

# Pulmonary Emphysema in Cystic Fibrosis Detected by Densitometry on Chest Multidetector Computed Tomography

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## Abstract

**Background:** Histopathological studies on lung specimens from patients with cystic fibrosis (CF) and recent results from a mouse model indicate that emphysema may contribute to CF lung disease. However, little is known about the relevance of emphysema in patients with CF. In the present study, we used computationally generated density masks based on multidetector computed tomography (MDCT) of the chest for non-invasive characterization and quantification of emphysema in CF.

**Methods:** Volumetric MDCT scans were acquired in parallel to pulmonary function testing in 41 patients with CF (median age 20.1 years; range 7–66 years) and 21 non-CF controls (median age 30.4 years; range 4–68 years), and subjected to dedicated software. The lung was segmented, low attenuation volumes below a threshold of –950 Hounsfield units were assigned to emphysema volume (EV), and the emphysema index was computed (EI). Results were correlated with forced expiratory volume in 1 s percent predicted (FEV1%), residual volume (RV), and RV/total lung capacity (RV/TLC).

**Results:** We show that EV was increased in CF (457±530 ml) compared to non-CF controls (78±90 ml) ( $P<0.01$ ). EI was also increased in CF (7.7±7.5%) compared to the control group (1.2±1.4%) ( $P<0.05$ ). EI correlated inversely with FEV1% ( $r_s=-0.66$ ), and directly with RV ( $r_s=0.69$ ) and RV/TLC ( $r_s=0.47$ ) in patients with CF ( $P<0.007$ ), but not in non-CF controls. Emphysema in CF was detected from early adolescence (~13 years) and increased with age ( $r_s=0.67$ ,  $P<0.001$ ).

**Conclusions:** Our results indicate that early onset emphysema detected by densitometry on chest MDCT is a characteristic pathology that contributes to airflow limitation and may serve as a novel endpoint for monitoring lung disease in CF.

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## Introduction

Cystic fibrosis (CF) lung disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and is the most common genetic form of chronic obstructive pulmonary disease (COPD) [1,2]. CFTR

malfunction results in airway surface dehydration and impaired mucociliary clearance leading to airway mucus obstruction, neutrophilic inflammation and bacterial infection [3–5]. It is well established that this pathogenic sequence lead to early onset bronchiectasis that contributes to progressive loss of lung function and disease burden in patients with CF [5–7].

Histopathological studies in necropsy specimens from patients with CF performed in the 1960s to 1980s also reported structural changes in the peripheral airways consistent with emphysema [8–10]. Our previous studies in mice with airway-specific overexpression of the  $\beta$ -subunit of the epithelial Na<sup>+</sup> channel (ENaC) demonstrated that CF-like airway surface dehydration does not only cause chronic mucus obstruction and inflammation, but also emphysema [11–14]. Further, recent studies showed that cigarette smoke decreases CFTR expression and function [15,16], and that CFTR protein expression correlates inversely with emphysema severity in lungs from patients with cigarette smoke-induced COPD suggesting that impaired CFTR function may be implicated in emphysema formation in humans [17]. However, in contrast to COPD, where emphysema has long been recognized as an important phenotype [18], limited imaging data on emphysema in CF is available [19,20] and the clinical relevance of emphysema in CF remains largely unknown.

Multidetector computed tomography (MDCT) of the chest is widely used for the quantification of emphysema in cigarette smoke-induced COPD and  $\alpha$ 1-antitrypsin deficiency [21,22], employing density masks generated by dedicated post-processing tools based on Hounsfield units (HU). Previous MDCT imaging and histomorphological studies of lung parenchyma defined a threshold density of -950 HU on inspiratory MDCT and demonstrated that values below this density are diagnostic for emphysema and correlate well with loss of lung function in COPD [21,23,24]. Further, previous studies also demonstrated that MDCT allows to distinguish emphysema from air-trapping [25–27].

Based on previous histopathological studies [8–10], potential pathophysiological commonalities with cigarette-smoke induced COPD [15–17] and our own results from a mouse model of CF lung disease [11–14], we hypothesized that emphysema is present and contributes to airflow limitation in patients with CF. To test this hypothesis, we used MDCT of the chest as a non-invasive method to study the frequency and severity of emphysema in CF. Emphysema indices were determined from thin-section MDCT employing computationally generated density masks and results obtained for CF patients were compared with non-CF controls. To study the relationship between emphysema and lung function, emphysema severity was correlated with pulmonary function testing (PFT). Finally, emphysema severity was correlated with age to determine the onset and progression of emphysema in patients with CF.

## Materials and Methods

### Ethics Statement

The study was carried out as a retrospective analysis of clinically indicated MDCT performed between April 2003 and January 2012 and has been approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg. Informed written consent for examination and further data processing was obtained from patients or legal guardians.

