European Review for Medical and Pharmacological Sciences 2014; 18: 1373-1378

Significance of the histological and ultrastructural features of elastic fibers in diagnosis of bronchioloalveolar carcinoma

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Abstract. – OBJECTIVES: The accurate identification of bronchioloalveolar carcinoma (BAC) from adenocarcinoma (AC) and other types of lung cancer is important from clinical perspectives; especially, when BAC is histologically-mixed with AC.

We hypothesized that the elastic fibers (EF) pattern could be used as a differential marker to identify BAC from other lung cancers. The aim of this study was to characterize the EF pattern in different types of lung cancer and evaluate its significance for differential diagnosis of BAC.

PATIENTS AND METHODS: Clinical samples of different types of lung cancers were collected. The samples were stained by hematoxylin-eosin (H&E) staining for histopathological comparison. Then, modified Weigert's staining of the EF was performed to characterize its patterns. The EFs were semi-quantified and compared among different types of lung cancer. Further, transmission electronic microscopy (TEM) was performed and ultrastructural features of the EFs were compared between BAC and adenocarcinoma (AC).

RESULTS: H&E staining histopathology could differentiate the most types of lung cancer except certain types, such as histologically-mixed BAC and AC. The EF pattern in BAC was uniquely different from other types of lung cancer as > 95% of BAC was + or ++ for EF staining while > 95% of other types of lung cancer were - or ± type. TEM study further confirmed the EF pattern difference between BAC and AC.

CONCLUSIONS: As the data show, as > 95% BAC specimens can be identified from other lung cancers based on EF (Weigert's) staining. The EF pattern in BAC is uniquely different from other types of lung cancer and, therefore, can be used as a differential clinical marker of BAC.

Key Words:
Elastic fiber, Bronchiole alveolar carcinoma, Diagnosis, Lung cancer.

Introduction

Lung cancers are usually the malignant tumors with high incidence and mortality rates, severely threatening the human health. Different types of lung cancers include large-cell carcinoma (LC), small-cell carcinoma (SC), adenosquamous carcinoma (AdC), adenocarcinoma (AC), etc. In clinics, hematoxylin-eosin (H&E) stained histopathology is considered the gold standard for diagnosing different types of lung cancer[1]. However, it may become difficult sometimes to differentiate between certain types, such as in case of mixed features of AC with bronchioloalveolar carcinoma (BAC)[2,3]. Though BAC was traditionally thought to be a subtype of AC, the treatment options and prognosis of BAC differ greatly from those of AC[4]. Therefore, the accurate differentiation of BAC from AC and other types of lung cancer becomes important in clinical practice; especially, when BAC is histologically-mixed with AC. The presence or changed pattern of elastic fibers (EF) was observed in different types of cancers as a remarkable feature of the disease. The EF pattern was suggested as a diagnostic feature for certain types of cancers, such as ependymoma or as a measure for identification of cancer progression, such as regression in melanoma[5,6].

Over the past decades, as though several clinical features and methods have been evaluated for the differential diagnosis of BAC from AC or other lung cancers[2,4]; little attention has been paid to the EF as a characteristic feature for clinical diagnosis. Herein, we characterized the EF patterns in different types of lung cancers (BAC and AC) using histological and ultrastructural analyses and evaluated its potential as a marker for clinical diagnosis of BAC.

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Patients and Methods

In this study, 239 tissue samples of lung cancer were analyzed. These samples were collected from the patients that were admitted to the General Hospital of People's Liberation Army (PLA) from February, 1998 to January 2010 and were subjected to the surgical ablation of lung tumors. These included 66 BAC, 90 AC, 27 squamous carcinoma (SqCa), 18 adenosquamous carcinoma (AdC), 14 large cell carcinoma (LC), 12 small cell carcinoma and 12 carcinoid samples, whereas 50 normal lung tissue samples were included as control.

Histopathology

Tissue samples were fixed in 4% paraformaldehyde and paraffin embedding was performed using standard technique[7]. Hematoxylin and eosin (H&E) stained tissue sections (4 μm thick) were microscopically examined to determine the types of lung cancer. Later, modified Weigert's staining of the EF was performed. Briefly, paraffin-embedded sections were deparaffinized and processed in 0.5% potassium permanganate solution for 5min and then bleached in 1% oxalic acid. After washing in distilled water and 70% alcohol, the sections were stained by Weigert's staining solution as instructed[8]. A positive staining was indicated by royal blue color. The abundance of EFs was graded semi-quantitatively from non-existent (-) to present (+) or abundant (++).

