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ORIGINAL ARTICLE

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Mild emphysema: a novel method using formalin-fixed lungs for computed tomography and pathological analyses

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Abstract We evaluated a novel method of computed tomography (CT) analysis using formalin-fixed lungs of autopsy cases with mild emphysema. Eight formalin-inflated lungs (FILs) obtained at autopsy were examined using CT after draining off the formalin and air inflation with an air pump, and subjected to pathological study including pathological scoring of emphysema and microscopic image analysis (MIA). Satisfactory CT examination was carried out within 5 h of lung fixation. The mean alveolar area determined by MIA correlated highly with the lung volume (r=0.845) and CT score (r=0.722). This method is simple compared with conventional polyethylene glycol fixation for CT and enables CT examination of resected lungs without anxiety about biohazards. Mild emphysema can be detected by MIA.

Key words Emphysema of FILs · Post-mortem examination · Computed tomography · Microscopic image analysis

Introduction

Emphysema is defined as "a condition of the lung characterized by abnormal permanent enlargement of air space distal to the terminal bronchiole accompanied by the destruction of their walls" [1]. Emphysema is diagnosed by pathological examination using both macroscopic and microscopic observations of the lung. However, "the destruction" in this definition is not actually defined and is a subjective finding, difficult to analyse quantitatively. In order to overcome this ambiguous definition, several methods have been developed to assess the severity of emphysema. Among them are the panel grading method (emphysema score; ES) [15], the mean linear intercept [2], the internal surface area at a volume of 5 l [14] and the destructive index [13].

Clinically, computed tomography (CT) has been advocated for the radiological diagnosis of emphysema. High-resolution CT (HRCT) has been reported to be particularly useful for the diagnosis of mild emphysema, because of the fine anatomical structures it can visualize [6]. We considered that a comparative study of CT and microscopic image analysis (MIA) would provide important information that would play a role in the assessment of mild emphysema. However, it is not yet clear how much we can see at HRCT and how it is pathologically correlated. For this purpose, we still need a good comparative study based on pathological specimens.

Histopathologically, postmortem quantitative study of emphysema using lung tissue processed by conventional histological procedures is difficult, because formalin-fixed lungs shrink easily and are not suitable for radiological examination. Markarian and Daily devised polyethylene glycol, ethanol and formalin (PEF) fixative solution for fixing lungs, followed by drying with an air pump [10]. This fixing method yielded good results in preserving the lung shape and air spaces, which could be visualized by CT. However, it takes a long time to complete fixation (over 48 h) and drying (over 48 h) of a lung because this fixative is viscid and difficult to volatilize. Furthermore, PEF solution permeates less well and has a poorer fixing capacity than fomalin fixative.

Herein, we introduce a simple method useful for CT and pathological study using formalin-inflated lungs (FILs). We report preliminary data on the quantitative analysis of mild emphysema.

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Table 1 Clinical histories of the autopsy cases

Autopsy number	Age	Sex	Clinical diagnosis	Pulmonary lesion	Occupation	Brinkmann Index
A93-23	84	F	Acute cardiac failure	Pulmonary oedema	None	0
A93-30	53	M	Hepatocellular carcinoma	Pulmonary oedema	Office worker	0
A93-43	55	F	Uterine cancer	Emphysema, mild	None	0
A93-26*	80	M	Gastric cancer	Bronchopneumonia	None	2,500
A93-28*	83	M	Multiple myeloma	Bronchopneumonia	None	1,000
A93-62*	67	M	Hepatocellular carcinoma	Emphysema	Office worker	1,400
A93-27#	81	M	Duodenal ulcer, shock	Emphysema	Metal worker	0
A93-48#	58	M	Hepatocellular carcinoma	Metastatic tumour	Driver	0