**Table 1.** Characteristics of study population.

	CONTROL	CF
Age [a]	30.4 (4-68)	20.1 (7-66)
Sex	13 ♂ / 8 ♀	22 ♂ / 19 ♀
MDCT n =	21	41
PFT n =	15	39
$\Delta$ PFT-MDCT [d]	1 (0 - 66)	0 (0 - 73)
FEV1 [l]	3.7 $\pm$ 0.9	1.6 $\pm$ 1.2 <sup>†</sup>
FEV1%	102 $\pm$ 16	46 $\pm$ 30 <sup>†</sup>
VC [l]	4.3 $\pm$ 1.0	2.4 $\pm$ 1.3 <sup>†</sup>
VC%	100 $\pm$ 15	64 $\pm$ 23 <sup>†</sup>
RV [l]	1.8 $\pm$ 0.6	2.8 $\pm$ 1.5*
RV%	107 $\pm$ 26	192 $\pm$ 71 <sup>†</sup>
TLC [l]	5.9 $\pm$ 0.8	5.3 $\pm$ 2.1
TLC%	101 $\pm$ 9	103 $\pm$ 12

Summary of age, gender and lung function data from patients with cystic fibrosis (CF) and non-CF controls (CONTROL), who underwent multidetector computed tomography (MDCT) and pulmonary function testing (PFT), including forced expiratory volume in 1 s (FEV1), vital capacity (VC), residual volume (RV), and total lung capacity (TLC). Data given as mean or median  $\pm$  SD or with data range in brackets as appropriate. \*  $P < 0.05$ , <sup>†</sup>  $P < 0.001$ .

### Study Population

Table 1 provides a summary of the clinical characteristics of our study population. The diagnosis of CF was established by clinical symptoms characteristic of CF, increased sweat Cl<sup>-</sup> concentrations and/or detection of disease causing mutations in the *CFTR* gene as previously described [28]. The *CFTR* genotypes of CF patients are provided in the online supplement (Table S1). All CF patients showed characteristic signs of CF lung disease such as bronchial wall thickening, mucus plugging and bronchiectasis of at least one lobe. The non-CF control group was recruited from non-smoking patients who obtained a diagnostic chest MDCT for various indications but showed no evidence of airway disease, emphysema or major parenchymal changes upon reading of the diagnostic MDCT scan. Additional information is provided in the online supplement (Methods S1).

### Multidetector Computed Tomography

Non-enhanced MDCT at end-inspiratory breath-hold in supine position and thin-section reconstructions with a medium soft kernel algorithm were performed as previously described [21,29]. Further details are provided in the online supplement (Methods S1).

### Quantitative MDCT Densitometry

The MDCT images were analyzed using a custom in-house software (YACTA) as previously described, and controlled for extra-corporal air attenuation [29,30]. After the segmentation of the lung from the stack of MDCT images, a lung voxel was assigned to emphysema if its density was equal to or below the threshold of -950 HU, as routinely used for the quantification of emphysema in COPD [21,31]. The volume of the segmented lung (LV) and emphysema (EV), EV/LV ratio (pixel index = emphysema index, EI), lung weight (LW), mean lung density in

HU (MLD) and the 15<sup>th</sup> percentile of the density histogram (15th) were calculated automatically. 15th is defined as the threshold value in HU for which 15% of lung voxels have a lower density. A manual correction of the results was carried out to exclude sacculations, abscesses, cysts or bronchiectases from emphysema voxels (Figure S1). This step became necessary in most CF patients and took around 15 min per patient. Additional information on densitometry is provided in the online supplement (Methods S1).

### Pulmonary Function Testing

The following lung function parameters (absolute and percent predicted values) acquired by whole-body plethysmography were chosen for correlation analysis: forced expiratory volume in 1 s (FEV1, FEV1%), vital capacity (VC, VC%), FEV1 to VC ratio (FEV1/VC, "Tiffeneau index"), residual volume (RV, RV%), total lung capacity (TLC, TLC%). To estimate the degree of hyperinflation, the RV to TLC ratio was calculated (RV/TLC). Additional information is provided in the online supplement (Methods S1 and Figure S2).

### Statistical Analysis

Data were analyzed using SigmaPlot® (Systat Software GmbH, Erkrath, Germany). Groups were compared by Student's t-test or Wilcoxon rank sum test, and the Pearson  $r$  (absolute values) or Spearman rank order correlation coefficient  $r_s$  (EI, percent predicted values) were calculated for selected MDCT vs. PFT parameters as appropriate. A  $P$ -value of  $<0.05$  or  $<0.05/m$  (number of tests) with Bonferroni's method to correct for multiple testing was accepted to indicate statistical significance [32].