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was performed for parallel analysis of the selected tissue samples as described in detail elsewhere[9]. Briefly, tissue samples were first fixed in glutaraldehyde, followed by post-fixation treatment with osmium tetroxide. Later, tissues were dehydrated in graded series of alcohol and embedded in epoxy resin. About 80nm thick sections were prepared and stained by uranyl acetate and lead citrate prior to examination at 60kV using a transmission electron microscope. Specific examination of the EF patterns was carried out.

Results

H&E Staining Characteristics of the EFs

H&E staining demonstrated that the histological characteristics varied among different types of lung cancers (Figure 1). Since it was difficult to clearly differentiate between histologically-mixed AC and BAC types, we resorted to modified Weigert's staining of the EFs and found that AC and other lung cancers were similar with respect to EF patterns. As shown in Figure 1, rare positive stained interstitium in the AC and other lung cancer samples could be observed by Weigert's staining. Further, quantification of the EF content showed that most of the samples were - or ± (~98% in analyzed samples; Table I and Figure 2). The apparent presence of EFs in AC samples was very rare (only one AC sample was graded as +). On the other hand, Weigert's staining of BAC specimens demonstrated the frequent presence of EFs as > 95% were stained as + or ++ (Table I and Figure 2). In this regard, 26/66 (~39.4%) samples were stained +, 39/66 (~59.0%) stained ++, and 1/66 (~1.5%) stained as ±. These EFs were mainly present in the alveolar wall and showed a tendency of hyperplasia. Morphologically, they were well-arranged and distributed continuously. The EFs in BAC specimens showed similarity with those present in the normal alveolar wall; however, the EF patterns in BAC specimens were typically different from those of other lung cancer types which was differentially revealed by Weigert's staining.

TEM Characteristics of the EFs

The TEM studies further revealed that the EFs in normal alveolar walls were mainly represented by two components i.e. a formless and homogeneous core and a surrounding layer of short microfibers. Compared with the collagenous fibers (CFs), EFs were of deeper color; and CFs were found to be abundant in the interstitium which appeared as intermittent light and dark bands. The EFs in BAC specimens demonstrated a certain degree of similarity with those from the normal alveolar wall. They were found scattered in the interstitium and intermixed with the CFs; and the EFs were more conspicuous in BAC than in normal specimens. Notably, the EF pattern in AC specimens was clearly different than that in BAC or normal specimens (Figure 3) wherein the EFs were rarely observed and most of the interstitium contained CFs. These findings were consistent with those of Weigert's staining and overall suggest that the histological features of BAC tissue specimens are different from those of other lung cancers and can be used as a marker for differential diagnosis of BAC.