* Smoker
# Dust-exposed cases

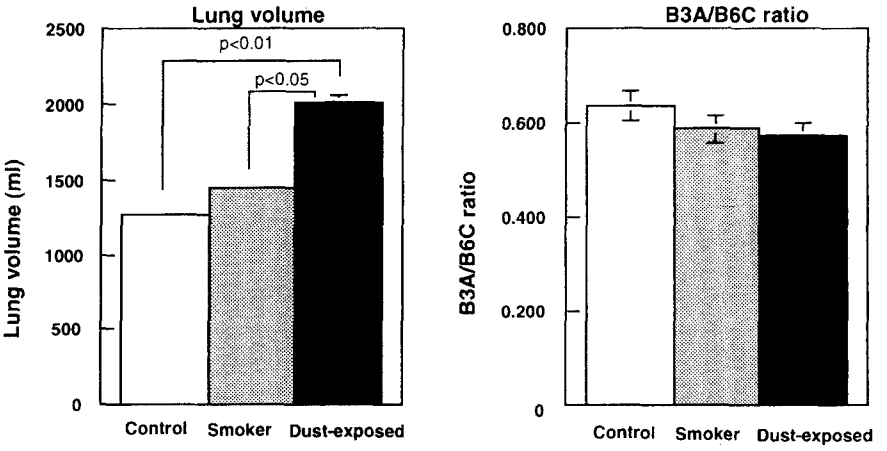

Fig. 1 Lung volumes of the control, smoker and dust-exposed groups. The mean lung volumes of the smoker ($P<0.05$) and the dust-exposed ($P<0.01$) groups are significantly larger than that of the control group. There were no significant differences in the B3A/B6C ratios of the three groups. *Error bars* show standard errors of the mean

Materials and methods

Eight left lungs obtained at autopsy were fixed by continuous perfusion of 15% v/v aqueous formalin at 25 cm water fixing pressure for at least 48 h using apparatus based on the model of Heard et al. [5]. The fixed lungs were photographed after macroscopic observation, and their volumes, weights and sizes had been measured. They were then semi-dried until their weights were similar to the prefixed ones at autopsy, using continuous air inflation with an air pump (a-1500, Nippon Suiso Industrial Company, Tokyo, Japan) for about 3 h. After this they were washed twice with tap water and rinsed with absolute ethanol by flushing them through the main bronchus.

A CT scan was performed on each FIL under continuous air inflation by the air pump, using a TCT 900S Scanner (Toshiba, Tokyo, Japan) with 2 mm collimation. Several slice-planes were scanned and one, a frontal slice at the level of main bronchus, was selected and the CT scores were determined by measuring the average CT values in two square 20 pixel×20 pixel areas of the anterior and hilar portions in this plane. The selected CT slice-line was marked on the pleura of each FIL using a magic marker to indicate the orientation for sectioning it.

To obtain an ES and carry out microscopic image analysis the lungs were cut along the plane equivalent to the CT slice-line and each cut surface was observed and photographed. The distances from the orifice of B3 to the apex (B3A) and from the orifice to B6 to the costo-phrenic angle (B6C) were measured whilst lung sectioning. Each whole-mount section of the paraffin block of lung tissue cut was stained with haematoxylin and eosin (H&E) and elastic van Gieson's (EVG) stains. The pathological ES based on the method of Thurlbeck were estimated by two surgical pathologists (T.H. and M.H.) using the EVG sections [15].

The area (AREA), maximum diameter (DMAX), minimum diameter (DMIN), circular diameter (DCIRCLE) and perimeter (PERIM) of each of 200–300 alveolar spaces equivalent to the portion used for CT scoring in the hilar and anterior portions of the lung were measured using 8 mm thick EVG sections at a magnification of ×1.25 using a computed MIA (IBAS, Kontron Electronic, Munchen, Germany). The computer programme employed for the input and analysis of alveolar images was designed so that other structures, such as blood vessels or exudates outside alveolar spaces could be omitted manually.

Pearson's method was employed to obtain relative coefficients and Scheffe's F test was used to evaluate the differences between the various parameters statistically. Differences at $P<0.05$ were considered to be significant.

Results

Clinical history of the cases

The eight patients (two females and six males) listed in Table 1 were 53–84-years-old (70.1±13.4, mean±standard deviation). Three of them (A93-26, A93-28 and A93-62; group S) were heavy smokers (Brinkmann index ≥1.000), two (A93-27 and A93-48; group D) had histories of occupational exposure to dust, but no history of smoking, and three (A93-23, A93-30 and A93-43; control group C) had histories neither of smoking nor dust exposure. None of the eight patients had any respiratory complaints before admission to hospital.