## Results

### Detection of emphysema in patients with CF by MDCT

Different patterns of emphysematous lesions were observed in CF patients with increasing age and severity of lung disease (Figure 1). In young CF patients with a low EI, emphysema voxels were mainly observed in the subpleural regions. With increasing EI, more voxels were found along bronchovascular structures with an emphasis on the lung periphery (Figure 1A,B). High EI resulted in extensive involvement of the parenchyma with a spread to the perihilar region (Figure 1C,D). Some CF patients with advanced lung disease showed a centrilobular and paraseptal emphysema pattern (Figure 1E–G).

### Quantification of emphysema in CF lung disease

For quantification of emphysema in our CF study population, we next determined LV, EV, EI, LW, MLD and 15th, and compared values obtained from CF patients with non-CF controls (Figure 2). These quantitative analyses of the density masks demonstrated that LV remained unchanged (Figure 2A), but that EV ( $P<0.01$ ) and EI ( $P<0.001$ ) were significantly increased in CF patients compared to non-CF controls (Figure 2B,C). LW was also increased in CF ( $P<0.001$ ), probably due to areas of increased density, e.g. due to mucus or

**Table 2.** Correlation analysis of densitometry with lung function.

		FEV1	FEV1% VC	FEV1/VC RV	TLC	RV/TLC		
CONTROL	LV	0.67	0.75*	0.67	-0.14	0.16	0.79*	-0.38
	LW	0.47	0.61*	0.40	0.19	0.13	0.29	-0.02
	EV	0.67	0.71*	0.70	-0.04	0.37	0.83*	-0.13
	EI	0.55	0.67*	0.62	0.01	0.33	0.78*	-0.04
	MLD	-0.47	-0.62*	-0.54	0.10	-0.37	-0.66	0.15
	15th	-0.48	-0.70*	-0.52	0.00	-0.39	-0.67	0.14
CF	LV	0.29	-0.46*	0.50*	-0.56*	0.75*	0.92*	0.37
	LW	0.15	-0.44*	0.35	-0.49*	0.80*	0.88*	0.35
	EV	0.24	-0.55*	0.40	-0.65*	0.59*	0.73*	0.48*
	EI	-0.27	-0.66*	0.05	-0.63*	0.69*	0.61*	0.47*
	MLD	-0.09	0.22	-0.23	0.38	-0.42	-0.46*	-0.19
	15th	0.16	0.56*	-0.01	0.64*	-0.62*	-0.49*	-0.50*

Summary of correlation analyses between densitometry on chest multidetector computed tomography (MDCT) and pulmonary function testing in patients with cystic fibrosis (CF) and non-CF control subjects (CONTROL). Pearson  $r$  or Spearman  $r_s$  rank order coefficient were calculated for lung volume (LV), lung weight (LW), emphysema volume (EV), emphysema index (EI), mean lung density (MLD), and 15<sup>th</sup> percentile of lung density (15th) with forced expiratory volume within 1 s (FEV1, FEV1%), vital capacity (VC), Tiffeneau index (FEV1/VC), residual volume (RV), total lung capacity (TLC), and RV/TLC ratio. \*  $P<0.05/7$  (Bonferroni's method, 7 tests per MDCT parameter).

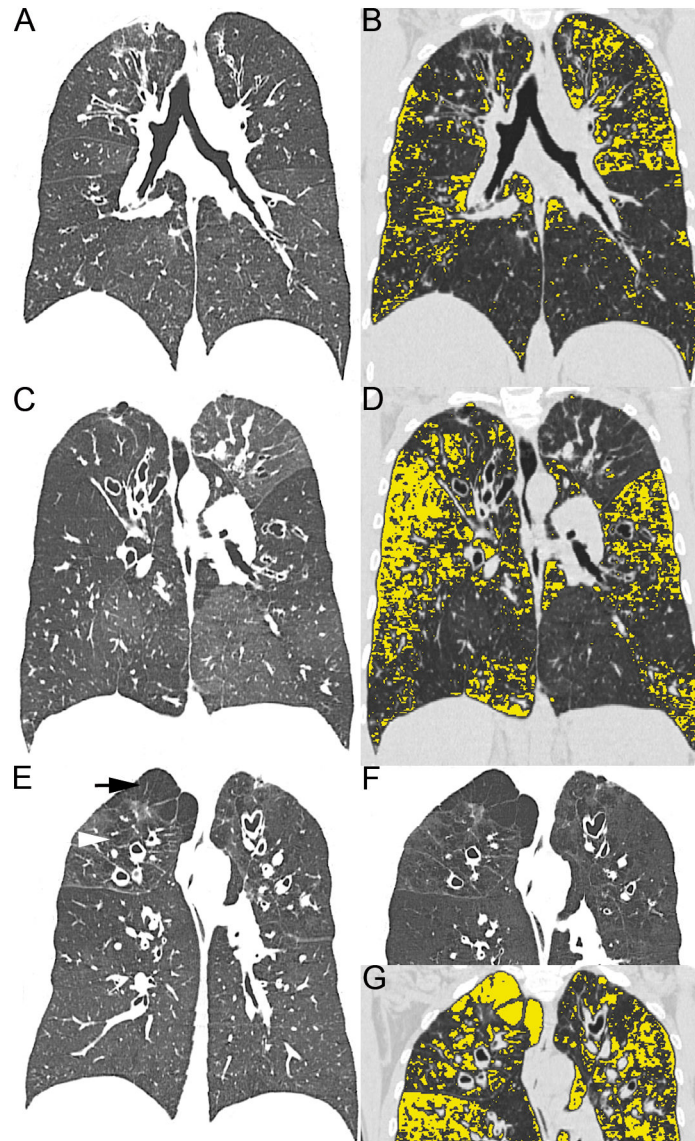
inflammation, whereas MLD was not different in CF compared to non-CF controls (Figure 2D,E). Finally, 15th was significantly reduced in CF patients versus controls ( $P<0.05$ ) (Figure 2F). Taken together, these results identify emphysema as a characteristic lesion in CF lung disease.

