern, and prognoses. We then classified the adenocarcinomas ≤ 20 mm in diameter as two types, BAC or non-BAC, defined according to the development pattern. BAC was defined as adenocarcinomas in which tumour cells progressed in a lepidic pattern on

the inner side of the alveolar wall, and did not destroy the alveolar components. Following the WHO classification, cases in which the cancer cells had invaded the interstitial component were classified as non-BAC.¹ As a result of the marked hyperplasia of the interstitial collagenous fibre inside the BAC and the progression of and increase in tumour cells in the alveoli, the conventional structure consisting of alveoli is destroyed, and the tumours lose air containment and develop a solid structure, thereby becoming non-BAC. On the other hand, some groups of adenocarcinoma show a papillary or tubular pattern of development, which may be marked even in the early phase. We also classified such groups as non-BAC.

In non-BAC lesions, it was possible to divide the area of the tumour into an alveolar replacing area at the most external part of the lesion and a solid area in which the conventional structure and alveolar component had been fully destroyed. We defined this solid area as the non-alveolar replacing area (NAA). We could also divide the tumour area into a high grade atypical area (HAA) and a low grade atypical area in the same cut face, because the differences in nuclear atypia in the same tumour were marked in many cases of adenocarcinoma (fig 1). We defined cases having features such as nuclear enlargement, eruption of nucleoli and rough chromatin as having high grade nuclear atypia, and defined the

Abbreviations: BAC, bronchioloalveolar cell carcinoma; CT, computed tomography; HAA, high grade atypical area; HAAR, high grade atypical area ratio; LBAC, localised bronchioloalveolar cell carcinoma; NAA, non-alveolar replacing area; NAAR, non-alveolar replacing area ratio

Figure 1 A case of non-BAC. High grade atypical area and low grade atypical area in the same small sized adenocarcinoma.

Figure 2 Marking the outline of the tumour and the borderline between the high grade atypical area (HAA) and the low grade atypical area on a digital image created by a scanner.

area in which most cells had high grade nuclear atypia and were crowded as the HAA. We also defined the NAA and HAA, each divided by the total area of the tumour, as the non-alveolar replacing area ratio (NAAR) and the high grade atypical area ratio (HAAR), respectively.

We directly took images of the maximum cross section of haematoxylin and eosin stained specimens using a film scanner (Dimage Scan Multi F-3000; Konica Minolta Photo Imaging Inc., Tokyo, Japan), confirmed the presence of the regional border using a microscope, and drew a border line between both groups on the display using imaging software (MacScope; Mitani Corp., Chiba, Japan) and a personal computer (Power Macintosh G3 350; Apple Computer, Inc., Cupertino, CA, USA) (figs 2, 3). We then measured and calculated the NAA, HAA, NAAR, and HAAR. This procedure was performed three times and the mean values used.

Figure 3 Methods for measuring an area.

Table 1 Characteristics of small sized adenocarcinomas

	BAC	non-BAC	Total
Age (years), mean (SD)	61.9 (8.5)	60.3 (10.8)	60.8 (10.2)
Sex (M/F)	13/18	35/47	48/65
Node metastasis			
N0	31	55	86
N1	0	9	9
N2	0	18	18
Intrapulmonary metastasis			
PM0	31	79	110
PM1	0	3	3
Recurrence			
(–)	31	43	74
(+)	0	39	39
Outcome			
Alive	25	39	64
Dead (lung cancer)	0	37	37
Dead (other disease)	6	6	12
Total	31	82	113

BAC, bronchioloalveolar cell carcinoma; non-BAC, non-bronchioloalveolar cell carcinoma.

Statistical analysis

The survival rate and recurrence free survival rate were calculated by the Kaplan-Meier method and calibrated by the generalised Wilcoxon test. We also used Student's *t* test for calibration between the means of two groups. Furthermore, we used χ^2 calibration or the Fisher direct probability method for independence between related factors. Calibration was significant when the *p* value was <0.05 . In addition, we used a Cox proportional hazard model for multivariate analysis. Before multivariate analysis, we excluded some factors using the Spearman hierarchy correlation coefficient and Pearson correlation coefficient. Furthermore, we cleaned up the rest of the factors by the stepwise method. StatView software for Macintosh (version 5.0; SAS Institute, Inc., Cary, NC, USA) was used for statistical analyses.

RESULTS

Characteristics and prognosis of BAC and non-BAC

We classified 113 cases of small sized peripheral adenocarcinoma into 31 BAC cases and 82 non-BAC cases based on the development pattern, and then investigated the patients' sex, age, metastases to lymph nodes, intrapulmonary metastasis, recurrence, and outcomes (table 1).