Table 2 Pathological diagnosis, emphysema score, computed tomography (*CT*) score and macroscopic data of each lung (*CLE* centrilobular emphysema, *PE* peripheral emphysema, *HU* Hounsfield units, *ant* anterior portion, *hilar* hilar portion)

Autopsy number	Pathological diagnosis	Emphysema score	CT score ant	(HU) hilar	Weight (g)	Volume (ml)	Length (cm)	B3A (cm)	B6C (cm)	B3/B6 ratio
A93-23	CLE, Mild	10	−690.0	−720.3	250	1260	22.3	8.0	13.5	0.59
A93-30	CLE, Trace	5	−658.6	−448.0	330	1270	23.8	7.5	11.0	0.68
A93-43	CLE, Trace	5	−773.8	−918.3	250	1270	24.1	7.6	12.0	0.63
A93-26*	CLE, Mild	15	−722.2	−617.5	390	1450	21.0	8.8	14.8	0.59
A93-28*	CLE, Mild	15	−423.1	−475.3	720	1470	25.0	8.8	15.0	0.59
A93-62*	CLE, Mild	10	−764.1	−623.6	440	1420	24.4	8.3	14.0	0.59
A93-27#	CLE and PE, Mild	20	−852.9	−888.4	350	2045	27.8	9.0	15.0	0.60
A93-48#	CLE, Mild	15	−828.8	−832.1	320	1980	25.1	7.6	13.5	0.54

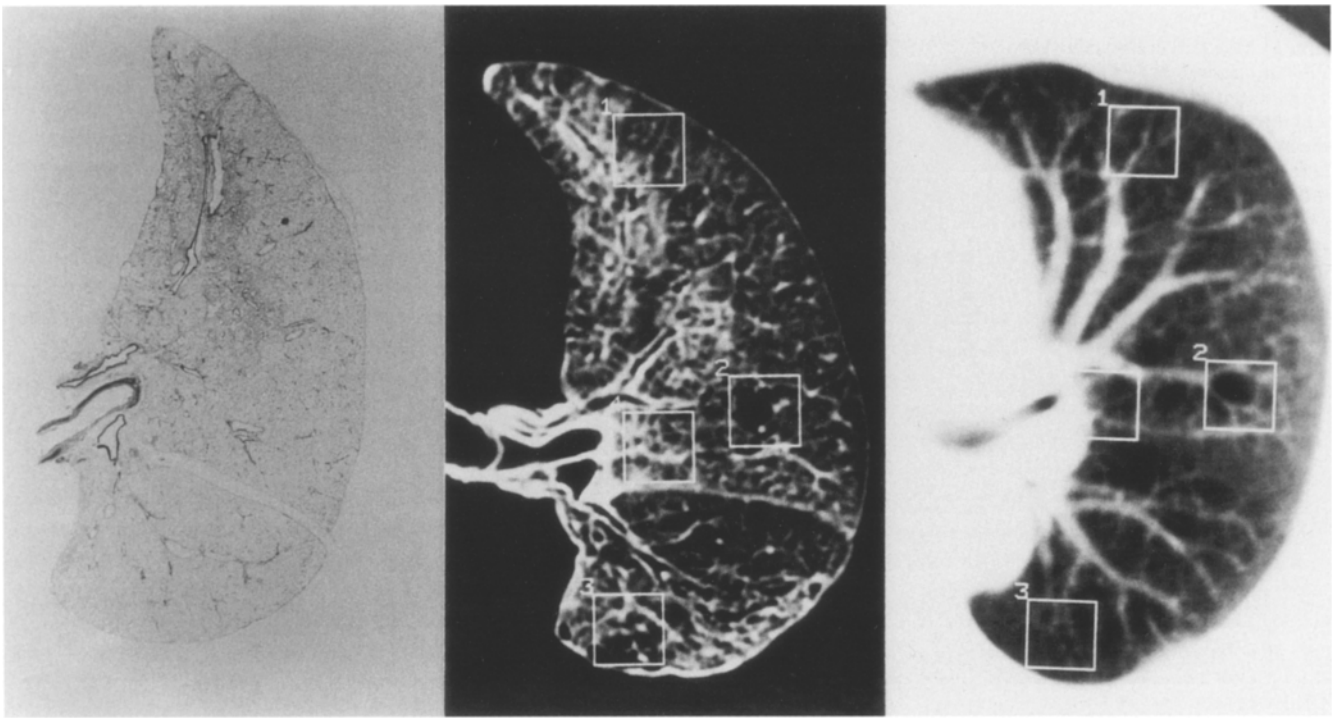

Fig. 2 Representative features (A93-62) of whole-mount section (**A**), post-mortem high-resolution computed tomography (CT; **B**) and pre-mortem CT (**C**) images of smoker's lung. The post-mortem CT image corresponds with both the pre-mortem image and the features of the whole-mount section (elastic van Gieson's stained). The CT scores in each post-mortem CT portion are similar to those of the pre-mortem scores (See Table 3)