### Correlation between emphysema severity and lung function in CF

Next, we studied the correlation between quantitative emphysema indices, as determined from MDCT densitometry and pulmonary function (Tab. 2 and Figure 3). As shown in Figure 3, EI showed a significant inverse correlation with FEV1% ( $r_s = -0.66$ ,  $P<0.05/7$ ) (Figure 3A), i.e.  $r_s^2 = 43\%$  of the decrease in FEV1% may be explained by variations in EI. Further, EI was directly correlated with total RV as well as RV/TLC (Figure 3B,C). This relationship between lung density and lung function was also confirmed by significant correlations of 15th with FEV1%, RV and RV/TLC in CF, but not in non-CF controls (Tab. 2). These results indicate that emphysema contributes to airflow limitation in CF.

### Timing of onset and progression of emphysema in CF lung disease

Plotting the EI against age demonstrated that normal lung ageing was associated with a small increase of EI in individuals from the non-CF control group. In the CF group, emphysema severity correlated significantly with patient age, and the slope of incline with age was significantly larger in CF (regression slope of 0.35) compared to non-CF controls (regression slope 0.04) ( $P<0.0001$ ) (Figure 4). The limits of the 95% confidence



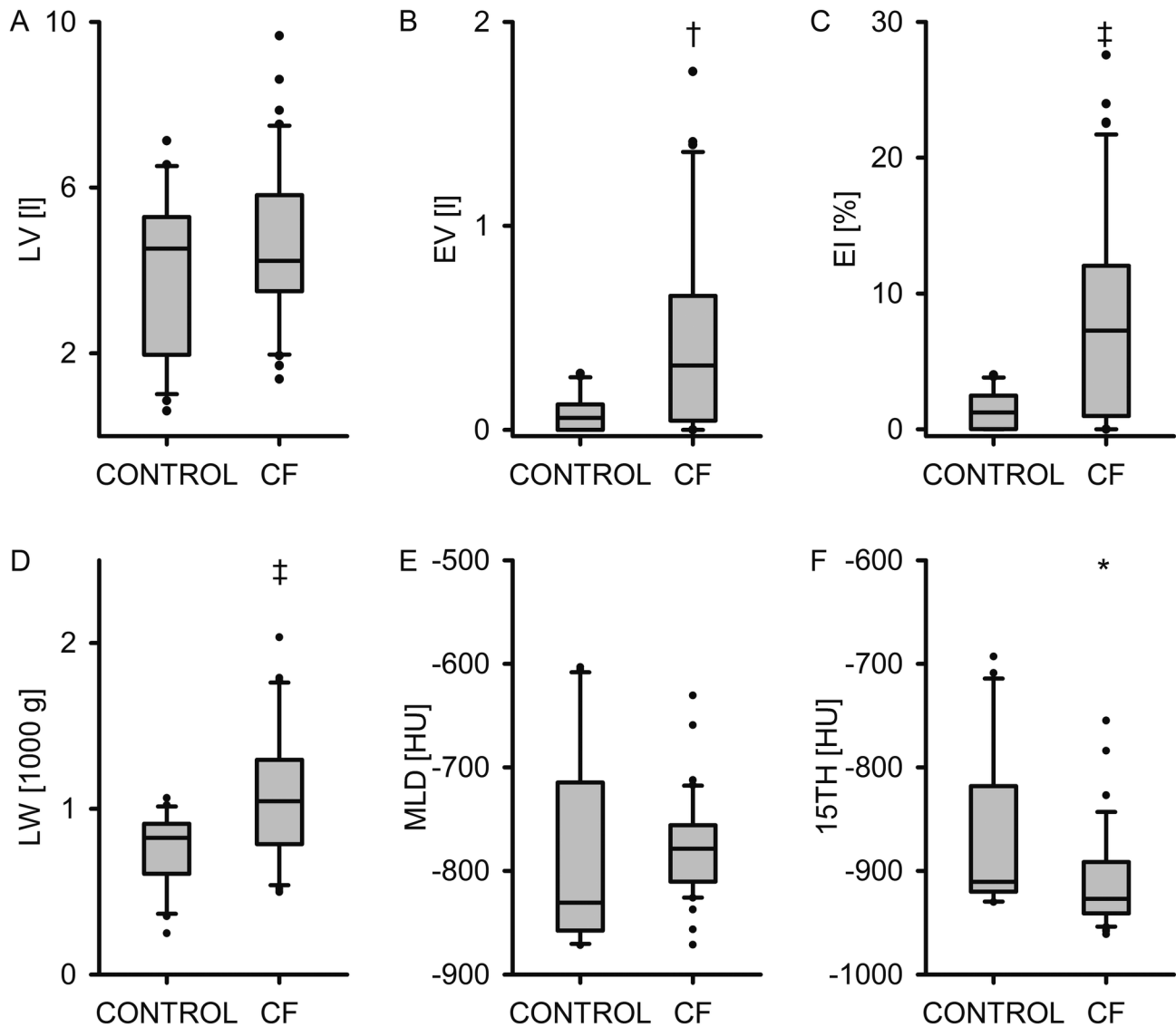
**Figure 1. Visualization of emphysema distribution in cystic fibrosis (CF) patients by chest MDCT density masks.** (A–G) Representative examples of morphologic images from non-enhanced multidetector computed tomography (MDCT) of the chest (left panels A, C, E) are complemented by density maps generated by dedicated software highlighting low attenuation areas below -950 Hounsfield units (HU) in yellow (right panels B, D, G). (A,B) MDCT image of a 36 year-old female CF patient with FEV1% = 48% showing bronchiectasis (A) as well as hypodense areas corresponding to emphysema (EI = 13.2%) mainly along subpleural and bronchovascular structures (B). (C,D) 38 year-old male CF patient with FEV1% = 29% with the lung parenchyma of the upper segments of the inferior lobes showing an overall hypodense texture and constricted vasculature (C). The density map shows extensive emphysema (EI = 24.0%) of both lungs with an emphasis on the lower lobes (D). (E–G) 46 year-old male cystic fibrosis patient (FEV1% = 55%) with marked bullous paraseptal emphysema of the right lung apex (black arrow) and centrilobular emphysema predominantly of both upper lobes (white arrowhead) (E). Note that these bullae do not possess walls differentiating them from cysts or sacculations (compare Figure S1). The minimum intensity projection (MinIP, 5 mm slice thickness) emphasizes emphysema visualization by accentuating low attenuation areas (F) with an overall EI of 18.0% (G).

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intervals for regression curves obtained from CF patients and non-CF controls intersected at ~13 years of age (Figure 4). These results suggest that the majority of CF patients develop significant emphysema beyond this threshold age.

## Discussion

Emphysema is a major disease phenotype that determines the morbidity and mortality of many patients with cigarette