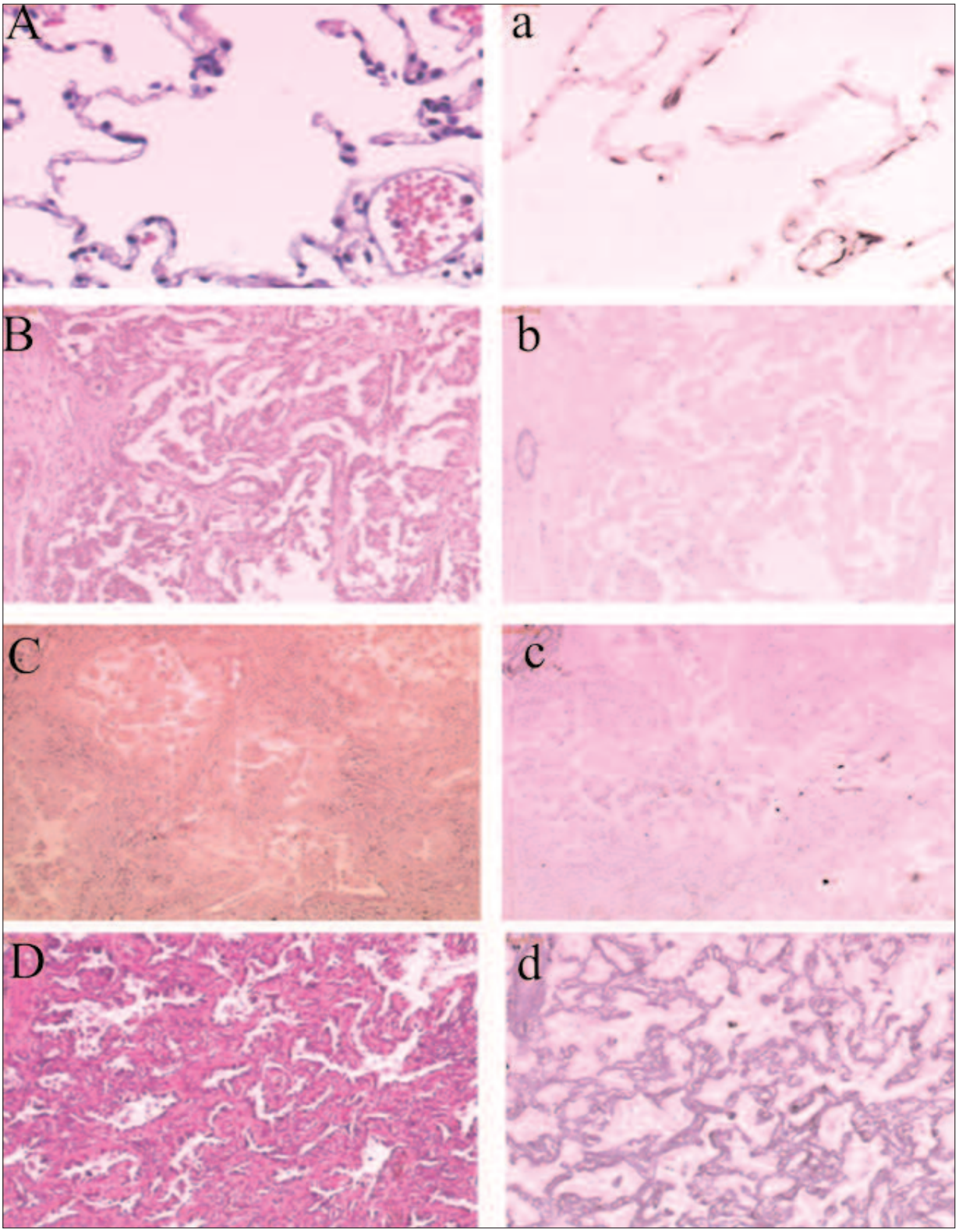

Figure 1. H&E and Weigert's staining (staining of the elastic fibers) of different types of lung cancer. H&E staining ***(A)*** and Weigert's staining ***(a)*** of the normal alveolar wall; H&E staining ***(B)*** and Weigert's staining ***(b)*** of papillary adenocarcinoma (AC); H&E staining ***(C)*** and Weigert's staining ***(c)*** of large-cell lung carcinoma (LC); and H&E staining ***(D)*** and Weigert's staining ***(d)*** of bronchioloalveolar carcinoma (BAC).

Table I. Weigert's staining and quantification of EFs in different types of lung cancer.

Cancer types	EF staining				Total
	–	±	+	++	
BAC†	0	1	39	26	66
AC‡	76	13	1	0	90
SqCa¥	26	1	0	0	27
Adenosquamous carcinoma	18	0	0	0	18
Large cell carcinoma	14	0	0	0	14
Small cell carcinoma	12	0	0		12
Carcinoid	12	0	0	0	12

Discussion

In this study, we found that the EF pattern in BAC specimens was typically different from that in other types of lung cancer. This histopathological feature enables an easy identification of the BAC among other types of lung cancer by simply performing EF staining (Weigert's staining). This finding is significant from the perspective of an accurate clinical diagnosis of BAC in order to allow sufficient time for therapy and prognosis. The typical features of EFs in BAC may be closely correlated with the growth pattern of the tumor[5,6]. As further revealed by TEM studies, cancer cells in BAC grew along the alveolar walls without destroying the core structure. Perhaps, this is why the interstitium in BAC specimens and the alveolar septum in normal tissue share some morphological similarity. The EF staining of BAC samples shows their similarity with the control specimens. However, we also observed that certain BAC specimens were more deeply stained by Weigert's staining as opposed to normal tissue samples which may be due to the following reasons: (1) the alveolar walls in BAC tissue were found to be shrunken slightly; and (2) significant hyperplasia of the EFs occurred in BAC.