Macroscopic and histopathological examinations of the lungs

The lung volumes and B3A/B6C ratios among the C, S and D groups described above were compared. The mean lung volume of group D was significantly higher than groups S ($P<0.05$) and D ($P<0.01$). The B3A/B6C ratio of group D was lower than the values for groups S and C, but the differences were not significant (Fig. 1). The pathological diagnosis and ES of each lung determined from whole-mount sections, are listed in Table 2. All the cases were diagnosed as having trace to mild emphysema (ES<20).

Table 3 Pre-mortem and post-mortem CT scores of the lung from a case with mild emphysema (*SD* standard deviation)

Lung portion	CT scores (HU)			
	pre-mortem		post-mortem	
	mean	SD	mean	SD
Anterior (1)*	−858.9	43.1	−764.1	177.4
Lateral (2)	−846.0	63.8	−893.1	191.3
Posterior (3)	−861.7	25.9	−782.8	232.9
Hilar (4)	−722.5	188.4	−623.6	234.7

* Numbers in parentheses corresponds to the square numbers in Figure 2

CT scores of the lungs

The CT scores of the anterior and hilar portions of each FIL are listed in Table 2. They correlated well ($r=0.881$). A pre-mortem CT was obtained and compared with the post-mortem CT in one case (A93-48), whose clinical di-

Table 4 Mean alveolar area (*AREA*), circular diameter (*DCIRCLE*), maximum diameter (*DMAX*), minimum diameter (*DMIN*) and perimeter (*PERIM*) measured with image analysis

Parameters	Portion	A93-23	A93-30	A93-43	A93-26*	A93-28*	A93-62*	A93-27#	A93-48#
AREA ($\times 10^3$ μm^2)	Anterior	123.62	126.57	129.61	157.79	157.66	143.24	259.08	165.51
	Hilar	147.18	152.38	143.02	167.96	137.91	125.50	294.14	132.82
DCIRCLE (μm)	Anterior	302.60	302.96	315.22	328.76	328.38	297.32	392.72	328.22
	Hilar	308.62	336.92	323.42	333.52	329.98	289.08	462.04	318.66
DMAX (μm)	Anterior	484.32	443.98	456.66	504.42	518.66	461.34	662.22	517.38
	Hilar	551.82	506.86	488.90	493.34	501.74	434.30	767.12	482.58
DMIN (μm)	Anterior	263.74	282.78	296.48	299.00	307.80	262.22	338.06	285.42
	Hilar	243.56	304.84	304.28	309.40	298.38	263.32	425.02	279.30
PERIM (μm)	Anterior	1712.08	1719.66	1874.16	1949.60	2036.22	1790.66	2866.48	1878.44
	Hilar	1839.44	1904.82	2061.96	1970.84	1844.60	1610.12	3436.70	1709.76