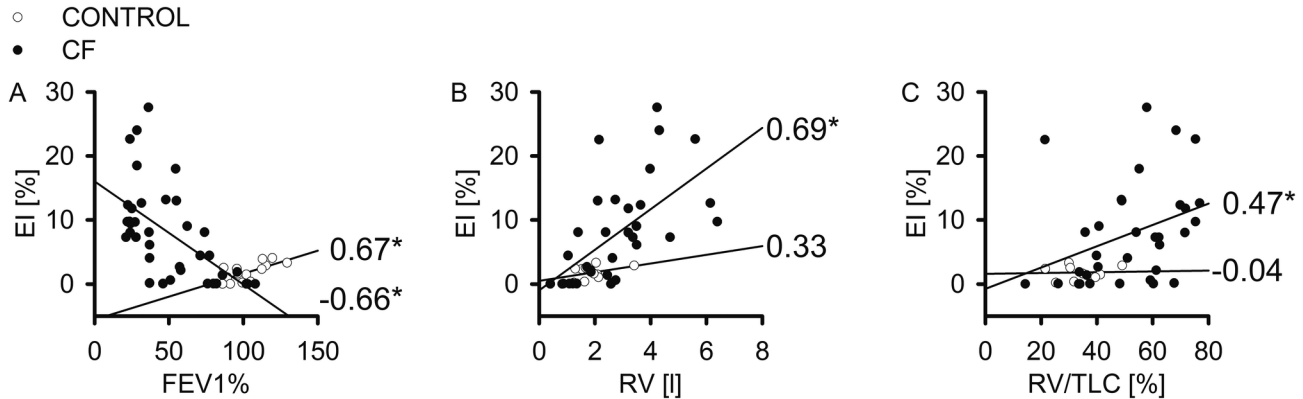


**Figure 2. Quantification of emphysema in cystic fibrosis (CF) lung disease by densitometry.** (A–F) Box-and-whisker plots for lung volume (LV) (A), emphysema volume (EV) (B) and emphysema index (EI) (C), lung weight (LW) (D), mean lung density (MLD) (E) and 15<sup>th</sup> percentile of the lung density histogram (15<sup>th</sup>) (F) in the non-CF control group (CONTROL) and patients with CF. The central line represents the median, the box encompasses the 25<sup>th</sup>-75<sup>th</sup> percentiles, whiskers show 10<sup>th</sup> and 90<sup>th</sup> percentiles, and closed circles (•) represent individual outliers. \*  $P<0.05$ , †  $P<0.01$  and ‡  $P<0.001$  compared to CONTROL.

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smoke-induced COPD [33]. Although it is well established that CF and COPD share key features including small airways mucus obstruction and chronic pulmonary inflammation [5,34], little is known about the frequency of occurrence and clinical relevance of emphysema in patients with CF [8,35,36]. Besides histopathological post-mortem studies [8–10], emphysema was depicted in CF in some previous MDCT imaging studies including a semi-quantitative visual scoring system developed for assessment of morphological changes of the CF lung [19,20]. However, these studies did not report any quantitative or densitometric data on emphysema. Furthermore,

subsequent work did not further assess the relevance of emphysema, but rather focused on the development of bronchiectasis [37,38] and the contribution of air-trapping to ventilation impairment [39,40] in CF lung disease. Air-trapping results in regional hypoperfusion on inspiratory MDCT, and may be diagnosed more sensitively by paired inspiratory/expiratory MDCT [40]. Of note, previous imaging studies in patients with COPD demonstrated that air-trapping is associated with a density range between -860 and -950 HU, which is higher than the density threshold defining emphysema [41].



**Figure 3. Emphysema severity correlates with impairment in lung function in cystic fibrosis (CF).** (A–C) Dot plots with linear regression curves for emphysema index (EI) plotted against forced expiratory volume in 1 s percent predicted (FEV1%) (A), residual volume (RV) (B), and RV as ratio of total lung capacity (RV/TLC) (C) for patients with CF and the non-CF control group (CONTROL). Spearman rank order correlation coefficients ( $r_s$ ) are given for each plot. \*  $P < 0.05/7$  (Bonferroni's method, see Table 2).