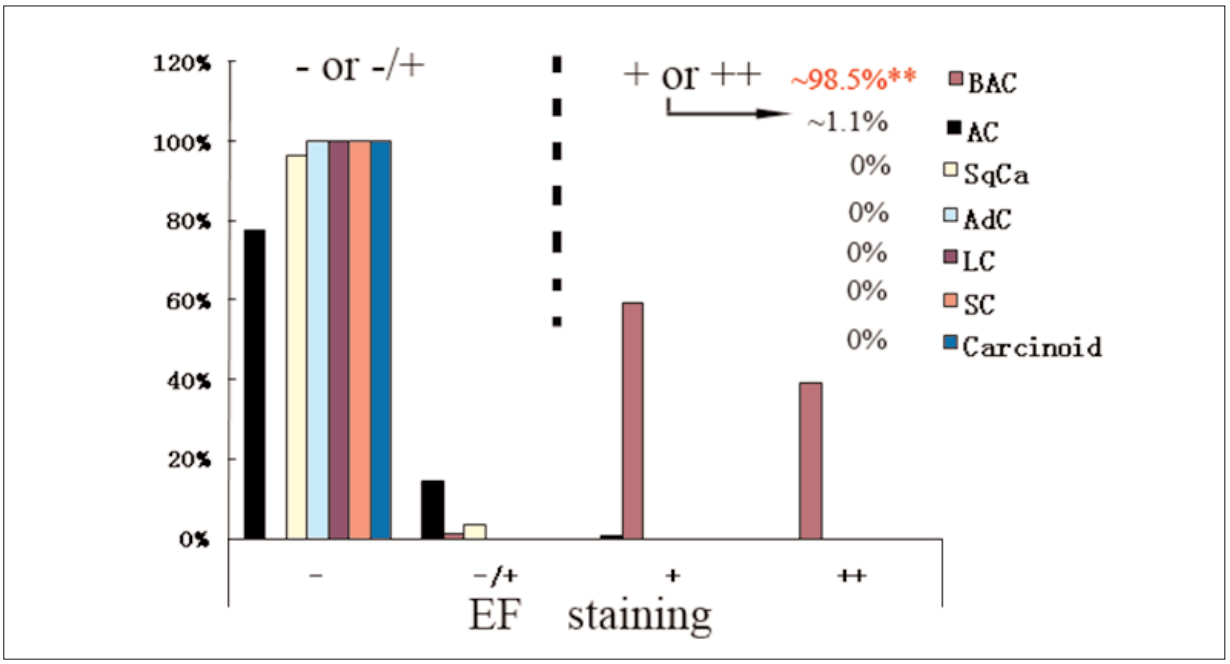

Figure 2. EF pattern distribution in different types of lung cancer. As shown, > 95% BAC samples demonstrate significant EF staining (+ or ++) while most of other types of lung cancer demonstrate a negative EF staining (-). BAC, AC, SqCa, AdC, LC, and SC represent bronchioloalveolar carcinoma, adenocarcinoma, squamous carcinoma, adenosquamous carcinoma, large cell carcinoma, and small cell carcinoma, respectively.