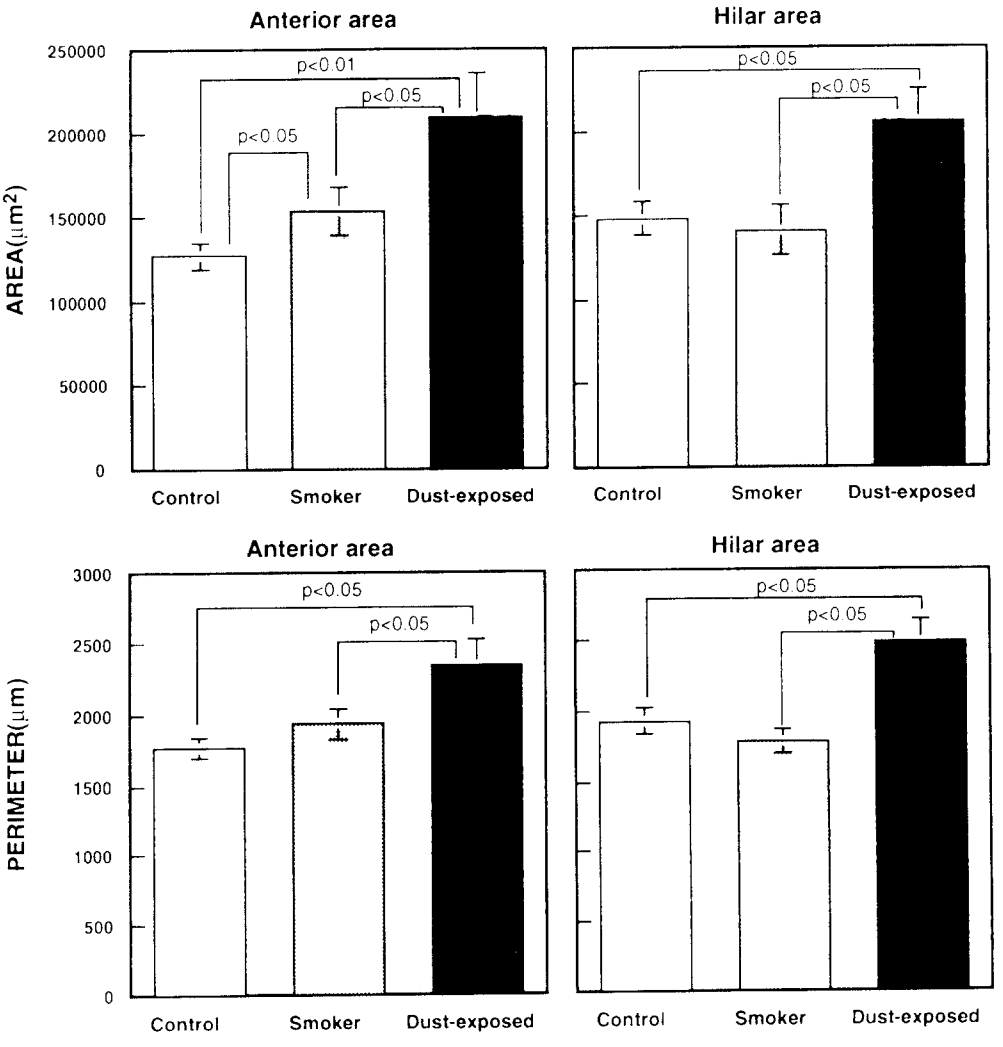

Fig. 3 Mean alveolar area (*AREA*) and perimeter (*PERIMETER*) in the control, smoker and dust-exposed groups. The mean area and perimeter of the anterior lung portion show more highly significant difference between controls and smokers than those of hilar portions. *Error bars* show standard errors of the mean

agnoses were shown, by CT, to be mild emphysema and lung metastases from hepatocellular carcinoma. The post- and pre-mortem CT images and CT scores were similar to each other (Fig. 2, Table 3).

Microscopic image analysis of the alveoli

The AREA, DMAX, DMIN, DCIRCLE and PERIM of each lung portion are listed in Table 4. The AREA correlated well with the PERIM (r=0.867), DMAX (r=0.972), DMIN (r=0.876) and DCIRCLE (r=0.975). The mean AREA (Ant-AREA) and PERIM (Ant-PERIM) of the anterior lung portion of group C were significantly lower than those of groups S (P<0.05) and D (P<0.01). The mean AREA (Hil-AREA) and PERIM (Hil-PERIM) of hilar lung portions of group C showed similar differences compared with groups S (P<0.05) and D (P<0.05); Fig. 3).

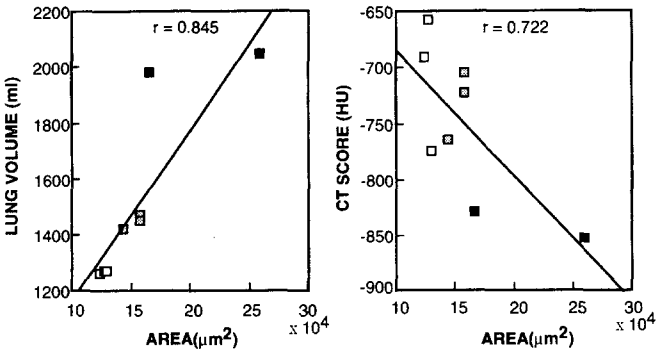

Fig. 4 Correlation of lung volume and CT score with AREA. There is a high degree correlation between AREA and both lung volume and CT score. (*Open square* control, *grey square* smoker, *black square* dust-exposed cases)

Comparative study of macroscopic examination, CT score and MIA data

Of the various macroscopic data evaluated, the best correlation was between Ant-AREA and lung volume (r=0.845). This parameter also correlated well with the CT score (r=0.722) of the anterior lung portion (Fig. 4). The CT score of the hilar portion showed little correlation with AREA (r=0.401).