The observation period was >5 years in all cases. We did not observe a significant difference in sex or age between patients with BAC or non-BAC, and we did not observe metastases to lymph nodes, intrapulmonary metastasis, postoperative recurrence, or death due to tumours for any BAC case. In the non-BAC cases, we observed metastases to lymph nodes in 27 cases, intrapulmonary metastasis in 3 cases, and recurrence in 39 cases, and all cases with intrapulmonary metastasis had metastases to lymph nodes.

The 5 year survival rate of the BAC cases was 90.3%, and was significantly better than that of the non-BAC cases at 62.1%.

Morphometric measurement and prognosis of non-BAC

We assessed 69 of the 82 non-BAC cases. The remaining 13 cases were not suitable for morphometric measurement because of the condition of specimens and other technical reasons. A study of the metastases to lymph nodes and postoperative recurrence revealed that the NAAR, HAA, and HAAR were higher in cases that were positive for recurrence (table 2).

Furthermore, we divided the non-BAC cases into two groups, a high level ($n = 33$) and a low level ($n = 36$) group, based on the median value of NAAR and HAAR (four groups altogether), and examined the recurrence free survival rates and survival rates. The values of NAAR and HAAR were not necessarily related, and we considered them to be mutually independent parameters (fig 4). The 5 year recurrence free rate of the high level NAAR and high level HAAR group was significantly lower than that of the low level group (fig 5). The 5 year survival rate of the high level NAAR and high level HAAR group was also significantly lower than that of the low level group (fig 6).

Multivariate analysis

Before performing multivariate analysis with a Cox proportional hazard model, we examined factors such as the maximal diameter of the tumours, their total area, metastases to lymph nodes, NAA, NAAR, HAA, and HAAR using the hierarchy Spearman and Pearson correlation coefficients. Factors with $p > 0.7$ were excluded because we assumed them to be well correlated to each other. We examined the rest of the factors using the stepwise method, and excluded factors deemed unsuitable for multivariate analysis. Finally, only metastases to lymph nodes and HAAR were suitable for multivariate analysis. As a result of the multivariate analysis, the hazard ratio of HAAR was larger at 5.03 than that of metastases to lymph nodes at 1.59 (table 3).

DISCUSSION

The mechanisms of oncogenesis and progression have been elucidated for at least some lung adenocarcinomas. Recent studies suggest that most adenocarcinomas progresses from low to high grade atypical adenomatous hyperplasia, early BAC, and overt cancer, and finally becomes invasive carcinoma (adenocarcinoma with mixed subtypes).²⁻⁴ We previously studied small sized peripheral adenocarcinoma of the lung, and reported that cases with poor central fibrosis and short neoplastic cells in their tumours had a good prognosis, and that there was no recurrence in lepidic growth types sized ≤ 10 mm in diameter.⁵⁻⁶ We performed intentionally limited surgery as part of a prospective trial for BAC with a maximum diameter of ≤ 15 mm, and showed that the prognosis was good.⁷

Table 2 Prognosis and morphometrical features of non-BAC

	TA (cm ²)	NAA (cm ²)	HAA (cm ²)	NAAR	HAAR
Nodal metastasis					
–	1.11 (0.49)	0.76 (0.47)	0.59 (0.56)	0.70 (0.28)	0.53 (0.39)
+	1.20 (0.54)	0.95 (0.50)	0.81 (0.60)	0.79 (0.22)	0.64 (0.27)
Recurrence					
–	1.12 (0.50)	0.73 (0.46)	0.49 (0.55)*	0.67 (0.28)†	0.43 (0.38)‡
+	1.17 (0.51)	0.94 (0.49)	0.87 (0.56)	0.80 (0.22)	0.73 (0.26)

TA, total area of tumour; NAA, non-alveolar replacing area; HAA, high grade atypical area; NAAR, non-alveolar replacing area ratio = NAA/TA; HAAR, high grade atypical area ratio = HAA/TA. Values are expressed as mean (SD). **p* = 0.006; †*p* = 0.0495; ‡*p* = 0.0003.

Figure 4 The values of NAAR and HAAR were not necessarily related. The left photograph shows a case for which the HAAR was high, and the NAAR was low. The right photograph shows a case for which the HAAR was low, and the NAAR was high.

Although the ability to detect of minute lung cancer of early BAC is increasing with the increasing prevalence of CT examinations, these discoveries are still rare, and most cases that we treat at our hospital are non-BAC with destroyed alveolar structure. Many of these cases are classified as “adenocarcinoma with mixed subtypes” according to the WHO classification. Their biological malignancies may vary, and some groups may have a relatively good prognosis.