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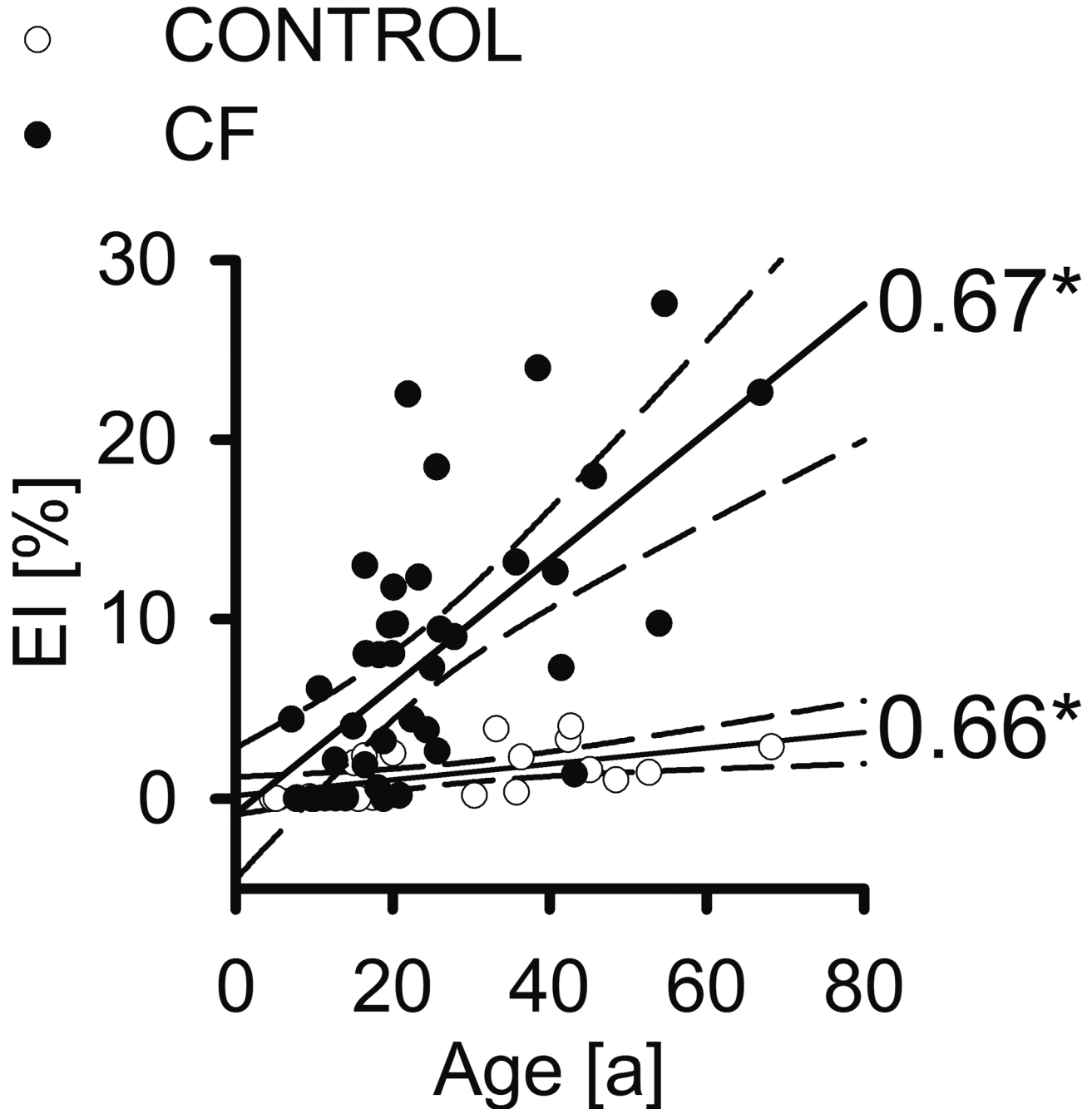
In phenotyping of patients with COPD, MDCT has long been accepted for the visual and computational quantification of emphysema [21,31,42], including recent large epidemiological trials (COPDGene, ECLIPSE) [43,44]. In the present study, we demonstrate that CF patients develop significant emphysema in addition to airway mucus plugging and bronchiectasis (Fig. 1). Using MDCT densitometry as a non-invasive method with a threshold value of -950 HU, and indices well established for the diagnosis and quantification of emphysema in patients with cigarette smoke-induced COPD [31], emphysema in CF patients was evidenced by a significant increase in EV and EI (Figures 1 and 2). Similar to previous studies in patients with COPD [26,29], emphysema severity in CF correlated significantly with airflow limitation and hyperinflation, as determined from FEV1%, RV and RV/TLC (Figure 3). Based on this correlation, we estimate that on average, emphysema accounted for ~43% of FEV1% reduction in the CF patients included in our study. These results show that emphysema is a clinically relevant phenotype contributing to the severity of lung disease in a subgroup of patients with CF. Further, our results suggest that chest MDCT densitometry might be a suitable non-invasive method for the diagnosis and quantitative monitoring of emphysema progression in individual patients with CF.

Compared to patients with COPD, overall emphysema severity was moderate in our cross-sectional study in children and mostly young adults with CF [29,45,46]. Values for EV and EI were on average less elevated in CF compared to the values previously reported for patients with advanced stages of COPD. Further, the