Discussion

Recently, CT has been found to be useful for the clinical diagnosis of emphysema, especially in centrilobular emphysema (CLE). Foster et al. reported that visual evaluation of CT images using the emphysema criteria of non-peripheral low-attenuation areas could provide clinical information about the presence and degree of CLE [3]. However, they pointed out that there was considerable overlap between normal lung architecture and trace to mild emphysema diagnosed using their method. Moderate to severe CLE can be diagnosed easily by radiological or physiological examination of a given patient. However, it is extremely difficult to assess mild CLE and differentiate between it and normal lung architecture. In the present study, a simple method using FILs enabled post-mortem and pre-mortem CT images of lungs to be compared within a short time after fixation. Furthermore, this examination could be carried out under safer conditions than using autopsy lung in a fresh state; full formalin-fixation can eliminate the risks of biohazards. For CT findings were measured CT values of certain portions of the lung and used them as CT scores. This may be problematic since the lung specimens are in a different condition from the lungs in a live patient. However, the lung specimens were kept inflated under continuous air pumping similar to breathing. In addition, in our patients, the post-mortem and pre-mortem CT scores were in good agreement.

Pathologically, alveolar destruction is notably non-uniform and is virtually impossible to estimate using conventional quantitative methods, especially in patients with mild CLE [7]. The destructive index, which represents alveolar destruction, was shown to correlate with the ES and some pulmonary function tests [11], but it does not represent the actual alveolar size and is an indirect measure of emphysema. It is reasonable to assume that morphological changes of alveoli, such as their area, reflect the structural destruction associated with emphysema directly. To our knowledge, no reports describing MIA of alveoli associated with emphysema have been published. The results of the present study show that EVG sections of lungs were more suitable than H&E sections for obtaining alveolar structure data for the image analyser input because EVG staining demonstrated alveolar septa with high contrast [18]. Morphometry carried out by Weibel demonstrated that although the normal alveoli had a mean diameter of 250 mm, the alveolar size within a lung varied considerably [17]. The DMAX and DMIN values of the control alveoli in our study ranged from 484 to 456 and 263 to 296 mm, respectively, and appear to be larger than the early morphometric values. The difference may be attributable to the high age of our cases and the use of inflated lungs [16].

With respect to the present results, it is noteworthy that the AREA determined using MIA correlated not only with the ES but also with the CT score in the same lung portion. Evaluation of CT scores is proved to be useful to grade mild emphysema. In a comparative study of CT scores and emphysema grades, the "density mask" CT score that showed a high degree of correlation with ES was reported to be – 910 Hounsfield Units (HU) [12]. Using this method, there was significant correlation between the extent of emphysema demonstrated by CT and forced expiratory volume/forced vital capacity percent of predicted, functional residual capacity percent predicted and Dsb percent predicted [8]. Although we measured CT scores on lung specimens and the data obtained on living patients could not be directly applied, our results showed that a CT score indicative of emphysema was about – 800 HU. A clinico-pathological study of mild emphysema using CT and pathology scores of resected lungs has been reported [9]. These authors concluded that HRCT could help to identify the presence and grade of mild emphysema as the CT and pathology scores showed a high degree of correlation. In our study, hilar CT scores were less useful than peripheral CT scores for diagnosing emphysema. Furthermore, of the macroscopic data evaluated, the AREA showed the best correlation with FIL volume. The FIL volume may reflect the grade of emphysema, as emphysematous lungs were found to be more distended than normal lungs when subjected to constant inflation pressure, due to the loss of elasticity [4].

Not surprisingly, AREA, DCIRCLE, DMAX, DMIN and PERIM showed high degrees of correlation with each other in each patient. However, it should be noted that PERIM was proven to show a high correlation with AREA. This finding indicates that it may be possible to diagnose mild emphysema using non-inflated lungs by

measuring PERIM, because this parameter is constant irrespective of whether the fixed lung is inflated or not.

Therefore, MIA of alveoli enabled mild emphysema to be detected and graded, but there were some limitations to diagnosing all kinds of emphysema using this method. First, moderate to severe emphysema could not be quantified accurately because markedly distended air spaces could not be included in the microscopic field of the image analyser. Second, massive amounts of intra-alveolar material, such as exudates, interfered with the accurate demonstration of alveolar structure.

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