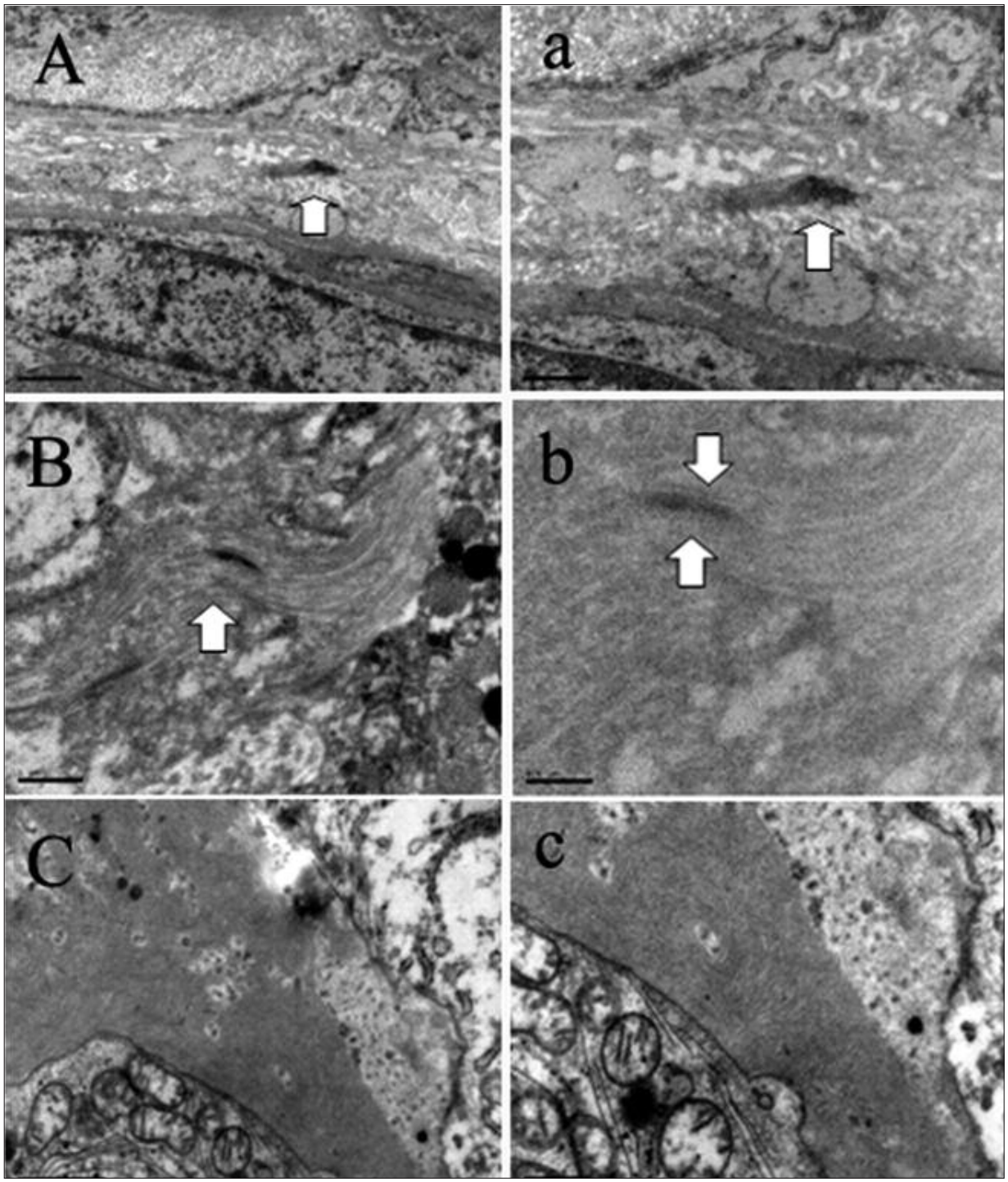

Figure 3. Ultrastructural (TEM) features of BAC and AC. Transmission electron microscopy revealed characteristic differences between BAC and AC. Mesenchymal elastic fibers are shown (*arrows*) in the normal lung at magnifications of 15K ***(A)*** and 30K ***(a)***; Mesenchymal elastic fibers are shown (*arrows*) in BAC specimen at magnifications of 15K ***(B)*** and 30K ***(b)***; Mesenchymal collagen fibers are visible in AC specimen at magnifications of 15K ***(C)*** and 30K ***(c)***.

As though BAC is conventionally thought to be a subtype of AC, the accurate diagnosis of BAC from AC is important in the clinical setting as the surgical and post-surgical treatments are different in each case. Herein, we performed both histopathological and ultrastructural (TEM) studies to distinguish the BAC from the AC. Of note, the characteristic EF pattern found in BAC samples was a useful feature for the differential diagnosis as > 95% BAC cases could be identified from other lung cancers based solely on EF staining (Table I and Figure 2).

A previous study by Honda et al[10] also studied elastic fibers and myofibroblasts in BAC and reported that myofibroblasts lay transversely and longitudinally in the interstitium, intertwined with the elastic fibers. The study concluded that myofibroblast proliferation was important in the alveolar wall folding and shrinkage observed in BAC. Similarly, Llanos et al[11] have also discussed the significance of anatomical and histopathological features, including fibroelastosis, in the cardiac manifestations of neonatal lupus. Of note, fibroelastosis was reported as the most significant diagnostic feature of endocardial disease[12,13].

However, Weigert's modified staining solution used in this study needs to be prepared fresh each time due to its relative instability and limited shelf life and, therefore, other staining methods such as immunohistochemical and molecular pathology techniques should also be included in the future studies to identify BAC from AC.

Conclusions

Elastic fiber pattern in BAC are typically different from that of other types of lung cancers. Hence, the characteristic EF pattern in BAC can be used as a potential feature for the accurate differential diagnosis of BAC in the clinical setting.

Acknowledgements

We thank all our colleagues from Pathology Department for critical comments and useful suggestions regarding this study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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