mean MLD, often used as an emphysema marker in COPD, did not differ and the estimated lung weight (LW) was increased rather than reduced in patients with CF compared to non-CF controls (Figure 2). We speculate that normal MLD and elevated LW in CF may result from areas with increased density due to regional mucus retention, inflammation and/or compensatory hyperperfusion, which may

all hamper the use of MLD and LW as emphysema parameters in CF. However, the values for 15th of lung density were significantly reduced in CF compared to age-matched non-CF controls (Figure 2). Taken together, these results support the notion that lesions with elevated tissue density and emphysema coexist in the CF lung, and suggest that the EI and 15th may be more reliable than MLD in estimating emphysema severity in CF.

Correlating the EI with age demonstrated that, in contrast to common early lesions of the conducting airways such as mucus obstruction associated with air-trapping, airway wall thickening and bronchiectasis [7,47], emphysema is rarely present in children with CF (Figure 4). However, emphysema formation was observed in early adolescence (~13 years of age) and emphysema severity progressed in adult patients with CF (Figure 4). In contrast, consistent with previous reports in healthy adults, little emphysema was observed in non-CF controls (Figures 2 and 4) [48]. This timing of occurrence and progression shows that early onset emphysema is a characteristic feature of CF lung disease, and suggests that emphysema develops secondary to chronic airways disease in patients with CF. The clinical relevance of this phenotype is highlighted by an increase in life expectancy of patients with CF with a median survival of ~40 years in North America and Western Europe [49,50].