Noguchi classified adenocarcinoma ≤ 20 mm or less in diameter as types A to F, and found a good prognosis for type A and type B tumours (table 4).⁸ Our BAC may be equivalent to types A and B, and most non-BAC may be equivalent to types C and D. Several studies focused on the two components of small sized adenocarcinoma, the BAC component and non-BAC component, and tried to elucidate

the relationship between the morphological features and prognosis.^{9–10} Higashiyama measured the BAC component and classified adenocarcinoma into four types, from type I, which contains no BAC component (0%), to type IV, which consists only of a BAC component (100%), and reported that the type with a high ratio of BAC component had a good prognosis.¹¹ Sakurai focused on the grade of stromal invasion, and classified the invasive growth into four grades, from grade 0, which had pure bronchioloalveolar growth and no stromal invasion, to grade 3, which had stromal invasion into the centre of a fibrotic focus. They reported that tumours with grade 2 or less of stromal invasion showed a good prognosis.¹² For indices for objectively showing the atypia of neoplastic cells, the nuclear size, nuclear DNA dosage, and ploidy of chromosomes have been studied, and their

Figure 5 Estimated overall survival (recurrence free) rate for non-BAC and the 5 year recurrence free rate. (A) Non-alveolar replacing area ratio (NAAR); (B) high grade atypical area ratio (HAAR).

Figure 6 Estimated overall survival rate for non-BAC and the 5 year survival rate. (A) Non-alveolar replacing area ratio (NAAR); (B) high grade atypical area ratio (HAAR).

Table 3 Multivariate analysis for non-BAC

Variant	RC	SE	RC/SE	χ^2	p	HR
Node metastasis	0.47	0.163	2.85	8.14	0.0043	1.59
HAAR	1.62	0.558	2.90	8.40	0.0038	5.03

RC, regression coefficient; SE, standard error of mean; HR, hazard ratio; HAAR, high grade atypical area ratio.

Table 4 Noguchi's histological typing of small adenocarcinoma of the lung.⁸

Type	Description
A	Localised bronchioloalveolar carcinoma (LBAC)
B	LBAC with foci of collapse of alveolar structure
C	LBAC with foci of active fibroblastic proliferation
D	Poorly differentiated adenocarcinoma
E	Tubular adenocarcinoma
F	Papillary adenocarcinoma with compressive and destructive growth

TAKE HOME MESSAGES

- In total, 113 cases of peripheral adenocarcinoma of the lung ≤ 20 mm in maximum diameter were classified into 31 BAC cases and 82 non-BAC cases. The BAC cases had no node metastases, intrapulmonary metastases, or recurrence. The prognoses of the BAC cases were significantly better than those of the non-BAC cases.
- The NAAR and HAAR were studied in 69 non-BAC cases, and were higher in the recurrent and metastatic cases.
- We divided the cases into two groups, high and low, based on NAAR and HAAR, respectively. The prognoses of the high NAAR and HAAR groups were worse than those of the low groups.
- The NAAR and HAAR may thus be more closely and accurately associated with the prognosis of small sized adenocarcinoma of the lung, especially of non-BAC, than any other factor.

relationships with prognosis were previously reported.¹³ The immunohistochemical characteristics of BAC and non-BAC have also been studied.¹⁴⁻¹⁵ Aoyagi found that the change in histology from localised BAC (LBAC) to LBAC with active fibroblastic proliferation is characterised by a significant rise in the incidence of allelic losses and that deletions of 3p, 17p, 18q, and 22q significantly increase during malignant progression.¹⁶

We focused on non-BAC that are difficult to classify as type C or D by Noguchi's classification. For many cases classified as type C, the high grade nuclear atypia of non-alveolar replacing cells is frequently in the centre of the tumour, but cells from the alveolar replacing pattern of the external part show low grade atypia. On the other hand, even in cases classified as type D, low grade nuclear atypia of cells and the alveolar replacing pattern may frequently appear in the most

external area. If we can quantify an element of BAC, it may be an objective marker for predicting a patient's prognosis. We speculate that the NAAR and HAAR could become good prognostic factors for small sized non-BAC.

The recent evolution of business machines such as personal computers and film scanners has been remarkable. Formerly, it was an extremely complicated procedure to measure the area of a tumour in its cross section, requiring much time and the use of expensive machines. However, it is now possible to measure one case in several minutes after making permanent specimens. Because of the low price of these machines, it is possible to perform this measurement even in private hospitals or clinics. Further evolution of hardware and software will make more accurate and easier measurement possible in the future.

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