In COPD, emphysema pathogenesis with structural damage and remodeling of distal airspaces has been linked to cigarette smoke-induced oxidative stress, inflammation, extracellular matrix proteolysis, alveolar cell death, and disrupted alveolar maintenance triggering apoptosis and autophagy [51]. We speculate that CFTR dysfunction may trigger several of these mechanisms and thereby induce emphysema formation in patients with CF. First, it is well established that airway surface dehydration caused by CFTR malfunction in airway epithelia is an important disease mechanism that impairs mucociliary clearance and triggers the pathogenetic cascade of airway



**Figure 4. Emphysema progresses with age in cystic fibrosis (CF).** Dot plots with linear regression curves for emphysema index (EI) plotted against patient age for patients with CF and the non-CF control group (CONTROL). Spearman rank order correlation coefficients ( $r_s$ ) are given for each plot. Dashed curves indicate 95% confidence intervals. \*  $P < 0.001$ .

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mucus obstruction, chronic inflammation and bacterial infection in CF lung disease [3–5]. Our previous studies in  $\beta$ ENaC-overexpressing mice demonstrated that mucus obstruction and airway inflammation caused by airway surface dehydration are associated with emphysema formation *in vivo* with increased lung volumes, distal airspace enlargement, increased lung

compliance and reduced density of lung parenchyma, as determined from volumetric CT studies [12,14,52]. Recent studies indicate that airway surface dehydration causes impaired *in vivo* clearance of inhaled particulates and bacterial products such as lipopolysaccharide (LPS), which trigger the recruitment of macrophages and neutrophils, and increase

secretion of elastolytic proteases such as macrophage elastase (matrix metalloprotease 12) and neutrophil elastase into the airspaces [53–55]. Hence, similar to cigarette smoke-induced COPD [56], proteolytic damage of distal airspaces due to a protease/antiprotease imbalance caused by proteases released in chronic inflammation, may also play an important role in emphysema formation in CF. A second link between CFTR dysfunction and emphysema formation was suggested by recent studies demonstrating i) that cigarette smoke exposure reduces CFTR expression and function [15,16,57] *in vitro* and *in vivo*; ii) that CFTR protein levels correlate inversely with ceramide accumulation and emphysema severity in lungs from COPD patients [17]; and iii) that CFTR controls cigarette-smoke induced apoptosis and autophagy in mice [58]. These studies suggest that, in addition to airway surface dehydration and mucostasis caused by impaired CFTR Cl<sup>-</sup> channel function, CFTR dysfunction may cause other abnormalities on the cellular level, such as altered ceramide metabolism, that may play an important role in alveolar inflammation and emphysema formation in CF [59–61]. However, further studies are required to determine the relative role of these mechanisms for emphysema formation in patients with CF.

In addition to further mechanistic studies on emphysema pathophysiology, it will also be important to assess the relationship between *CFTR* genotypes, as well as treatment regimens, and emphysema development in CF [1,5,28]. Due to the limited number of patients available for analysis, we were not able to address these issues in this retrospective study. Hence, future longitudinal studies in larger patient cohorts are necessary to determine the impact of different classes of *CFTR* mutations, differences in treatment regimens and adherence to therapy, as well as other environmental and genetic factors on emphysema in patients with CF.

In summary, we demonstrate that early onset and progressive emphysema is a characteristic feature of CF lung disease. Emphysema severity determined by chest MDCT correlated with airflow limitation, suggesting MDCT densitometry as a non-invasive method for detection and monitoring of emphysema progression in individual patients with CF. Our results also suggest that emphysema contributes to disease severity and may therefore serve as a novel endpoint for monitoring of lung disease in patients with CF.

## Supporting Information

**Figure S1. Necessity of manual adaptation of density maps.** Coronary reconstructions of a multidetector computed

tomogram of the chest of a 22 year-old female cystic fibrosis patient without density map (A), with the density map (emphysema depicted in yellow color) generated by the automatic software algorithm (B), and after manual adaptation to exclude cystic lesions and bronchiectasis in the right superior lobe (black arrows). Emphysema severity may be overestimated by the automatic software algorithm, if they are not connected to the airway tree or airway segmentation was interrupted. The emphysema index of the right lung was calculated as 15.3% without manual correction (B) and 13.6% after manual correction (C).

(TIF)

**Figure S2. Validation of segmented lung volume from inspiratory computed tomography (CT) against pulmonary function testing.** Dot plot with linear regression curve for lung volume (LV) determined from CT images plotted against total lung capacity (TLC) as derived from whole-body plethysmography. Data from cystic fibrosis (CF) patients are shown as closed circles and data from non-CF controls (CONTROL) as open circles. The Pearson correlation coefficient (*r*) for pooled analysis is indicated. \* *P*<0.001.

(TIF)

**Table S1. *CFTR* genotypes of patients with CF.**

(DOC)

**Methods S1. Supplementary methods section.**

(DOC)

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## Author Contributions

Conceived and designed the experiments: MOW OW ME MW JB HUK CPH MAM MP. Performed the experiments: MOW OW ME CPH MP. Analyzed the data: MOW OW ME MW MAM MP. Contributed reagents/materials/analysis tools: OW MW HUK CPH MAM. Wrote the manuscript: MOW OW ME MW JB HUK CPH MAM MP.

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